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HIGH CONTENT SCREENING (HCS) OF THE EFFECTS OF 47 EXTRACTS ON AUTOPHAGY, A CELLULAR PROCESS COMMONLY INVOLVED IN THE ONSET OF NEURODEGENERATIVE DISEASES

(scientific disciplinary field BIO/15)

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Abstract

The end of the "era" of synthetic drugs and the development of techniques and technologies of investigations based on computational methods, such as High Content Analysis (HCA), have allowed natural products (NPs) to come back as protagonists in the drug discovery scenery. Autophagy, a physiological cellular process for the maintenance of the cellular homeostasis, is currently one of the most investigated cellular process because involved in many common diseases of the modern age, for example cancer, neurodegeneration and inflammation. In this work an analytical approach based on High Content Analysis methods for the investigation of the effects of NPs on autophagy is presented. The effects of forty-seven extracts from different organisms, like algae, invertebrate and plants, have been investigated by setting up fluorescence and immunofluorescence assays based on autophagy markers; quantitative data describing autophagic phenotypes obtained from the resulting images were then used to define qualitatively their effects on autophagy.

Key words: Autophagy, Lysosomes, LC3B protein, Natural products (NPs), High Throughput Screening (HST).

Long Abstract

Natural products are coming back to cover the role of leading actors in the current drugs discovery landscape, after several years in which the research has preferred to focus on the chemical synthetic drugs. Despite the historical problems associated to the development of drugs based on natural products (such as the high costs, the low yield and the and the long times requesting for production) also the benefits brought by such drugs are currently emerging: in fact, natural products can provide a wide plethora of compounds, having a wider range of specific activities and also offering a starting point for the drug discovery and development processes. Moreover, in support of this new trend, the classical drug discovery approaches have been presently joined by new important techniques and technologies mostly based on computational methods, like the High Content Analysis, which allows to further improve the drug discovery programs based on natural products.

In this context the relatively recent discovery about autophagy process and its involvement in the onset of most of the pathological conditions at the base of the development of many different diseases, such as cancer, neurodegeneration and inflammation, have pushed the researchers to find out new remedies against such pathologies. Autophagy is a physiological cellular pathway which ensures the maintenance of the cellular homeostasis by conveying unused, aged or damaged cytoplasmic material and/or organelles towards the lysosomal degradation through a specific pathway strictly regulated and highly conserved. As autophagy is a dynamic pathway that can lead to many different pathological conditions when its basal flux results to be alterated, compounds which results to be able to perturb the flux rate, increasing or decreasing it, can be an interesting starting point for developing drugs to the diseases associated to the alteration of this physiological pathway.

The present thesis work was focussed on the investigation of the effects produced by the extracts of 47 different organisms (46 plants and just 1 ascidian), received form researchers working in different Countries in the world, on the neuronal cell line SH-SY5Y, with particular interest for the analysis of the autophagic pathway. The investigations of the effects of the extracts consisted in a screening aimed to the selection of those showing the ability to interfere with the autophagy physiological state. The selection was performed on the base of the comparison of the different autophagic phenotypes performed through a High Content Analysis (HCA) approach developed and fine-tuned specifically for this purpose. The phenotypes were detected by fluorescence and immunofluorescence assays specifically designed and the most interesting extracts were then selected by comparing the quantitative results extrapolated from the analysis of the phenotypes produced by the administration of different concentration of each extract with those produced by samples used as controls. The selection of the extracts and their most interesting dilutions, provided the starting material for further deeper investigations aimed to the detection and the isolation of molecules or molecular complexes responsible of the perturbing activity and hence interesting because potentially involved in the development of new drugs for the treatment of the “autophagy-related diseases”. In addition, besides to being simply detected, the most interesting treatments were also sorted depending on their ability in inducing or inhibiting the autophagic rate, thus directly addressing the subsequent analysis by specifying what kind of activity they exert on the autophagic process.

Therefore, during the present thesis work an analytical approach, based on the High Content Analysis (HCA) method, was developed and, in the meanwhile, it was also assessed by performing the primary screening of the activities of 47 extracts of different organisms on a neuronal cell model, after testing also HeLa cells to verify if the method provided reliable results using different cell types as investigation model. Further studies will need to deeper investigate the

selected extracts and to identify and characterize the molecules or the molecular complexes responsible of the detected activities.

Summary

Summary of the figures and tables

1 Introduction

1.1 Natural Products (NPs)

Natural Products (NPs) are compounds derived from natural sources, e.g. plants, animals and microorganisms, which have biological activities (Baker et al. 2007). NPs are also known as secondary metabolites, which are the end-products of the gene expression, generally not essential for the reproduction, the growth or the development of an organism but produced as the results of the adaptation to the environment or as a possible defensive mechanism against predators; in both cases, secondary metabolites are produced to assist and to improve the survival of organism (Dewick 2009; Molyneux et al. 2007, p. 422). The secondary metabolites, and therefore NPs, are produced from biosynthetic intermediates (like Acetyl Coenzime A (acetyl-CoA), shikimic acid, mevalonic acid and 1-deoxyxylulose-5-phosphate) of the most basic cellular processes, such as photosynthesis, glycolysis and Krebs cycle, after modification through numerous different mechanisms and reactions (e.g. alkylation, decarboxylation, aldol, Claisen and Schiff base formation) (Dewick 2009), that allow to produce an infinite number of secondary metabolites even starting from a limited number of intermediates (Dias et al. 2012). The wide diversity of natural chemicals is due to different factors, mostly the countless biodiversity of marine and terrestrial organisms, which produce different chemical structures having an array of biological activities (Dias et al. 2012). Further, the chemical diversity is also the result of millions of years of evolution, that have modified the biosynthetic pathways in response to various biotic and abiotic stresses caused by natural (e.g. viruses and enviromental changes) or unnatural events (e.g. chemicals or radiations) (Sarker et al. 2006). For the reasons listed above, NPs are an essential, reputable source of successful drug leads which originate from Earth's bio-diverse flora and fauna (David et al. 2015).

During time plant NPs have been used as traditional medicines, remedies, potions and oils without any knowledge about the bioactive compounds contained inside but just considering results of hundreds of centuries of man experimenting (Hicks 2014; Kinghorn et al. 2011). The plant of the genus *Salvia* was used by the Indian tribes of southern California in childbirth and male new-born babies were literally "cooked" in the hot ashes of *Salvia* in order to promote their growth as the strongest and healthiest member of the respective tribes and to preserve them from any kind of respiratory ailments for the whole life (Hicks 2014). The plant *Alhagi maurorum* Medik (Camel thorn) secretes a sweet gummy material called "manna", consisting mostly of melezitose, sucrose and invert sugar, which has been documented and claimed to have an important activity to contrast anorexia, constipation, dermatosis, epistaxis, fever, leprosy and obesity by Ayurvedic people (Duke et al. 2007). In addition, *Alhagi maurorum* was also used by other populations: Israelis boiled roots and drank extracts to stop bloody diarrhea, Konkani people smoked it to treat asthma while Romans used plant against nasal polyps (Duke et al. 2007). Another example is *Ligusticum scoticum* Linnaeus, typical plant of Northern Europe and Eastern America, eaten crude as first meal in the day in order to gain a protection from daily infection, while their roots were believed to be a cure for flautolence, an aphrodisiac and used as a sedative in the Faeroer Islands (Beith 1995).

Besides to plants also other sources have provided very important NPs used as drugs in folk medicine. Among fungi, for example, *Piptoporus betulinus*, which grows on birches, was cooked to produce carchoal that had an antiseptic and disinfectant activity (Swanton 1915) or used in stripes to staunch bleeding (Swanton 1932). Another example is *Agaricus campestris* Linnaeus ex Fries (field mushroom), typical of northern and southern temperate zones and the Caribbean, which was used stewed in milk to soothe cancer of the throat (Hatfield 2002). Even if to date there aren't any medicinals derived from lichens, they are largely used in folkloristic applications (Müller 2001).

Well known examples of lichens as therapeutics are *Usnea dillenius* ex Adanson, used to treat diseases of the scalp and as an ingredient in anti-dandruff shampoos and in Ireland to treat sore eyes (Purvis 2000) and *Parmelia omphalodes* (Linneaus) Acharius, abundant in British Isles, where it's sprinkled on stockings before to start a long walk on foot in order to prevent inflammation of the feet (Cameron 1900; MacFarlane 1929), and in Ireland, where is used to cure for bad sores under the chin, burns and cuts (Allen & Hatfield 2004).

Although at a much lower rate, even the marine organisms have provided different therapeutic remedies. The red algae *Chondrus crispus* and *Mastocarpus stellatus* were used to produce a beverage for the cure of colds, sore throats and chest infections, including tuberculosis, and, further, after boiling in milk or water, they were found to be active against kidney trouble and burns (Moloney 1919; Vickery 1995). Another example is the red alga *Porphyra umbilicalis* (Linnaeus) Kützing, that was used against breast cancer (Borlase 1758) or, as in the Aran Islands, for easing indigestion (Ó hEithir 1983).

All the folkloristic knowledges about the therapeutic activity of natural substances have provided the basis for the investigation and the subsequent production of the NPs-based drugs. Following the progress in the chemistry techniques occurred at the dawn of the nineteenth century, plant formulations with therapeutic potential have been well investigated and, in the mid-twentieth century, crude therapeutic formulations have been replaced by partly purified NPs pharmaceuticals (Mishra and Tiwari 2011). Following, a chronological history of natural substances used as therapeutics is reported in order to understand how NPs are become so important for the modern production of drugs.

1.1.1 An historical overview

NPs have been used as therapeutic agents for millennia. The first traces of the use of NPs as medicinals were depicted on clay cuneiform tablets from Mesopotamia (2600 B.C.), which report an elaborated medicinal system consisting in about 1000 plant derived medicines including documented oils from *Cypressus sempervirens* (Cypress) and *Commiphora* species (myrrh) still used today to treat coughs, colds and inflammation (Cragg & Newman 2005). In Egypt medicine knowledge was dated back to about 2900 B.C. and the information in our hands were recorded in the Ebers Papyrus (about 1550 B.C., 9th year of Amenhotep 1 reign), which contains over 700 plant-based drugs (Borchardt 2002; Cragg & Newman 2013; Sneader 2005) ranging from gargles, pills, infusions and ointments. The Traditional Chinese Medicine (TCM) has been extensively documented over thousands of years (Unschuld 1986) and it is collected largely in the Chinese Materia Media (1100 B.C.) (Wu She Er Bing Fang, containing 52 prescriptions), the Shannong Herbal (around 100 B.C., containing 365 drugs) and the Tang Herbal (659 A.C., containing 850 drugs) (Cragg & Newman 2005). Documentation about the Ayurveda system dates back to the 1st millennium B.C. (Patwardhan 2005).

The knowledge about plant-based therapeutics in the Western world is mainly based on the Greek and Roman culture. In particular, compendia written by the Greek and Roman philosophers and natural scientists: the Greek Theophrastus (around 100 B.C.) wrote about medicinal herbs, the Roman Pliny the Elder (1st century A.D.), the Greek (working at Rome) Galen (2nd century A.D.) (Sneader 2005) and the Greek physician Dioscorides (1st century A.D.) reported information about the collection, storage and the uses of medicinal herbs (Atanasov et al. 2015).

During the Dark and Middle Ages (5th to 12th centuries) English, Irish, French and German monasteries preserved the Western plant-based therapeutic knowledge whilst Arabs enshrined the Greco-Roman knowledge, complementing it with their own resources and with herbs from the Chinese and Indian traditional medicines, unknown to the Greco-

Roman world (Cragg & Newman 2013). Further, in the 8th century the Arabs contributed much to the science of pharmacy and medicine introducing the private pharmacies with the Persian pharmacist, physician, philosopher and poet Avicenna and with works such as the *Canon Medicinae* (Cragg & Newman 2005). Around the 10th century, in the south of Italy the Arab culture met the Greco-Roman plant medicinal culture, leading to the development of the famous Salerno school, sponsored and pushed by the emperor Federico II and considered like a precursor of the modern University (Bostock 2002, p. 20).

In the 15th and 16th century a resurrection of the Greco-Roman knowledge occurred, due to the invention of the letterpress by Johannes Gutenberg which allowed to press and distribute in the whole Europe herbal books such as *The Mainz Herbal* (*Herbarius Moguntinus* 1484) and the *German Herbal* (1484), both edited by Gutenberg's partner Peter Schöffer, the *Herbarium Vivae Eicones* (Otto Brunfels 1530), the *Kreütter Buch* by Hieronymus Bock (1546) written in German, *De History Stirpium* by Leonhart Fuchs published in Latin (1542) and also in German during the following years (Sneader 2005).

In more recent times a very important milestone for rational drug discovery from plants was the isolation of the analgesic and sleep-inducing agents *morphium* (morphine, from the Greek God of dreams Morpheus) from opium around 1804 (Dias et al. 2012) by the German apothecary assistant Friedrich Sertürner, who discovered a new class of drugs, the alkaloids. After morphine isolation an intensification of the investigation of other medicinal herbs started and during the 19th century a very wide range of NPs, mostly alkaloids (e.g. quinine, caffeine, nicotine, codeine, atropine, colchicine, cocaine, capsaicin), were isolated and purified form their natural sources (Corson & Crews 2007; Felter & Lloyd 1898; Hosztafi 1997; Kaiser 2008; Sneader 2005; Zenk & Juenger 2007). Compounds like alkaloids were isolated and purified by the apothecaries, who were the progenitors of the modern pharmaceutical companies, and the first example was H.E. Merck in Darmstadt (Germany) that started to extract morphine and other alkaloids in 1826 (Kaiser 2008). Efforts were then focused on the chemical synthesis of NPs, in order to optimize the production, improve the quality and reduce the costs. The first natural compound produced by chemical synthesis was the Salicylic acid in 1853 (Kaiser 2008).

At the beginning of 20th century the most important development in the field of natural substances has been brought by Alexander Fleming, who discovered penicillin (1928), a molecule with antibiotic activity produced by *Penicillium chrysogenum*, at the base of the modern anti-lactam antibiotics. Due to this discovery, most of the efforts of scientific research were focused on the isolation of NPs from microbial source and the scientific and financial foundations support such programs before and during the World War II (Davies 1999), contributing to the war time efforts. After the World War II this trend led to the birth of the modern pharmaceutical industry, that focussed efforts on the research of new molecules with antibiotic activity (Baker et al. 2007). During these years streptomycin, gentamicin, tetracycline and other antibiotics were discovered and the pharmaceutical companies increased the efforts in the development and enlargement of their research and programs concerning natural product discovery and mostly microbial fermentation based technologies (Baker et al. 2007). During the second half of the 20th century every pharmaceutical companies developed a NPs discovery program focused on the treatment of a wide variety of diseases (e.g. anti-bacterial, anti-fungal, infectious diseases). In the 1970s the discovery of two new molecules, compactin (Brown et al. 1976) and mevinolin (Endo et al. 1976), able to inhibit cholesterol biosynthesis, opened a new important research field based on the production of "statins", also called "everyday medication" (Verpoorte et al. 2005), which even today are a very successful medical application, important also for pharmaceuticals business (Baker et al. 2007).

The large success of the NPs derived drug discovery programs, supported by an increment of the patent activity from 1980 to the 90's (Koehn & Carter 2005), created enthusiasm and simultaneously generated pressure to increase the number of new drugs (Dickson & Gagnon 2004; McChesney et al. 2007). In this context, the challenges related to the investigation of NPs along with the development of combinatorial and computational chemistry techniques led most of the pharmaceuticals companies to shift their efforts from the investigation of NPs to the laboratory bench, starting to produce drugs by chemical synthesis (Cragg & Newman 2013), that were collected in even larger libraries and used together with combinatorial chemistry techniques in order to meet the increasing demand for new drugs and to increase the rate of "drug-like" chemical compounds discovery (Baltz 2006). Nevertheless, the NPs based drug discovery programs continue to be an interesting research field and it is carried out especially by academic research within the universities and start-up (Beutler 2009; David et al. 2015; Ortholand & Ganesan 2004; Sheridan 2012).

1.1.2 The issues behind the decline of the NPs-based drug discovery programs

There were many reasons behind the decline of the interest in NPs investigation: high costs, difficulty of sourcing/harvesting biological starting material in adequate quantities for the production of pharmaceutical preparations, the intrinsic variability of biological material itself, the redundancy of the discovered molecules, the low economic return, long times of testing programs and low yields, impracticality of scale-up, difficulties in the isolation and/or purification procedures, high toxicity of some active compounds, ecological and legal considerations, government policies, lack of infrastructures and insufficient capital investments (Bhatnagar & Se-Kwon 2010; Lamottke et al. 2011; Paterson & Anderson 2005; Thomas & Johannes 2011), and, lastly, the development of combinatorial and computational chemistry techniques which promised to overcome most of the issues listed above and led to the production of drugs by chemical synthesis, shifting the drug discovery efforts from nature to the laboratory bench (Cragg & Newman 2013). Below, some of these reasons will be thoroughly discussed.

Since plant sources are usually collected directly from their natural habitat, it is difficult to get a correct identification and to assign them the right nomenclature. This could be a huge problem because the identification represents the first step and the essential starting point for the NPs based drug discovery process (Baker et al. 2007). To achieve a good identification a combination of different methods is needed, such as morphological and anatomical characterization flanked by genetic and chemical analysis (Bucar et al. 2013). This important issue became a challenge considering that plant taxonomy is continuously modified, synonyms are even more frequently introduced and it is not possible to automatize all the tasks (such as plant material collection, accurate documentation, botanical identification and preparation of herbarium vouchers) (David et al. 2015) but, rather, there would be needing of specialists, who instead are becoming rarer (Bucar et al. 2013). Even if the identification of the harvested plants could be perceived as a tedious and unnecessary work, it enables to know the material under analysis and allows a fruitful usage of databases, such as the Dictionary of Natural Products (CRC Press Taylor & Francis Group 2017) that correlates the plant "*Latin name*" with their chemical content and constitutes a very precious and powerful tool for the NP chemist (Erkens 2011).

Another problem related to the use of plants as a source for identification of bioactive compound is the accessibility of the starting material and the subsequent recollection of the plants which will result to be more interesting (Atanasov et al. 2015). Often, the available amount of natural products is too low for testing for a wide range of biological activities and the situation becomes even worse when a certain extract looks like to have a promising bioactivity and it's

considered as a pharmaceutical lead, so needing of much larger quantities for characterizing of pharmacological activities of its constituents. Factors affecting the availability of the plant material are, mainly, the amount of the plant in nature, the need to respect the habitat of the wild plants to collect (especially for protected species), natural seasonal cycles and also the change in chemical composition due to the life cycle of the plant, natural catastrophes and, sadly, the anthropic pressure, including deforestation, wildcrafting, local wars and changing of paradigm of legal regulations for travelling and export of plant material (Atanasov et al. 2015). The importance of plant material accessibility has been described in a recent study by Amirkia & Heinrich (2014), in which the authors investigated the correlation between the abundance of species in which alkaloids occur and their use as pharmaceutical drugs. Considering the species distribution proposed by the Global Biodiversity Information Facility (GBIF), the authors concluded that 93% of the alkaloids used in medicines occur more than 50 times in the GBIF database, while only two have less than 10 occurrences, suggesting that NPs contained in many species are the most favourable for medicinal uses and that the supplying of constraints are a huge obstacle for the research, development and commercialization of NPs. On the other side, it is also true that when a plant starts to be commercialized as a herbal medicine or used to produce pharmaceutical drugs, its populations, subjected to extensive wildcrafting and unsustainable harvesting techniques, become threatened, (Cordell 2011; Vines 2004). An example was the so-called "taxol-supply crisis" due to the increasing demand for the bark of the western yew (*Taxus brevifolia L.*), with a consequent ecological impact of the intensive bark collection (Cragg et al. 1993; Kingston 2011). However, although the cultivation could be a good, accessible and sustainable alternative route to get starting plant material, approximately two third of 50,000 medicinal plant species are still wildcrafted (Canter et al. 2005). Because of these conflicting aspects, the institutions developed guidelines in order to promote sustainable plant collection techniques and to reduce the ecological problems produced by wildcrafting of medicinal plants (Atanasov et al. 2015): such rules are contained in the "Guidelines on Good Agricultural and Collection Practies (GACP)" published by World Health Organization (World Health Organization 2003) and the "Committee on Herbal Medicinals Products (HMPC)" published by European Medicines Agency (European Agency for the Evaluation of Medicinal Products 2006).

An important aspect about the accessibility of medicinal plants is the respect of the international, national and local laws concerning plant access, sharing of benefits and patentability of the plant material (Atanasov et al. 2015). A significant step of the legal regulation of the plant material usage occurred on the 29th December 1993, when the responses of the Convention of Biological Diversity (CBD) were applied and all the genetic resources moved from a common heritage of mankind to the sovereignty of the States where they live (David et al. 2015). The United Nation's CBD was an agreement signed in June 1992 by the international community gathered in Rio de Janeiro, Brazil, that has established three main rules: 1) conserving the biodiversity; 2) sustainably using its genetic resources; 3) sharing the benefits from their use in a fair and equitable manner (Cragg et al. 2012; Kingston 2011; Soejarto et al. 2004). Article 2 of the CBD defines biodiversity as – "*the variability among living organisms from all sources of terrestrial, marine and other aquatic ecosystems and ecological complexes.*", including the ecosystem diversity and the diversity between and within species, and according with this article the CBD applied its three rules to the genetic resources, defined as "*any material of plant, animal, microbial or other origin containing functional units of hereditary of actual or potential value*" (United Nations 1992). With these rules CBD provide an instrument for Countries to regulate and define the exploitation of the plant material as source for drug discovery, however many issues remained open, especially on the access and benefit sharing (Cragg et al. 2012; Kingston 2011). In particular, many developing Countries maintain the skepticism towards bioprospecting and, on the other hand, the expectations of the biodiversity-rich Countries on the potential profits related to the pharmaceuticals developed from their genetic resources have been betrayed since from

12,000 species investigated (112,000 extracts) by the US National Cancer Institute (NCI) over decades only taxol and comptothecin are currently used as pharmaceuticals drugs (Atanasov et al. 2015). Such issues hampered the access to the plant materials and at the beginning of the new century the Big Pharma industries decommissioned their NPs-based drug discovery programs (Cragg et al. 2012; David et al. 2015; Kingston 2011). Following these events, the international community gathered in Nagoya in October 2010 produced the Nagoya protocol, a legally binding document published in 2011 and applied in October 2014, in which the rules on the genetic and non-genetic resources were taken out and shared by 50 Countries in order to clarify issues about access and benefit sharing (Burton & Evans-Illidge 2014; Oliva 2011). The protocol clarifies that all public or private researchers that would enter in a Research & Develompment process must request a Prior Informed Consent (PIC) and negotiate a Mutually Agreed Term (MAT) about the condition of access and benefit sharing with the representative of the source Country (David et al. 2015). The establishment of these regulations, helped also by the implementation of the national laws, should lead to revitalize the interest in the investigations of plants from biodiversity-rich Countries with a careful eye to preserving biodiversity (Burton & Evans-Illidge 2014; Cragg et al. 2012) however the protocols could lead to stricter regulations and limit drug discovery and biodiversity conservation (Gilbert 2010). These concerns are actually reflected in reality: the daily applicability of the rules, in fact, is very far from the theoretical concepts included in the protocol (Kingston 2011). Further, there is a very huge distance between the expectation of the biodiversity-rich Countries and academic or industrial users in the field of pharmacy and cosmetology about benefit sharing and, in addition, the negotiations of the access permits to the biodiversity-rich Countries are very difficult and take several years of uncertain processes (David et al. 2015).

Besides the accessibility, also the quality and the chemical composition of the plant materials are crucial because they can interfere with the determination of therapeutic claims and can make problematic the re-isolation of NPs problematic and, therefore, these issues should be taken into consideration more often with acuity (Kusari et al. 2012). The chemical composition depends on a lot of factors, such as species identity, harvesting time, soil composition, altitude, actual climate, possession and storage conditions; in addition, also extraction and isolation processes can introduce transformation and degradation of compounds (Bucar et al. 2013; Jones & Kinghorn 2012). A determining aspect influencing the chemical composition of the starting plant material is represented by the presence of endophytic organisms, such as fungi and bacteria, which inhabit inside plants and produce their own secondary metabolites. As a consequence, plant extracts may contain NPs derived from endophytic organisms or produced by plants as a results of the interaction with them (David et al. 2015). The wide chemical complexity of the NP structures, which contain numerous oxygen-containing substituents and chiral centers, is also another important issue which can discourage the development of methods for total synthesis or derivatization, needed for a proper optimization of the drug candidates. In this view, pharmaceutical leads originated from synthetic libraries are usually preferred because their development as well as their modification are very easier to realize (Feher & Schmidt 2003) and can be achieved using simpler chemical approaches (Butler 2004; Henrich & Beutler 2013; Li & Vederas 2009).

Important obstacles to the investigation of plant extracts were also the determination of the precise molecular mechanism of action of the natural products, even if a detailed knowledge of the interaction of a drug candidate compound with its molecular target was available, as well as the conduction of rigorous clinical trials in order to approve NPs as drugs, since the patentability of the NPs not synthetically modified is a very controversial matter (Corson & Crews 2007). Guidelines regarding the patentability of NPs were issued the last time on 4th of march 2014 by the United States Patent and Trademark Office with the publication of the document known as “Guidance For

Determining Subject Matter Eligibility Of Claims Reciting Or Involving Laws of Nature, Natural Phenomena, & Natural Products". In this document it was established that in a patent claim a "marked difference" from a known natural law, material or phenomenon must be demonstrate and it needs to be approved by two relevant high-profile Supreme Court decision: the Association of Molecular Pathology versus Myriad, which ruled that isolated and purified DNA is not patentable, and Mayo versus Prometheus, which ruled that methods for determination of optimal drug doses based on levels of naturally occurring metabolite is not eligible for patenting (Harrison 2014).
In this context, next to the issues regarding patentability of NPs, pharmaceutical companies decided to shift their interest from small molecule-based drug discovery toward big biological molecules, such as proteins or nucleic acids, known as "biological" (Appendino et al. 2010, p. 221). Anyhow, as the cost of biologicals for patients are much higher than small molecules drugs, their use increases the pressure on national health insurance and a cost decreasing isn't expected in the near future, then a rapid turn back to the old small molecule-based approaches is probable (Appendino et al. 2010, p. 221).

One of the most important cause of the decline in the interest for NPs was due to the development and the always wider use of the combinatorial and computational chemistry techniques, in particular High Throughput Screening (HTS) (Koehn & Carter 2005). HTS techniques allowed to increase the number of samples analyzed per day, even if the investigation of plant extracts using this technology becomes very challenging. The samples to investigate should not decompose or precipitate, do not interfere with assays reagents or show unspecific effects, however very often plant extracts fail in fulfilling these requirements (Atanasov et al. 2015). Crude plant extracts are a complex and sometimes high viscose mixture of many different compounds that tend to aggregate or precipitate; they contain components that unspecifically bind proteins, resulting in misleading assay outcomes and therefore necessitating of sophisticated sample preparation and fractionation techniques (Coan et al. 2011; Johnson et al. 2011; Maes et al. 2012; Schmid et al. 1999; Tu et al. 2010).

Other issues limiting the applicability of HTS techniques to the investigation of crude plant extracts are the presence of fluorescent or fluorescence quenching compounds, which interfere with fluorescent and/or colorimetric HTS endpoint measurements (Gul & Gribbon 2010; Henrich & Beutler 2013; Zou et al. 2002). Moreover, plant extracts may contain organic and inorganic molecules able to lead analysis to false positive or false negative results: highly apolar compounds such as fatty acids (Balunas et al. 2006) and common polar molecules such as polyphenols, flavonoids (Zhu et al. 1997; Zhu et al. 2011; Zou et al. 2002) and chlorophyll (Henrich et al. 2006), as well as metals (Hermann et al. 2013), commonly concentrated by the plants from the environment (Fernando et al. 2013) and present in commercially available plant samples (Eisenberg et al. 2011), can interfere with a wide range of different assays. Furthermore, compounds with cytotoxic activity can also mask the detection of other possible bioactive compounds in the plant extracts: saponins, for example, hold a detergent effect which can interfere in cell-based assays, leading to the lysis of the cells (Henrich & Beutler 2013).

1.1.3 Back to the NPs-based drug discovery

As previously introduced, most of the pharmaceutical companies moved their interest toward the development of drugs based on synthetic molecules grouped in very large libraries promising to overcome all the problems and limitations reported above and to ensure a strong increment in the rate of discovery and marketing of new drugs.

However, despite forecasts, chemical synthesis didn't satisfy the expectations and the rate of new pharmaceuticals reaching the market is decreased. For example, the failure of the chemical synthesis results evident considering that of the 1,135 new drugs approved from 1981 to 2010 only 36% were purely synthetic, whereas more than a half were molecules from natural sources, derivatives or analogues (Newman & Cragg 2012). More specifically, considering the period between 1981-2002, 61% of the 877 novel medicines based on small molecule came from NPs: 6 % were NPs, 27% were NPs derivatives, 5% synthetic compounds with NPs-derived pharmacophore and 23% synthetic compounds designed from NPs, or NPs mimic (Newman et al. 2003; Yuliana et al. 2011). Further, instead to increase the approval rate of new drugs, the number of pharmaceuticals reaching the market decreased: from the 45 new drugs approved by US FDA in 1990, only 21 new molecules were approved in 2010 (David et al. 2015; Kingston 2011).

The reasons of such declining trend are very complex (Kola & Landis 2004) and the most important evidence is that the chemical diversity of the compounds contained in the synthetic libraries is often nearly or completely absent (Bauer et al. 2010). Most of the HTS-compounds libraries have been generated by similar strategies and can contain the same molecules, resulting in an overlapping effect known as "attrition rate", because of which the compounds are often selected from the libraries very quickly, basing on their potency values (Scannell et al. 2012), but at the same time they can have a negative correlation concerning ADME/T (Adsorption, Distribution, Metabolism, Excretion/Toxicity) (Gleeson et al. 2011).

The statistics described above led the researchers to renew the interest in the investigation of NPs. This new trend, probably destined to grow and to lead a substantial amount of NPs to successfully reach the market in the near future (Lamottke et al. 2011), is also supported by the objective truth that plant kingdom includes a very high number of species, each of which able to produce a wide range of bioactive compounds based on different chemical scaffolds. In 2015 the International Union for Conservation of Nature (IUCN) reported that around 310,000 plant species have been described so far (International Union for Conservation of Nature 2017). Of the total plant species known today only around 60,000 species, approximately 20%, have been already screened and they have provided 135 known drugs (David et al. 2015; Farnsworth 1990; Newman et al. 2003): as a consequence, making a quick projection, these numbers suggest that the screening of the remaining plant species could lead to detect around 700 new drug candidates. Moreover, most of the 60,000 already screened plants were investigated for their effects against a limited number of disease targets and there are still chances to find other or new effects against neglected disease targets (Miller 2011); in addition, they can be useful as molecular probes to identify disease relevant targets (Schmitt et al. 2011). The picture become even more promising considering that the total world's biodiversity consists of 2 million species of plant, animals, fungi, and micro-organisms and alike and more than 95% of the biodiversity in the world has not been evaluated for any biological activity (David et al. 2015); as a consequence, it could be very important to try to efficiently access and take advantage to this natural reservoir of chemical diversity (Dewick 2009; Mishra & Tiwari 2011; Molyneux et al. 2007, p. 422).

Aside these numbers, many other evidences have contributed to the renewal of the interest in NPs investigation. For example, as previously said NPs are made from living organisms and possess properties optimized during and by evolution for specific biological functions, such as binding to specific target proteins or other biomolecules (Appendino et al. 2010, p. 210; Hunter 2008). Unluckily, such wide variety of properties and functions implies also a high structural complexity with respect to molecules created by combinatorial synthesis, designed to have a simpler structure, and this structural complexity means more difficult and expensive isolation methods and techniques. Examples of elements that contribute to increase structural complexity of NPs are a high molecular weight, higher number of chiral centers and freely rotatable bonds, lower chain lengths, higher number of rings, more oxygen and less nitrogen, sulfur, and halogen atoms, a higher number of Lipinski-type H-bond acceptors and donors, and lower calculated octanol-water partition

coefficient (cLogP values), as well as the complexity of the ring system and the degree of saturation (Feher & Schmidt 2003; Koehn & Carter 2005; Lee & Schneider 2001; Stahura et al. 2000). Nevertheless, all the apparently negative structural features described above, in particular the lower number of chiral centers and the higher size and flexibility, contribute for stronger drug candidate molecules and more specific activities compared to synthetic compounds (Feher & Schmidt 2003; Klebe 2009), confirming that NPs have selective biological activities, major chemical diversity and complexity developed during the biosynthesis (Clardy & Walsh 2004; Koehn & Carter 2005) in addition to more advantageous ADME/T properties. It is also important to consider that "natural drugs", unlike synthetic drugs, imply also an important reduction of harmful side effects and, furthermore, NPs in medicinal plants resulted also to be able to efficiently mitigate side effects of serious illnesses or severe therapy, such as onco-chemotherapy or radiotherapy, although "natural" is not a synonymous of "harmlessness" (David et al. 2015). The evidences just described are known because medicinal plants are often accompanied by well documented ethnopharmacological information about the traditional use of the plant, that can provide feedback for detection of compounds effectively active in humans (Corson & Crews 2007; Heinrich & Gibbons 200; Heinrich 2010; Kinghorn et al. 2011). Supporting these knowledges, the analysis of 122 compounds derived from plants, selected because globally used as drugs, have revealed that 80% of them were contained in plants having an ethnomedical use identical or similar to the prescription of the respective pure compounds (Fabricant & Farnsworth 2001; Farnsworth et al. 1985). An important threat for this advantage is that more information regarding knowledge about traditionally used medicines risks to be lost forever, since they are disappearing even faster than the biodiversity of the plant species, especially with the modern phenomenon of globalization (Appendino et al. 2010, p. 224). Anyhow, at current time herbal phytopharmaceuticals constitute an important share of the total pharmaceutical market, that consists in US 60$ billion with an annual growth rates of 5-15% (Naoghare & Song 2010), also due to their wide and increasing application in psychosomatic, metabolic and minor disorders. In addition, medicinal plants and/or herbs are essential for more than 70% of the world population which has not access to the overpriced Western medicine: for these reasons, traditional medicine is highly recommended by WHO, as endorsed in Beijing declaration in 2008 (World Health Organization 2008), especially for local conditions and the future of health in both developing and industrialized Countries, which are facing a dramatic increment of the health costs mostly due to the "*single molecule*" medicine (David et al. 2015).

Nowadays, after the failure of chemically synthesized drugs, the market is expanding in direction of natural, traditional medicine, available at relative low costs (Lawson 2013). In this context, NPs extracts (botanicals or phytopharmaceuticals) can play an important role in therapy because they can be used in the form of dietary supplements, drugs or botanical drugs (Schmidt et al. 2008) and could eventually get the status of current registered pharmaceuticals, released by competent organizations such as the FDA in case they overcome clinical trials and demonstrate efficacy and safety (David et al. 2015). Supporting this new trend, in 2012 the Dutch Medicine Evaluation Board approved the introduction of the first TMC product (Traditional Chinese Medicine) in a European Union Country, a dry extract of *Dioscorea nipponica* commonly used in Chinese botanical tradition to give relief from headache, muscle pain and cramps (Gilbert 2012).

An important issue to consider in this context is that these new medicine formulations, like *Dioscorea nipponica*, have led to overcome the previously mentioned concept of "*single molecule*" medicines, that has always accompanied the production of drugs in the last centuries. Indeed, in many cases it is not possible to explain the efficacy of such plant preparations by the presence of a single active molecule because traditional medicines work by taking a complicated mixture of different NPs that can potentially act on multiple targets (Gertsch 2011). As a consequence, presently the development of drugs is based on the production of plant extracts with defined phytopharmaceuticals (EU) or botanicals

(USA) composition (Chen et al. 2008; Hoffman & Kishter 2013), and their status depends from the health claims made in the different Countries: they can be registered either in the form of dietary supplements or as a drugs in the case that the clinical studies are performed and successfully surpassed (David et al. 2015). A recent example of this kind of products has been the approval of Vergen® (an enriched extract of tea polyphenols), a useful remedy for the treatment of genital warts caused by human papilloma virus (Scheinfeld 2008) currently under clinical trials against various cancers as both preventative and as a direct agent (Newman & Cragg 2012).

One of the problem linked to the use of plant extracts per se as drugs is the possible presence of pesticides or heavy metals, as in part permitted by the Good Agricultural Practices (GAP) (Zhang et al. 2010). The presence of these toxic constituents (e.g. hepatotoxic compounds such as pyrrolizidine alkaloids or aristolochic acids) involves a risk that must be identified before to human administration (Chen et al. 2012; Stickel et al. 2005). For this reason, all the plants useful for drug formulations should be Generally Recognised As Safe (GRAS), a classification recognized and regulated by authorities such as the Food and Drug Administration (FDA), the European Medicines Agency (EMA) or the European Food and Safety Authority (EFSA) (Nicoletti 2012). In order to carry on the production of drug based on plant extracts it's important to manage, define and authenticate the chemical composition of the proposed natural mixtures. Standardization procedures currently available consist in the quantification of active principle(s) (when they are known), the detection of chemical marker(s) for correctly establishing the botanical origin of the material, the determination of the complete metabolite profile (metabolome) and a comprehensive estimation of the biological variability of the extracts (van der Kooy et al. 2009) in addition to various analytical validations to verify the absence of toxic or allergenic compounds (Ribnicky et al. 2008).

The use of plant extracts as drug formulations led to consider the important advantage that most of the NPs exist into the plants in form of conjugates with sugar moieties, called glycosides, in order to store secondary metabolites in a form not directly active on therapeutic targets. The processes of glycosides formation have been optimized during evolution in order to activate these molecules when needing and only after their metabolization; they can be used as pro-drugs activated by enzymatic metabolization. For example, as in the case of laxative herbs (e.g. Aloe), glycosides activated by the reductive environment of the intestine and reach the target in their active form as anthrones (Bruneton 2009). The achievement of the concept of NPs as pro-drugs is very important since can explain why a huge portion of NPs already known need to be chemically modified to gain their optimal efficacy and to minimize their toxic effects (Newman & Cragg 2012).

The longstanding and extensive usage of traditional medicines combined with herbal medicines with respect to isolated single molecules has made necessary to find a rationale for their observed pharmacological and therapeutic superiority (Wagner & Ulrich-Merzenich 2009). The superior efficacy can be attributable to a synergistic effect caused by the different bioactive NPs simultaneously present in plant extracts, although is very difficult to experimentally confirm. Anyhow, to support this hypothesis, synergistic effects can be ascribable to different possible occurrences: 1) NP extract consisting compounds affect different targets; 2) compounds can interact together thus improving the bioavailability of one or several active molecules; 3) compounds activity/efficacy could result enhanced by the effect of agents that antagonize eventual mechanisms of resistance (Wagner & Ulrich-Merzenich 2009). The only possibility to verify a supposed synergistic effect is the analysis of the isobole curves

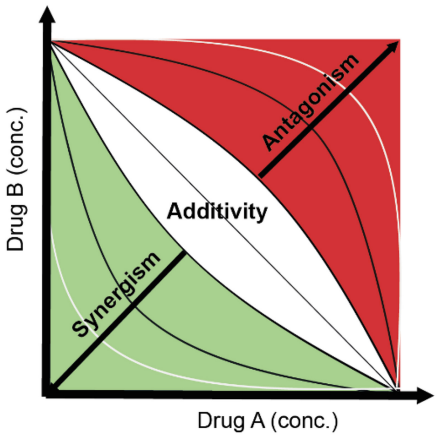


Figure 1. Example of generic Isobole Curves graph.

of the pharmacological effects of the mono-substances versus the mixture, considering data from several dose combinations: this analysis allows to discriminate between simple additive effects, antagonistic interactions or real synergism with potentiated or over-additive effects, as shown in Figure 1 (Berenbaum 1989; Yang et al. 2013).

Despite the evolutionary traces about molecular synergism in nature and though many studies and investigations have shown evidences supporting the therapeutic superiority of plant extracts over single isolated molecules, experimental data confirming that such clues are still lacking and new concepts such as poly-pharmacology and pharmacology network are emerging in the context of the pharmacology of botanical drugs (Gertsch 2011). Complicated aspects, such as multi-component mixtures acting in complex within biological systems, may be resolved by the emerging system biology approaches (Fitzgerald et al. 2006; Verpoorte 2012; Wang et al. 2005); in particular, poly-pharmacology and synergism are building the new paradigm in NPs drug discovery and "omics" approaches have been recently applied to extensively investigate TCMs (Buriani et al. 2012). However, in spite of the rapid development of system biology, only a few studies have been currently published and inclusive approaches combining phytoprofiling and metabotyping are emerging (Xie et al. 2013). In a futuristic view it is possible to speculate that these holistic approaches could prove the efficacy of personalized medicine, which is intrinsically correlated to the traditional medicines commonly carried out by healers (Verpoorte et al. 2005) thus confirming that the evidence-proved phytotherapy can provide a way to differentiate placebo effects from real pharmacological efficacy (David et al. 2015).

Furthermore, in this scenery characterized by the emerging interdisciplinary approaches, all the information coming from traditional medicine or concerning clinical trials on NP extracts are potentially useful to find new targets by the reverse pharmacology methodology ("target based drug discovery") or explain the mode of action of specific botanicals. Also "Bedside to bench" approaches combined with system biology could be very interesting, as well as the application of ethno-pharmacological know-how to modern in silico tools could lead to the discovery of new NPs (Rollinger et al. 2006; Rollinger 2011). Another possible approach consists in the use of "virtual screening" software for the analysis of libraries of compounds to test panel of target (e.g. antitumor) in order to identify selectivity and specific pharmacological activities (Lauro et al. 2012).

1.1.4 Historically important Natural Products

Traditional medicine has provided the basis for most of the modern medicines. Probably, the most famous and well known example of NPs derived drugs is the anti-inflammatory agent acetylsalicylic acid (Figure 2. A) (aspirin), derived from salicin, that is found in the bark of the willow tree *Salix alba* L. (Der Marderosian & Beutler 2002). Another example of widely known and used NPs is the alkaloid morphine (Figure 2. B), isolated from *Papaverum sonniferum L* (opium poppy) e firstly reported in 1803. This alkaloid is currently very important as commercial drugs and has been also used to produce other famous molecules since in the 1870s, after boiling in acetic anhydride, crude morphine was converted in diacetylmorphine (heroin) and found to be readily converted to codeine (painkiller) (Dias et al. 2012).

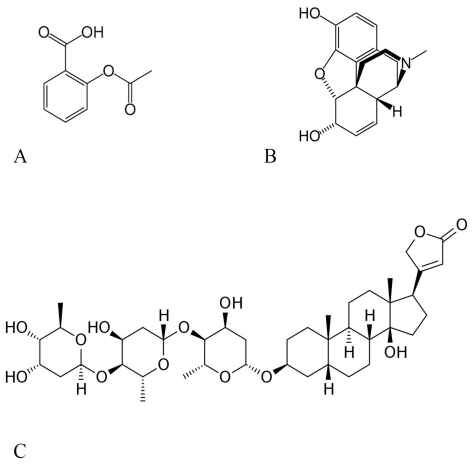


Figure 2. Structure formulas of Acetylsalicylic acid (A), Morphine (B) and Digitoxin (C).

Historically, it's documented that poppy extract was medicinally used by Sumerians and ancient Greeks, and Arabs described opium as addictive (Der Manderosian & Beutler 2002). Even if *Digitalis purpurea* L. (foxglove) was present in Europe in 10th century, only after 1700s its active constituent digitoxin (Figure 2. C) was found to be a cardiotonic glycoside and thereby able to enhance cardiac conduction and to improve strength of cardiac contractibility. Digitoxin (Figure 2. C) and its analogues have long been used in the treatment of congestive heart failure and, due to their possible long term detrimental effects, then replaced by other medicinals (Der Manderosian & Beutler 2002).

A very famous NPs-based drug widely used in the treatment of breast cancer is paclitaxel (Taxol®) (Figure 3. A), isolated for the first time from the bark of *Taxus brevifolia* (Pacific Yew) during 1960s, after their collection by United States Department of Agriculture (USDA) within the exploratory plant screening program at the National Cancer Institute (NCI) (Cragg 1998). The first of several FDA approvals for various application of Taxol® was announced in 1992 (Cseke et al. 2006) but due to the very low yield of extraction from the bark and, at the same time, the very high demand for the drug, paclitaxel is currently produced synthetically (Dewick 2009), though this process is high challenging and expensive (Nicolaou et al. 1994).

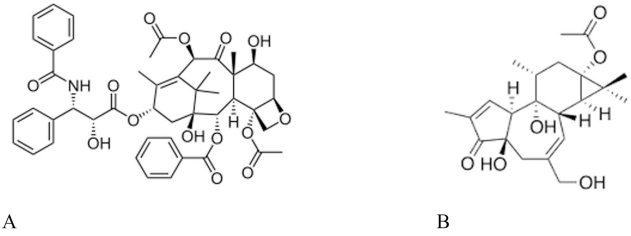


Figure 3. Structure formulas of Paclitaxel (Taxol®) (A) and Prostratin (B).

Several NPs are currently used also to treat Acquired Immune Deficiency Syndrome (AIDS). After the pandemia in 1980s, the National Cancer Institute (NCI) and other Organizations started to explore extracts of plant and marine organisms in order to find potential drug candidates for the treatment of lymphoblastic cells infected with HIV-1. This researching work has led to select a compound called prostratin (Figure 3. B), a protein kinase C activator isolated from the bark of *Homalanthus nutans* (mamala tree of Samoa), which surpassed Phase I of human clinical trials carried out in 2010 by AIDS ReSearch Alliance in Los Angeles, California (Dias et al. 2012).

In addition to NPs derived from plant sources, also other natural organisms have provided important compounds with therapeutic activity. A well-known example is penicillin, a NPs produced by fungus *Penicillium notatum* and discovered by Fleming in 1929 (Mann 1994). Penicillin was produced in high yield using a countercurrent extractive separation technique developed by Fleming, Chain and Florey that allowed to support the *in vivo* experimentation leading them to save a huge number of lives and to the victory of the 1945 Nobel prize in Physiology and Medicine (Stamets 2002). This discovery is a milestone in the NPs history because it led to the production and commercialization of synthetic penicillins in the early 1940s revolutioning the drug discovery research (Elder 1970; Mann 1999; Lax 2004; Wainwright 1990). In fact, after the publication of the first clinical data on penicillin G (Figure 4. A) in 1942-1944, there was a worldwide effort in the discovery of new antibiotics from microorganisms (Buss & Waigh 1995, pp. 868-884; Williams 1999) until to

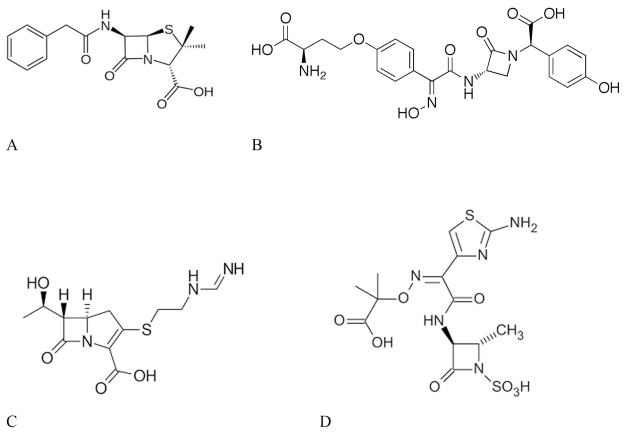


Figure 4. Structure formulas of Penicillin G (A) and Norcardicin (B), Imipenem (C) and Aztreonam (D).

introduction in the 1970s of new screening methods which allowed the discovery of novel antibiotics structural classes such as norcardicins, carbapenems and monobactams represented by the isolated antibiotics, respectively, norcardicins (Figure 4. B), imipenem (Figure 4. C) and aztreonam (Figure 4. D) (Fabbretti et al. 2011).

In the context of modern NPs-based drug discovery a predominant role is attributed to marine environment. Being the 70% of earth's surface covered by oceans, it's possible to consider this environment, consisting in a very high and unique biodiversity, as an important source for potential drug candidates (Haefner 2003). In the wake of these consideration, exploration of marine environment and organisms (algae, sponges, ascidians, tunicates and bryozoans) is started in 1970s and still continue today by exploiting various techniques and technologies, such as the SCUBA (1970s), the use of manned submersibles (1980s) and the more recent of remotely operated vehicles (ROVs, 1990s) as well as the modern snorkeling, resulting in the isolation of thousands of structurally unique bioactive marine natural products and consisting in three FDA approved drugs, one EU registered drug, 13 NPs (or derivatives thereof) in different phases of clinical trials and many other compounds in pre-clinical trial (Mayer et al. 2010). Some examples of these NPs coming from marine organisms are Plitidepsin (Aplidin®, PharmaMa) (Figure 5. A), a depsipeptide isolated from the Mediterranean tunicate *Aplidium albicans*, resulted effective against various kind of cancers, including melanoma, small cells and non-small cell lung, bladder as well as non-Hodgkin lymphoma, acute lymphoblastic leukemia and currently in Phase II of clinical trials (Mayer et al. 2010; Henríquez et al. 2005), or Ecteinascidin 743 (ET743; Yondelis^{TM}), isolated in very low yield from ascidian *Ecteinascidia turbinate* (Rinehart et al. 1990; Wright et al. 1990) and for this reason today produced as semisynthesis molecule using well established procedures (Cuevas & Francesch 2009; Henríquez et al. 2005; Manzanares et al. 2001). Ecteinascidin 743 is also known as Trabeactidin (Figure 5. B) and in October 2007 has become the first marine anticancer drug approved in EU, then approved by the European Agency for Evaluation of medical Products (EMEA) and is completing key Phase III of clinical trial for approval in the US (Mayer et al. 2010).

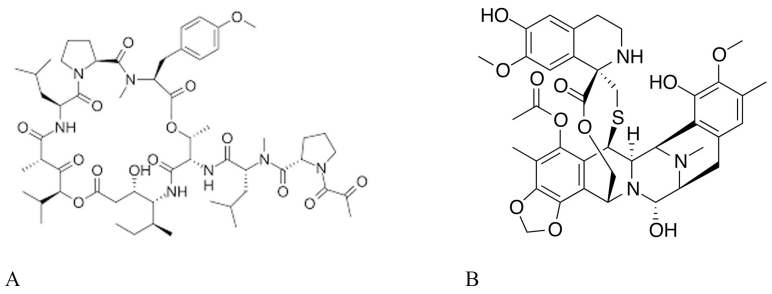
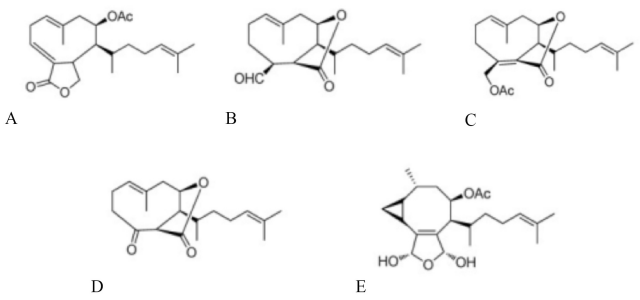


Figure 5. Structure formulas of Plitidepsin (A) and Trabeactidin (B).

Green, brown and Red algae also represent an important source of NPs, mostly with antimicrobial and antifungal activities (Baslow 1969), and grouped in different classes deriving from the class of terpenoids, such as brominated, nitrogen and oxygen heterocycles, phenazine derivatives, sterols, amino acids, amines and guanidine derivatives (Bhakuni & Rawat 2005). Examples of these compounds are diterpenes 4-acetoxydictylolactone (Figure 6. A), dictyolides A (Figure 6. B) and B (Figure 6. C) and nordictyolide (Figure 6. D), isolated from the brown alga *Dictyota dichotoma* for their antitumor activities (Faulkner



***F*igure 6.** Structure formulas of 4-acetoxydictyololactone (A), Dictyolides A (B), Dictyolides B (C), Nordictyolide (D) and Crenuladial (E).

1988; Ishitsuka & Kusumi 1988), or crenuladial (Figure 6. E), found in the brown alga *Dilophus ligatus* and selected for its antimicrobial activity against *Staphylococcus aureus*, *Micrococcus luteus* and *Aeromonas hydrophyla* (Faulkner 1988; Tringali et al. 1988).

Concluding, it's important to remember that the first notable biologically active compounds derived from marine organisms were the C-nucleosides Spongouridine (Figure 7. A), and Spongothymidine (Figure 6. B), isolated from the Caribbean sponge *Cryptotheca crypta* in the early 1950s (Watanabe et al. 1989). These two natural compounds were found to have an antiviral activity and the synthesis of structural analogues led to the development of Cytarabine or cytosine arabinoside (Ara-C) used as potent clinical antileukemic agent and to the production 15 years later of Vidarabine or 9-β-D arabinofuranosiladenina (Ara-A), produced as antiviral agent (Watanabe et al. 1989).

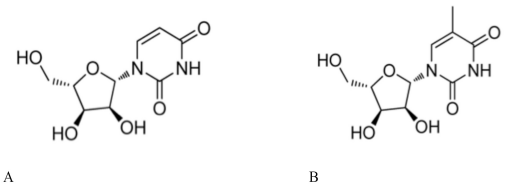


Figure 7. Structure formulas of Spongourdine (A) and Spongothymidine (B).

1.1.5 Approved NPs-based new drugs

The list of the FDA approved drug is reported in many different archives available on the web as for example US Food and Drug Administration (n.d.) or Drugs.com - Know more. Be Sure (n.d.), that collect all the drugs without distinguish between NPs derived and chemical synthesis derived drugs. In its M. Sc. Thesis work, Karlsdóttir (2016) have selected all the 279 NP and NP derivative NCEs (New Chemical Entities), which represents the18.4% of the total 1515 NCEs approved by the FDA by the end of 2015 (Kinch et al. 2014; Us Food and Drug Administration 2015 n.d.). Many papers (and references therein) can be also consulted for detailed information about specific NPs and respective derived drugs, some examples among these are Cragg & Newman (2013), Harvey (2014), Kurkov & Loftsson (2013), Miller & Lanthier (2015), Newman & Cragg (2016), Rangel & Falkenberg (2015).

1.2 Autophagy

Autophagy, or autophagocytosis (from the ancient Greek αὐτόφαγος autóphagos, meaning "selfdevouring" and κύτος kýtos, meaning "hollow") is a general term used to define the digestion of cytoplasmic material and organelles in strictly regulated and highly evolutionarily conserved lysosomal pathway (Cuervo 2004; Eskelinen 2008; Klionsky 2005; Klionsky 2007; Kundu & Thompson 2008; Levine & Klionsky 2004; Mizushima & Klionsky 2007; Shintani & Klionsky 2004).

Autophagy was observed for the first time by Keith R. Porter and Thomas Ashford at the Rockfeller Institute in the early '60s: they reported that an increment of the number of lysosomes in rat liver cells occurred after addition of glucagon, and that some of these lysosomes containing other organelles, such as mitochondria, shifted from the center of the cell (Ashford & Porter 1962) and called this autolysis. However, they got an incorrect interpretation of their data about lysosomes formation because they ignored the pre-existing organelles. One year later, a detailed ultrastructural description of "focal cytoplasmic degradation" clarified that there were three different stages of maturation of the sequestered cytoplasm to lysosomes, and that the process was not limited to injury but instead it was active and

functional under physiological conditions for “reutilization of cellular materials” and for “disposal of organelles” during differentiation (Hruban 1963). Based on this new evidence, in occasion of the *Ciba Foundation Symposium on Lysosomes* that took place in London in 1963, the Belgian biochemist Christian de Duve coined the term “autophagy” to define this phenomenon (Klionsky 2008). Few years later, during investigation of the role of glucagon in the induction of cell degradation in the liver together with his student Deter, de Duve assessed autophagy as a part of lysosomal function and establish the responsibility of lysosomes in glucagon-induced autophagy (Deter et al. 1967; Deter & de Duve 1967): for the first time lysosomes were considered as the site of intracellular autophagy (De Duve 1983; Klionsky 2008; William et al. 2013, p. 3-4).

After these discoveries, autophagy has come back to the fore just in the ‘90s, when several autophagy related genes (Atg) were discovered in budding yeast Saccharomyces cerevisiae. Presently, genetic investigations has provided 32 different Atg, many of them highly conserved in higher organisms such as slime mould, plants, worms, flies and mammals, underlining the importance of autophagic pathways across phylogeny (Nakatogawa et al. 2009). After determination of Atg, increasing interest in this pathway led the researchers to study in deep the mechanism of autophagy: two important milestones were the investigation of the starvation-induced non-selective autophagy carried out by Yoshinori Ohsumi and Michael Thumm (Takeshige et al. 1992; Thumm et al. 1994; Tsukada & Ohsumi 1993) and the simultaneous discovery of the selective autophagic pathway Cytoplasm-to-Vacuole Targeting (CTV) by Daniel J Klionsky (Harding et al. 1995; Klionsky et al. 1992). In 1999, Beth Levine’s group published a research that linked together autophagy and cancer (Liang et al. 1999), enhancing enthusiasm on the investigation of the autophagy related pathologies, currently comprising a wide range of diseases. Other important and recent milestones regarding autophagy are the first conference on autophagy that was held at Waterville in 2003, the scientific journal “Autophagy” launched by Daniel J Klionsky in 2005 and still very important in the scientific scenery and, lastly, the Nobel Prize in Physiology or Medicine to Japanese autophagy researcher Yoshinori Ohsumi.

Presently, autophagy is a cellular mechanism widely investigated and many issues about it were disclosed. Right now, it was clearly demonstrated that autophagy is involved in a wide range of physiological and pathophysiological processes, among which some of them are adaptation to starvation conditions, clearance of old or misfolded intracellular protein and/or old, damaged or dysfunctional organelles, turn-over of proteins and/or organelles during development, anti-aging processes, fighting and removal of microorganisms, cell death, cancer induction and tumour suppression, and antigen presentation (Mizushima 2005). The way by which such a bulk degradation system exerts so many functions is explainable considering that the rate of autophagy is determinant for its role: too much destruction without construction would be very harmful (Mizushima 2007). This it’s particularly true considering cultured cells, while autophagy rate seems to be strictly regulated in vivo, because it was observed that the autophagic activity, and the relative the digestion of protein, tends to decreases during prolonged starvation periods (de Waal et al. 1986; Mizushima et al. 2004; Mortimore et al. 1983). Basing on the correlation between the autophagy rate and its role, it may be very important to distinguish two different subclasses: the “basal autophagy”, that means the physiological rate, and the “induced autophagy”, in case of alterations of the autophagy rate (Mizushima 2005).

Depending on the delivery route and the cytoplasmic material on the lysosomal lumen, three different autophagy pathways are known in mammals, each mediated by own Atg and their associated enzymes: macroautophagy, microautophagy and chaperone-mediated autophagy (Lee et al. 2012; Mizushima et al. 2004; Mizushima et al. 2002; Xie & Klionsky 2007). Macroautophagy, commonly referred simply as autophagy, is physiologically present at low levels but can also be induced under stress conditions (nitrogen or energy starvation), in order to promote cell survival (Yorimitsu & Klionsky 2005) and consists in the wrapping of damaged organelles or unused protein (Levine et al. 2011) marked for destruction (Česen et al. 2012; Mizushima et al. 2002) by an intermediary double membrane bound

vesicles, called autophagosome (Klionsky et al. 2014), which travels in towards lysosomes and fuse with them (Levine et al. 2011) for cargo digestion via acidic lysosomal hydrolases (Shen & Mizushima 2013). Microautophagy, instead, consists in the engulfment of a portion of cytoplasm directly in the lysosomes through (Castro-Obregon 2010; Česen et al. 2012) invagination of lysosomal membranes, in a process similar to pinching off of phagosomes or pinosomes from the plasma membrane (Ahlberg et al. 1982); microautophagy can be activated by nitrogen starvation or rapamycin (Mijaljica et al. 2011). Both macro and microautophagy can act through both selective and non-selective mechanisms (Glick et al. 2010). Chaperone-mediate autophagy (CMA) is a very complex and specific autophagy pathway, by which targeted proteins containing chaperone specific signalling sequence are bound by Hsc-70 (Bandyopadhyay et al. 2008; Česen et al. 2012; Cuervo & Dice 1996) and drove to lysosomal membrane, where they are recognized by the lysosomal membrane receptor lysosomal-associated membrane protein 2A (LAMP-2A) (Bejarano & Cuervo 2010; Cuervo et al. 2005; Kiffin et al. 2004; Napolitano et al. 2015), resulting in their unfolding, shift inside lysosomal lumen (helped by Hsc-70) and degradation (Lee et al. 2012; Mizushima et al. 2002). Unlike other autophagic pathways, CMA translocates proteins in one by one way and it's extremely selective about the cargo (Levine et al. 2011; Saftig et al. 2008); CMA can be activated by oxidative stress and exposure to toxic materials (Bejarano & Cuervo 2010; Kiffin et al. 2004; Massey et al. 2006a,b). Usually, the pathway mainly took in consideration is the macroautophagy, because it is the most important and common autophagy mechanism and all the following consideration will be referred to macroautophagy pathway.

1.2.1 Autophagic molecular pathway

The molecular pathway of autophagy is still being fully defined, but many processes and involved molecular complexes have already been identified in yeast, and the respective homolog proteins have been detected in mammals. As already quickly introduced, macroautophagy pathway is strictly regulated and proceeds in several steps involving several molecular complexes that lead from the phagophore membrane formation to the fusion of autophagosomes with lysosomes, before the cargo digestion (Mehrpour et al. 2010). Following it has been provided an overview on the vesicles flux progression of the pathway and its signaling regulation ways, including a graphical representation of the whole cellular reported in figure 8.

1.2.1.1 Vesicles flux

Four steps and three main molecular complexes can be identified and used to describe the vesicles flux at molecular level: induction step (ULK1/2 (Atg1) complex), nucleation step (Class III PtdIns3K (Vps34) complex), elongation step (ATG 12-ATG5-ATG16 (Atg12-Atg5-Atg16) conjugation complex; LC3 (Atg8) conjugation system) and lastly the fusion with lysosomes.
Before to start to describe the autophagy pathway is very useful to provide a well-defined nomenclature about the vesicles involved (Klionsky et al. 2014):

- the new membrane growing around cargo is termed Phagophore (PG; double-membrane structure) and after its complete closure (to form a vesicle) this structure is named Autophagosome (AP; double-membrane structure), that fuses with a Lysosomes resulting in a vesicle known as Autolysosome (AL; single-membrane structure) (Klionsky et al. 2014);

- in some cases, it's possible that an AP fuses with an Endosome (single-membrane structure) producing an Amphisome (AM; single-membrane structure for digestion of AP inner membrane) that subsequently fuses with a Lysosome generating an AL (Klionsky et al. 2014);
- during phagocytosis a single membrane phagosome can fuses directly with a Lysosomes resulting in a vesicular structure named Phagolysosome (PL; single-membrane structure) (Klionsky et al. 2014);
- during the LC3-associated phagocytosis the fusion of LC3-decorated phagosome (containing a microbe) with a Lysosome produces a structure termed Autophagolysosomes (APL; single-membrane structure); microbes can also be contained in phagosome then wrapped by a PG resulting in a AP-sequestered Phagosome that fuses with a Lysosome producing a double-membrane APL (Klionsky et al. 2014).

Induction

The induction step consists in the *de novo* membrane formation designed to form the phagophore, which is the primary double-membrane sequestering compartment (He & Klionsky 2009). The phagophore formation in yeast begins at a single specific perivacuolar site called Phagophore Assembly Site (PAS) (Chen & Klionsky 2011) while in mammals this process seems to start at several sites involving structures known as omegasomes (Hayashi-Nishino et al. 2009; Ylä-Anttila et al. 2009); in both models, the sources for the new raising membranes seems to be derived mainly form Endoplasmic Reticules (ER) (Hayashi-Nishino et al. 2009; Ylä-Anttila et al. 2009), in dynamic equilibrium with other possible sources (Mizushima et al. 2011; Weidberg et al. 2011) such as *trans*-Golgi and late Endosomes (Axe et al. 2008; Mizushima 2007; Mizushima & Klionsky 2007; Simonsen & Tooze 2009; Takahashi et al. 2011), plasma membrane (Ravikumar et al. 2010a,b), mitochondria (Hailey et al. 2010) and possibly even derived membrane from nuclear envelope under restricted conditions (English et al. 2009).
The induction step is based on the activity of a kinase complex that is highly conserved from yeast to mammals and is regulated by mammalian Target Of Rapamycin Complex 1 (mTORC1) depending on the induction signals (Hosokawa et al. 2009a). In yeast the induction complex consists of 5 molecules named Atg1 (Ser/Thr kinase), Atg13, Atg17, Atg31, Atg29 (He & Klionsky 2009) which assemble depending on growth factor signalling and nutrient availability (Glick et al. 2010): in nutrient rich conditions TOR kinase phosphorylates Atg13 preventing its interaction with Atg1 (Diaz-Troya et al. 2008) and Atg17, thus inhibiting the activation of the complex and the recruitment of the Atg9 transmembrane protein, that is required to promote lipid recruitment for phagophore expansion (Klionsky 2007; Kundu & Thompson 2005; Simonsen & Tooze 2009). The mammalian homolog molecules have been identified: unc-51-like Kinases 1 and 2 (ULK1 and 2) (Atg1 homolog), ATG13 (Atg13 homolog) and RB1-inducible coiled-coil 1 (RB1CC1/FIP200) (Atg17 ortholog) (Ganley et al. 2009; Hara et al. 2008; Hosokawa et al. 2009a; Jung et al. 2009) are constitutively grouped in the ULK complex, which is stable regardless of nutrient status (Hosokawa et al. 2009b; Jung et al. 2009), and involves also the protein C12orf44/ATG101 (no yeast homolog is known), that is essential for macroautophagy (Hosokawa et al. 2009b; Mercer et al. 2009). In mammals mTORC1 regulates autophagy induction by binding ULK complex. More specifically, in nutrient-rich conditions mTORC1 associates with the complex and phosphorylates ULK1, ULK2 and ATG13 while ULK1 and 2 phosphorylate ATG13 and FIP200 (Hosokawa et al. 2009a; Jung et al. 2009); under starvation conditions, or in cells treated with rapamycin, mTORC1 is inhibited and it dissociates form the complex leading to the dephosphorylation of ULK1, ULK2 and ATG13 (unknown phosphatases) and to the increment of ULK 1 and 2 activity, resulting in the phosphorylation of ATG13 and FIP200 and in autophagy induction (Hosokawa et al. 2009a; Jung et al. 2009). Another way to induce autophagy (probably in parallel with mTORC1) way involves AMPK (ortholog of Snf1 yeast conserved protein), which can essentially phosphorylate ULK1

thus preventing the interaction between mTORC1 and ULK Complex and its inhibition (Egan et al. 2011; Kim et al. 2011a; Lee et al. 2010a; Shang et al. 2011).
It is still not clear if ULK1 or ULK2 functions analogously Atg1 in promoting autophagy in mammals (Glick et al. 2010): it's thought that in some tissues ULK2 can balance possible ULK1 deficiency (Yang and Klionsky 2010). Moreover, a third ULK protein (ULK3), with a role mechanistically different from the other two, has been identified for its participation in autophagy induction in oncogene-induced cell senescence (Young et al. 2009).
Although the molecules involved in the induction step have been relatively well characterized, the role of the ULK/Atg1 complex need to be further investigated in order to define how the activation of ULK/Atg1 kinase activity induces the phagophore formation and thus leads to the autophagy induction (Parzych & Klionsky 2014; Yang & Klionsky 2010).

Nucleation

After the ULK complex begins to form the new membrane, during the nucleation step the Class III Phosphatidylinositol 3-kinase (PtdIns3K or PI3K), mammalian correspondent of Vps34 complex in yeast, generates phosphatidylinositol (3)-phosphate (PtdIns3P or PI3P), which results to be essential for autophagy progression in both mammals and yeast (Burman & Ktistakis 2010) allowing the recruitment of other Atg proteins at the induction sites in mammals and at the PAS in yeast (Yang & Klionsky 2010) needed for phagophore elongation (Glick et al. 2010). In yeast the PtdIns3K complex is composed by several Vps (Vacuolar protein sorting) proteins: Vps34 (PI3-kinase), Vps15, Atg6 and Atg14, which is a protein specific for PtdIns3K complex I involved in autophagy, or Vps38, a protein specific for PtdIns3K complex II involved in autophagy and also in endocytic pathway (Yang & Klionsky 2010). In mammals several correspondent proteins which are member of Class III PtdIns3K complex have been described: PIK3C3/VPS34 (also known as hVps34; homolog of Vps34), PIK3R4/p150 (homolog of Vps15) and Beclin1 (homolog of Vps30/Atg6) are conserved and composes the complex (Furuya et al. 2005; Itakura et al. 2008; Kihara et al. 2001; Liang et al. 1999; Yan et al. 2009), while the orthologs ATG14L (or Barkor; ortholog of Atg14) and UVRAG (Ultraviolet irradiation Resistance-Associated Gene; ortholog of Vps38) (Itakura et al. 2008; Liang et al. 2006; Sun et al. 2008) are not constitutively comprised in the complex (Itakura et al. 2008; Liang et al. 2006). All these proteins are important for the correct functioning of the complex: for example Vps34 is the only PI3-kinase that uses PI as a substrate to produce PI3P (Glick et al. 2010); further, it has been observed that ATG14L, normally localized on the ER, moves on ATG16L-positive and LC3-positive structures in starvation conditions, independently from its interaction with PIK3C3 and Beclin1 (Itakura et al. 2008; Matsunaga et al. 2009), suggesting a possible role in directing Class III PtdIns3K complex to the phagophore to initiate Atg machinery recruitment (Yang and Klionsky 2010). The most important and well characterized regulatory protein of the Class III PtdIns3K complex activity is the protein Beclin1 (Atg6), that interacts by its BH3 domain with the antiapoptotic protein Bcl-2 (or Bcl-X_L) at the ER (Maiuri et al. 2007a) in nutrient-rich conditions, preventing/disrupting the interaction with PIK3C3 (Vps34) (Maiuri et al. 2007a; Pattingre et al. 2005) and thus inhibiting autophagy (Hara et al. 2008; Liang et al. 1998b; Pattingre et al. 2005). In response to starvation-induced signalling, several regulator proteins can interfere with this interaction allowing autophagy to proceed (Maiuri et al. 2007a): dephosphorylated BAD protein, containing a BH3 domain, can compete with Beclin-1 for binding Bcl-2/Bcl-X_L, thus triggering the activation of Class III PtdIns3K complex (Adachi & Imai 2002); Jnk-1 can mediate a phosphorylation of Bcl-2 disrupting its interaction with Beclin-1 and allowing its activation; DAPK1 (Death-Associated Protein Kinase 1) can phosphorylate and activate Beclin-1 promoting its dissociation from inhibitor Bcl-2 (Wei et al. 2008). Further, Bcl-2 protein results to be very important because plays a dual role depending on its sub-cellular

localization: it has a pro-survival function at mitochondria, inhibiting cytochrome *c* release and blocking apoptosis, and an autophagy-inhibitory activity at the ER by interacting with Beclin-1 and leading to a non-apoptotic cell death (Pattingre et al. 2005). Based on these important involvements, Bcl-2 results to be a very important molecule in balancing autophagy and apoptosis activation in response to specific stress, with important impact on progression and treatment of many diseases, currently representing a very interesting research area that still needs to be resolved (Maiuri et al. 2007b). In addition, Class III PtdIns3K complex can be obviously activated by the regulator proteins AMPK and mTORC1: more specifically, AMPK activates pro-autophagy Class III PtdIns3K complex by phosphorylating Beclin-1 and simultaneously inhibits non-autophagy Class III PtdIns3K complex via phosphorylation of Thr163/Ser165 of PIK3C3 (not showed in figure 8) (Kim et al. 2013); on the other hand mTORC1 inhibits PIK3C3 lipid kinase activity by phosphorylating ATG14L leading to the inhibition of autophagy (Yuan et al. 2013). Moreover, autophagy can be regulated at the nucleation step also by modulating the transcriptional activity and phosphorylation-dependent cytoplasm-to-nucleus shuttling of TFEB (Transcriptional Factor EB) (Settembre et al. 2011), a master transcriptional regulator of lysosomal and autophagy genes (Settembre et al. 2013): mTORC1 directly phosphorylates TFEB on Ser142 and Ser211 resulting in cytoplasmic sequestration of the Transcriptional Factor (Martina et al. 2012; Settembre et al. 2012) while Rag GTPase proteins can directly bind and sequester TFEB into lysosomes depending on nutrient availability, thus inhibiting its transcriptional activity (Martina et al. 2013).

Recent studies have suggested that many other molecules can interact with Class III PtdIns3K complex and that the precise molecular composition of the complex at the ER is determined by signalling events and can determine its stimulatory or inhibitory activity (Fimia et al. 2007; Glick et al. 2010; Liang et al. 2006; Matsunaga et al. 2009; Pattingre et al. 2005; Parzych & Klionsky 2014; Yang & Klionsky 2010; Zhong et al. 2009). More specifically, PIK3C3, PIK3R4 and Beclin-1 are always represented in the complex while and can interact with several other molecules: the previously introduced activator protein UVRAG can compete with the activator protein ATG14L for binding to Beclin-1, determining the involvement of the Class III PtdIns3K complex in autophagic or endocytic pathway and, possibly, its involvement in later steps of autophagosomes formation (Liang et al. 2006; Parzych & Klionsky 2014; Yang & Klionsky 2010); the protein AMBRA (Activating Molecule in Beclin-1 Regulated Autophagy) directly binds Beclin-1 thus activating autophagy in a not determined manner (Fimia et al. 2007; Miller et al. 2010; Takahashi et al. 2009); the positive regulator protein SH3GLB1/Bif-1 (Bax interacting factor 1) binds Beclin-1 via UVRAG and contains a N-BAR domain by whose binding and bending activity can contribute to the membrane deformation (Fimia et al. 2007; Miller et al. 2010; Takahashi et al. 2009), probably during the membrane elongation step considering that it was found to colocalize together with Atg5, LC3 and Atg9 during starvation (Liang et al. 2008a); the Class C Vps/HOPS proteins interacts with UVRAG and can accelerate delivery and degradation of autophagic cargo by promoting autophagosome fusion with late endosomes/lysosomes (not reported in figure 8) (Liang et al. 2008a); lastly, the KIAA0226/Rubicon (RUN domain and cysteine-rich domain containing, Beclin-1 interacting) protein binds UVRAG-containing Class III PtdIns3K complex localized at late endosomes/lysosomes and inhibits PIK3C3 activity, thus negatively regulating autophagosome maturation (Matsunaga et al. 2009; Zhong et al. 2009).

There are some other proteins that have been found to be involved in the nucleation mechanism (not showed in figure 8). In yeast, the Atg 18 and Atg21 proteins localize at the PAS and have a role in autophagy by binding PI3P generated by Vps34 (Krick et al. 2008). Two mammalian orthologs of Atg18 have been identified and named WIPI1 and WIPI2, and associates with phagophores by binding PI3P during amino acid starvation confirming its involvement in autophagy progression (Jeffries et al. 2004; Polson et al. 2010; Proikas-Cezanne et al. 2004). Another mammalian protein PI3P-binding named ZFYVE1/DFCP1 (Zinc-finger, FYVE domain containing 1) has been found to associate with PI3P-enriched omegasomes, which are particular membrane compartments rich of PI3P, that rise from ER after induction of

amino acid starvation and are characterized by a typical shape similar to the Greek capital letter omega (Ω) (Axe et al. 2008). However, the precise roles of these proteins in autophagy are still unknown (Parzych & Klionsky 2014).
Another protein important for induction and nucleation steps is the six steps transmembrane protein mAtg9, an highly conserved protein (Atg9 in yeast) previously briefly introduced. In mammals this protein is normally located on the *trans*-Golgi network and late endosomes (Young et al. 2006) but moves to GFP-LC3 positive autophagosomes after starvation induction or rapamycin treatment, in a cycling-manner dependent to both ULK complex and Class III PtdIns3K complex and negatively regulated by MAPK14/p38α (not reported in figure 8) (Webber & Tooze 2010; Young et al. 2006). The role of the yeast Atg9 protein is still unclear, while mAtg9 potentially provides membranes to autophagosomes growth (Yang & Klionsky 2010; Young et al. 2006).

Elongation

During the phagophore elongation step, two ubiquitin-like (UBL) proteins (Atg12 and Atg8/LC3) and their respective and partially overlapped conjugation systems, are very important in both yeast and mammals for phagophore expansion (Kirkin et al. 2009a ; Mizushima 2007; Weidberg et al. 2011).
The first conjugation system involves Atg12, Atg5 and Atg16 proteins in yeast and the ortholog proteins ATG12, ATG5 and ATG16L in mammals (Mizushima et al. 2003; Ohsumi 2001) maintaining the same function in either organisms. In mammals, ATG12 is activated by Atg7, that works like an E1-like activating enzyme by biding its carboxyterminal glycine residue in an ATP-dependent manner, and then transferred to Atg10, an E2-like conjugating enzyme that covalently binds ATG12 to ATG5 in an irreversible manner (Geng & Klionsky 2008; Glick et al. 2010; Kim et al. 1999; Ohsumi 2001; Shintani et al. 1999; Yang & Klionsky 2010). Upon starvation induction, ATG12-ATG5 complexes are noncovalently conjugated with ATG16L proteins, which dimerize to form larger multimeric complexes called ATG16L complexes (Kuma et al. 2002; Yang & Klionsky 2010) that associate with phagophore and promote membrane curvature through asymmetric recruitment of processed LC3B-II (Glick et al. 2010), dissociating immediately after autophagosomes completion (Barth et al. 2010; Mizushima et al. 2001; Mizushima et al. 2003; Yang & Klionsky 2010). This conjugation system is regulated mostly by two proteins: the Golgi protein RAB33A can bind and inhibit ATG16L and, in addition, ATG5, ATG7 and ATG12 are inhibited through acetylation by the acetyltransferase KAT2B/p300 (Lee & Finkel 2009).
The second conjugation system is responsible for the processing of the yeast protein Atg8, as well as the mammalian homolog microtubule-associated protein light chain 3 (LC3B), working in the same manner in either organisms (Geng & Klionsky 2008). LC3B is expressed in most of the cell types as a full-length cytosolic protein (Glick et al. 2010). Under starvation conditions LC3B is proteolytically cleaved at its C-terminus by the cysteine proteinase Atg4 (Kirisako et al. 2000), then used by activated by the E1-like ATP-consuming enzyme Atg7 and transferred to the E2-like conjugating enzyme Atg3 (Ichimura et al. 2000), that uses the free carboxyl-glycine to binds LC3B-I to phosphatidylethanolamine (PE), thus producing LC3B-II (Geng & Klionsky 2008) that is attached to both inner and outer membrane faces, where it plays a role in both membrane hemifusion and cargo selecting (Glick et al. 2010). Anyhow, LC3B-II is removed from the outer membrane after autophagosome completion and before fusion with late endosomes/lysosomes (Kirisako et al. 2000; Yang & Klionsky 2009) as a result of a second Atg4-mediated cleavage, an event named deconjugation, whose regulation is still unknown but absolutely required for autophagy progression (Nair et al. 2012). Looking at what already said, the two conjugation system are closely connected: Atg12-Atg5 conjugate can act as a novel E3-like ligase, determining the side of LC3B lipidation (Fujita et al. 2008; Glick et al. 2010; Hanada et al. 2007; Ichimura et al. 2000) and, simultaneously, the LC3B conjugation machinery, particularly Atg3 enzyme, results to

be essential for the formation of ATG16L complex and for its dissociation from the complete phagophore (Yang & Klionsky 2010). The synthesis and lipidation of LC3 in mammalian cells is increased under starvation conditions or other type of stress (Glick et al. 2010; Kabeya et al. 2000), and based on this well-defined event LC3B-II is considered a key readout of autophagy levels in cells (Barth et al. 2010).

Despite the autophagy process is very similar in both yeast and mammals, in mammals there are four isoforms of ATG4 and several Atg8-like proteins, whit the latter divided into two big subfamilies: LC3 and GABARAP (γ-aminobutyric type A ($GABA_A$)-receptor associated protein) subfamilies (Hemelaar et al. 2003; Mariño et al. 2003, Weidberg et al. 2010). Since both of them undergo similar processing during autophagy and localize with autophagosomes (Kabeya et al. 2004), it has been speculated that they may function at different moment during phagophore elongation and completion, with LC3B subfamily acting before the GABARAP subfamily (Weidberg et al. 2010). These proteins represent also an important control point of autophagy, as evident considering that PKA-mediated phosphorylation of LC3 can negatively regulate the progression of autophagy step (Cherra et al. 2010).

Although the critical role of Atg5- and Atg7-dependent autophagy in cell surviving during starvation periods following the birth, nowadays it's clear that exists an alternative Atg5-/Atg7-independent autophagy (Nishida et al. 2009). This autophagy pathway, which is also LC3B-independent, has been determined during investigation of mitochondrial clearance in reticulocytes (Zhang et al., 2009) and involves autophagosomes that seems to originate from late endosomes and *trans*-Golgi (Nishida et al. 2009). Several studies have shown that ULK1 is essential for both mitochondria clearance in reticulocytes (Kundu et al. 2008) and, along with Beclin-1, for Atg5/Atg7-independent autophagy (Nishida et al. 2009), even if the molecular mechanisms of this pathway is still to be cleared (Glick et al. 2010).

Fusion with lysosomes

The last step of the autophagosome maturation is the fusion with lysosomes and the following digestion of the cargo in the final structure called "autolysosomes" (Mizushima 2007). As previously introduced, several evidences have suggested that prior to the fusion with lysosomes, autophagosomes fuses with early and late endosomes that provide cargo and membrane fusion machinery components, as well as lower the pH of autophagic vesicles before to the intervention of lysosomal acidic proteases (Eskelinen 2005). Although this aspect of autophagy is relatively understudied, it is clear that it requires the small G protein Rab7 in its GTP-bound state (Gutierrez et al. 2004b; Jäger et al. 2004), and the Presenilin protein, that is implicated in Alzheimer's disease (Eskelinen 2005).

Anyway, whatever the order in which the vesicles blend, their movements are guided by microtubules of cytoskeleton (Monastyrska et al. 2009; Webb et al. 2004) and the fusion events require several proteins: fusion autophagosomes-endosomes requires the intervention of VTI1B protein (Atlashkin et al. 2003) while fusion autophagosomes-lysosomes depends to the already cited G protein Rab7 activated by the UVRAG-containing Class III PtdIns3K complex (Jäger et al. 2004; Liang et al. 2008a). Other studies have revealed that components of the SNARE machinery have a role in fusion: in addition to VAM7 and VAM9 (Fader et al. 2009; Furuta et al. 2010), syntaxin 17 was found to localize on completed autophagosomes and is essential for the fusion of autophagosome with endosome/lysosome through its interaction with SNAP29 and the endosomal/lysosomal SNARE VAMP8 (Itakura et al. 2012).

Also several lysosomal protein are essential for autophagy progression: cathepsin proteases B and D are required for autophagosomes turnover and, consequently, for autolysosome maturation (Koike et al. 2005) as well as LAMP-1 and LAMP-2, which have been shown to be critical for functional autophagy progression (Tanaka et al. 2000).

Specific cargoes and selective autophagy

In addition to the non-selective autophagy, which appears to involve random portion of cytosol and is enabled for cell survival in conditions of nutrient/energy deprivation, autophagy can also be highly specific, especially when is dedicated to cell maintenance and homeostasis (Cherra et al. 2010; Isakson et al. 2013), selecting cargoes such as, among the others, peroxisomes, mitochondria, and ubiquitinated proteins (Lee et al. 2012; Till et al. 2012; Weidberg et al. 2011). The importance of selective autophagy is highlighted considering its significance for neuropathies, cancer and heart diseases (Glick et al. 2010). Some examples of selective autophagy are briefly reported below.

The selective autophagic degradation of peroxisomes is defined pexophagy and it works under normal growth conditions, being responsible for the 70-80% of peroxisomes degradation (Yokota & Dariush Fahimi 2009), and also under starvation conditions, with the specific cargo recognised by binding of LC3B-II to PEX14, a component of peroxisomal translocon complex located on the peroxisomal membrane (Hara-Kuge & Fujiki 2008).

Another important type of selective autophagy is the mitophagy, which concerns the degradation of mitochondria not only for basal turnover (Tal et al. 2007), when allows to maintain the integrity of these organelles and to limit the production of reactive oxygen species (Kim et al. 2007), but also during development of certain cell types and for clearance of damaged mitochondria (Kim et al. 2007; Kundu et al. 2008; Schweers et al. 2007). More specifically, it has been observed that during cell development and maturation of mammalian red blood cells a mitochondrial outer membrane protein called BNIP3L/NIX allows the recognition of mitochondria by interacting through its WXXL-like motif (also known as LC3-interacting region) (Sandoval et al. 2008; Schweers et al. 2007) with LC3 and GABARAP protein located on the expanding phagophore (Youle & Narendra 2011). On the other hand, the process regarding the clearance of damaged mitochondria involves other protein: the mitochondrial outer membrane kinase PINK1, present on damaged mitochondria, recruits the cytosolic E3 ubiquitin ligase PARK2/Parkin which ubiquitinates mitochondrial substrate activating mitophagy (Youle & Narendra 2011); in the healthy mitochondria PINK1 is imported into inner membrane, where is cleaved by mitochondrial processing peptidase (PMPCB) and degraded by presenilin associated, rhomboid-like protease (PARL), thus preventing its accumulation on the outer membrane and the possible onset of mitophagy of healthy mitochondria (Jin et al. 2010; Meissner et al. 2011). The importance of mitophagy in clearance of damaged mitochondria can be emphasized considering that PINK1 and PARK2 genes are mutated in autosomal recessive Parkinson disease (Kitada et al. 1998; Valente et al. 2004).

Also ribosomes can be selectively degraded under starvation conditions in a process called ribophagy that is dependent on the catalytic activity of the Ubp3p/Bre5p ubiquitin protein (Kraft et al. 2008).

A fourth very important mechanism for cargo identification in selective autophagy involves the ubiquitin-binding protein SQSTM1/p62, which labels intracellular bacteria and guides them to autophagic degradation in a process called xenophagy (Zheng et al. 2009). Further, protein SQSTM1/p62 is involved also in the process for the clearance of ubiquitinated protein aggregates by interacting with LC3B-II protein to lead protein aggregates to degradation in a process named aggrephagy (Bjørkøy et al. 2005; Øverbye et al. 2007; Vadlamudi et al. 1996). Other proteins like SQSTM1/p62, working in the targeting of ubiquitinated proteins or pathogens for selective autophagy digestion are NBR1 and OPTN (Kirkin et al. 2009b; Wild et al. 2011). More specifically, protein SQSTM1/p62 works by binding poly-ubiquitinated proteins and/or aggregates through its ubiquitin-binding domain (UBD) (Pankiv et al. 2007) and LC3B-II protein through its LC3-Interacting Region (LIR), and can also regulates NF-kB signalling pathway by interacting with Traf-6 (Duran et al. 2008), thus playing an important role for the degradation of intracellular aggregates and in the onset of many diseases.

For example, in mice with autophagy deficiency caused by an Atg7 depletion, protein aggregates bound to SQSTM1/p62 protein accumulates, as in case of Mallory bodies observed in the liver in human hepatocellular carcinoma (Komatsu et al. 2007a; Mathew et al. 2009). Intracellular protein aggregates accumulation plays an important role also in the onset of neurodegenerative diseases, such as dementia, Alzheimer's (different forms of tau), Huntington's (polyglutamine-expansion repeats), Parkinson's (mutant form of α-synuclein) and Creutzfeldt-Jackob/prion diseases (Levine & Kroemer 2008; Rubinsztein 2006; Yue et al. 2009). It has been observed that the specific inactivation of Atg5 and Atg7 genes in neuronal cells produces an intracellular aggregates accumulation and neurodegeneration in mice (Hara et al. 2006; Komatsu et al. 2007b).

1.2.1.2 Regulatory molecular signalling pathways

Autophagy is a physiological highly conserved pathway active at basal level in most cell types and play a housekeeping role in cellular homeostasis, especially in maintenance of integrity of organelles and proteins (Jin 2006). For this reason, autophagy pathway is strictly regulated by a very complicated net of cellular ways in order to modulate the huge number of signals related to both physiological needs and intra-/extracellular stresses (He & Klionksy 2009). In such complicated scenery, two principal critical points can be identified and used as starting point to describe such regulatory ways: mTORC1 complex, which acts downstream the signals for autophagy inhibition as growth factors (GFs), insulin, presence of essential aminoacids and nutrient-rich conditions, and AMPK, which is the final executor of autophagy-inducing signals coming from endoplasmic reticule (ER) stresses (metabolic stresses), genotoxic and oncogenic stresses, cytokines (CKs), hypoxia and nitrogen/carbon starvation conditions (He & Klionksy 2009; Kim & Guan 2015). Following, both inhibiting and inducing signalling paths were analyzed in order to provide a complete overview about the regulation of the autophagic process.

The first regulatory element is the mammalian Target of Rapamycin (mTOR), existing in two different complexes composed of several proteins and with a common molecular core to both: along with mTOR (Ser/Thr kinase), DEPTOR, mLST8 and Tti1/Tel2, which constitute the core (Kaizuka et al. 2010), mTOR1 contains the regulatory subunits RAPTOR (positive regulator) and PRAS40 (negative regulator) (Hara et al. 2002; Wang et al. 2007) whereas mTOR2 contains RICTOR, mSin-1 and PROCTOR1/2 (Frias et al. 2006; Jacinto et al. 2006; Pearce et al. 2007; Sarbassov et al. 2004; Thedieck et al. 2007; Yang et al. 2006). Both mTOR1 and 2 complexes are involved in autophagy regulation but mTORC1 has a central role while mTOR2 is just partially involved and exerts its role also in several other cellular activities, such as cell survival, metabolism and cytoskeletal organization (Cybulski et al. 2009). Further, only mTORC1 is resulted to be sensitive to the activity of Rapamycin, while its derivative molecules named "Rapalogs" can affect both mTORC1 and 2 (Rubinsztein et al. 2007). mTORC1 activity is closely related to the activities of other two proteins, which are directly responsible of the activation/inhibition of the complex: Rheb (Ras homolog enriched in brain), that is a GTPase able to bind and thus activate mTORC1 just in its GTP-bound form (Kim & Guan 2015), and its negative regulator TSC1/TSC2 (Tuberous Sclerosis Tumour Suppressor Complex 1 and 2), GTPase-activating proteins (GAP) which senses and integrates most of the stimuli and the signalling networks for autophagy regulation. Briefly, mTORC1 is considered a negative regulator of autophagy because it's resulted to be activated in presence of autophagy-inhibitor stimuli and, conversely, is inhibited as a results of pro-autophagic signals (Inoki et al. 2002; Ma et al. 2005; Shaw 2009), particularly depending on the cellular levels of nitrogen energy derived from the presence of aminoacids (Bar-Peled & Sabatini 2014; Jewell et al. 2013). The autophagy-inhibitory activity of mTORC1 is exerted on two different step of the vesicles flux: at one hand mTORC1 binds ULK complex and

phosphorylates ATG13 and ULK1/2 subunits, preventing the essential phosphorylation of ULK1 on Ser758 by AMPK (Kim et al. 2011a), thus inactivating its kinase-activity, required for ULK complex activity (Ganley et al. 2009; Hosokawa et al. 2009a; Jung et al. 2009); on the other hand, mTORC1 can phosphorylate ATG14L in Class III PIK3 complex inhibiting the activity of the of PI3K activity and leading to the subsequent inhibition of autophagy progression (Yuan et al. 2013).

The second autophagy regulatory element is the AMPK (AMP-activated protein kinase), which is considered as the most important cellular energy-sensing kinase and is involved in regulation of a lot of cellular processes (Alers et al. 2012; Meley et al. 2006). Concerning its involvement in autophagy, AMPK works as a positive regulator acting directly or indirectly by inhibiting mTORC1 in response to a lot of different autophagy-activator stimuli, as for example the high AMP/ATP levels in case of carbon starvation (Glick et al. 2010; Parzych & Klionsky 2014; Yang & Klionsky 2010). More specifically, AMPK can inhibit mTORC1 by phosphorylating its positive regulator subunit RAPTOR, or by increasing GAP activity of TSC2 thus inducing the TSC1/2-dependent inhibition of Rheb and consequently activating autophagy (Corradetti & Guan 2006; Gwinn et al. 2008; Høyer-Hansen & Jäättelä 2007; Inoki et al. 2003; Sarbassov et al. 2005; Yang & Klionsky 2010). On the other hand, AMPK can activate autophagy by phosphorylating ULK1/2 subunits thus leading to ULK complex activation (Egan et al. 2011; Kim et al. 2011a; Lee et al. 2010a; Shang et al. 2011) or by phosphorylating Beclin1 in order to activate Class III PI3K complex (Kim et al. 2013). Moreover, if stimulated by stress conditions via LKB1 protein, AMPK can activate $p27^{kip1}$ inducing autophagy and simultaneously arresting cell cycle progression (Liang et al. 2007).

Further, both mTORC1 and AMPK can be upstream regulated also by PKA (Protein Kinase A Camp-dependent), which responds to intracellular levels of cAMP (produced by adenylate cyclase depending on glucose and ATP levels) (Blancquaert et al. 2010; Djouder et al. 2010; Mavrakis et al. 2006; Parzych & Klionsky 2014) and is thought to be a cross-talk point between carbon- and nitrogen-sensing signals. In particular, PKA can phosphorylates AMPK to promote its inactivation (Djouder et al. 2010), mTORC1 leading to its activation (Blancquaert et al. 2010; Mavrakis et al. 2006) and also LC3B interfering with the elongation step, thus acting as a negative autophagy-regulator in nutrient rich-conditions (Cherra et al. 2010).

As introduced above, such regulatory proteins act downstream several inducing/inhibiting stimuli, modulating these signals in order to produce a resulting effect. Several autophagy-inhibiting stimuli are received and modulated mainly by mTORC1, as well as their upstream acting proteins TSC1/2 and Rheb, in consequence of several signalling events. Growth factor binding to its specific tyrosine-kinase receptor activates the Raf/MEK/ERK pathway (via Ras-GTP activity), which directly phosphorylates TSC1/2 and in parallel activated p90 ribosomal S6 kinase 1 (RSK1) that phosphorylates TSC2, thus leading to TSC1-TSC2 dissociation (Yang & Klionsky 2010) and to inactivation of TSC2 GAP activity (Ma et al. 2005; Roux et al. 2004), respectively. The inhibition of TSC1/2 activity allows Rheb protein to exert its inducing activity on mTORC1 (Saucedo et al. 2003; Stocker et al. 2003), thus promoting autophagy inhibition. Another autophagy-inhibitor stimulus that activates mTORC1 is Insulin (Liu et al. 2009; Yin et al. 2008): the binding of Insulin to its specific tyrosine-kinase receptor activates Class I PI3K complex via Insulin Receptor Substrate 1 (IRS1) induces the production of PI3P for membrane recruitment of Akt (PKB) and its activator PDK1 (Liu et al. 2009; Mammucari et al. 2007). After activation Akt protein phosphorylates and inhibits TSC1/2 and, in parallel, phosphorylates PRAS40, a negative regulator subunit of mTORC1, thus activating mTORC1 activity to inhibits autophagy progression (Choi et al. 2013; Kim & Guan 2015; Sancak et al. 2007; Vander Haar et al. 2007). The whole Class I PI3K complex pathway is critical for mTORC1 activity regulation because is also involved in the regulation of mTORC2, involving this molecular complex in the autophagy regulation (Sarbassov et al. 2005), even if the molecular mechanisms are still unknown (Kim & Guan 2015). However, is clear that Class I PI3K signalling axis is an upstream

activator of mTORC2 and it has been proposed that the activation consists in the promotion of the association between the kinase complex with ribosomes (Zinzalla et al. 2011). Activated mTORC2 complex can phosphorylate turn motif and hydrophobic motif in order to respectively stabilize and activate (Kim & Guan 2015) a subset of AGC family kinases (PKA, PKG and PKC), which act downstream as regulator for cell survival, metabolism and cytoskeletal organization (Cybulski et al. 2009). However, the most important and well characterized substrate of mTORC2 is Akt protein, which is phosphorylated in its hydrophobic motif (Ser473) in order to reach its maximal activation (Cybulski et al. 2009) that is essential for an appropriate phosphorylation of forkhead box O (FoxO3) transcription factor, which has been found to stimulate autophagy in muscle cells independently from mTORC1 (Mammucari et al. 2007). Moreover, due to its involvement in both mTORC1 and mTORC2 regulation, Akt mediates a positive cross-talk between the two kinase complexes (Kim & Guan 2015). To close the parenthesis regarding mTORC2 it is important to mention some important negative feedback from mTORC1 to mTORC2. One of the downstream effector of mTORC1, the ribosomal protein S6 kinase 1 (S6K1), can negatively regulates IRS1, thus downregulating the whole Class I PI3K way (not showed in figure 8) (Harrington et al. 2004; Um et al. 2004), and can in parallel phosphorylates RICTOR subunit of mTORC2 thereby affecting kinase activity of the complex (not showed in figure 8) (Sarbassov et al. 2005). Other important negative feedback involves mTORC1, which can inhibit Class I PI3K way by phosphorylating IRS1 and the growth factor-bound protein 10 (Grb10) by its self (Tzatsos & Kandror 2006; Yu et al. 2011). Another autophagy-inhibiting stimulus that activate mTORC1 is the intracellular presence of essential aminoacids, which are key elements for protein synthesis (Bar-Peled & Sabatini 2014; Jewell et al. 2013), and are sensed by a family of Ras-like small GTPase protein (Rag A/B/C/D) (Kim et al. 2008a; Sancak et al. 2008). Rag A and B are homologous and are functionally redundant, as well as Rag C and D, when expressed in the same cells (Kim & Guan 2015). Rag A (or Rag B) heterodimerizes with Rag C (or Rag D) (Sancak et al. 2008) and the complex is kept on the lysosome by the lysosomal protein complex Ragulator (Sancak et al. 2010): in presence of aminoacids, and when Rag A (or B) and Rag C (or D) are bound to GTP and GDP, respectively, the Rag GTPases are activated and bind RAPTOR subunit to recruit mTORC1 on lysosomal surface, thus promoting its colocalization and interaction with its activator Rheb (Sancak et al. 2010). As reported above, Akt signalling way also contribute to localize TSC1/2 on lysosomes, and this evidence makes lysosomes as the central mTORC1 activation platform (Menon et al. 2014).

Generally, all the autophagy-inducing stimuli converge on AMPK, which can acts directly or by modulating the activity of mTORC1 complex, as already discussed above. AMPK activity is stimulated by several type of cellular stresses, metabolic (ER), genotoxic/oncogenic, oxidative and carbon nutrient stresses, via different signalling ways. Nutrient stress is regulated by two different paths: the first path involves PKA, a protein which is activated by the high levels of cAMP in nutrient rich conditions, as already introduced before, and lead to autophagy-inhibition by phosphorylating mTORC1, AMPK and LC3B (Blancquaert et al. 2010; Cherra et al. 2010; Djouder et al. 2010; Mavrakis et al. 2006; Parzych & Klionsky 2014); the second regulatory path, instead, depends to LKB1 protein, which is activated by high levels of AMP/ATP ratio, typical in starvation conditions, and activates AMPK (Corradetti et al. 2004; Shaw et al. 2004 a,b). Metabolic stress also activates AMPK in response to the increment of cytosolic free Ca^{2+} concentration, typical in consequence of ER stress (Høyer-Hansen et al. 2007). More specifically, AMPK activation is mediated by Ca^{2+}/calmodulin-dependent kinase kinase-β (CaMKKβ), that is required for Ca^{2+}-autophagy induction (Høyer-Hansen et al. 2007). Another similar autophagy-activating pathway is dependent on the increment of cytosolic cytokines (such as TRAIL), which activates AMPK downstream activation of transforming growth factor-β-activating kinase 1 (TAK1) (Herrero-Martin et al. 2009). Genotoxic stress leads to activation of AMPK through p53 following two ways: p53 can activate autophagy by activating AMPK, which then acts TSC1/2 inducing the inhibition of mTORC1 (Feng et al. 2005), or can also upregulate the damage-regulated autophagy modulator (DRAM) leading to autophagy induction

(Crighton et al. 2006). However, is still not clear if p53 acts as a positive or negative regulator for autophagy induction because it has been observed that several stimuli, including starvation or ER stress, can induce HDM2 protein to mediate the proteasomal degradation of p53 thus promoting autophagy and placing p53 among the negative regulator of autophagy (Tasdemir et al. 2008). Also hypoxia is comprised among the autophagy-inducing stimuli (Semenza 2010). Low O_2 cellular levels can induce autophagy through three main ways, which are dependent on target genes induced by hypoxia inducible factor (HIF) effects or also through HIF-independent effects, as well as in consequence of unfolded protein response. Specific HIF targets include two non-canonical members of the Bcl-2 superfamily, BNIP3L/Nix and BNIP3, which have a role in mitophagy (Tracy et al. 2007; Zhang & Ney 2009). BNIP3L/Nix has already been cited for its involvement in mitochondria clearance of maturing reticulocytes (Sandoval et al. 2008; Schweers et al. 2007) while BNIP3 appears to have a similar role in cardiac and skeletal muscle in response to oxidative stress (Hamacher-Brady et al. 2006; Mammucari et al. 2007). More specifically, BNIP3 induces mitophagy by interacting with Rheb (Li et al. 2007) while BNIP3L/Nix promotes mitophagy by interacting with GABARAP (Youle & Narendra 2011; Schwarten et al. 2009). Moreover, it has been proposed that BNIP3 can act on Beclin1 by disrupting its interaction with Bcl-2 (not showed in figure 8) (Zhang et al. 2008). HIF-independent effects, instead, can inhibit mTORC1 directly or indirectly downstream AMPK or DNA damage response 1 (REDD1) protein which promote TSC1/2 activation by modulating its TSC2 subunit (Brugarolas et al. 2004; DeYoung et al. 2008; Shaw 2009). Lastly, hypoxia can induce ER stress through the unfolded protein response (UPR): UPR, together with the reduced function of mitochondria in oxidative phosphorylation under hypoxia conditions, leads to the autophagy induction allowing the elimination of compacted portions of ER and the reduction of mitochondrial mass, thus preventing wasteful ATP consumption at ER and limiting production of reactive oxygen species (ROS) at mitochondria, and allowing generation of ATP from catabolism induced by autophagy (Glick et al. 2010).

Obviously all the stimuli analyzed above can act simultaneously and are triggered by the critical protein AMPK and mTORC1, interfering with the autophagy activity and modifying its basal levels in order to ensure the correct rate depending on the conditions. For this reasons, alteration in the autophagy regulation can easily lead to the onset of a wide range of diseases with very dangerous consequences (Arroyo et al. 2014; Bhutia et al. 2013; Bravo-San Pedro et al. 2017; Levine and Kroemer 2008; Shi et al. 2013).

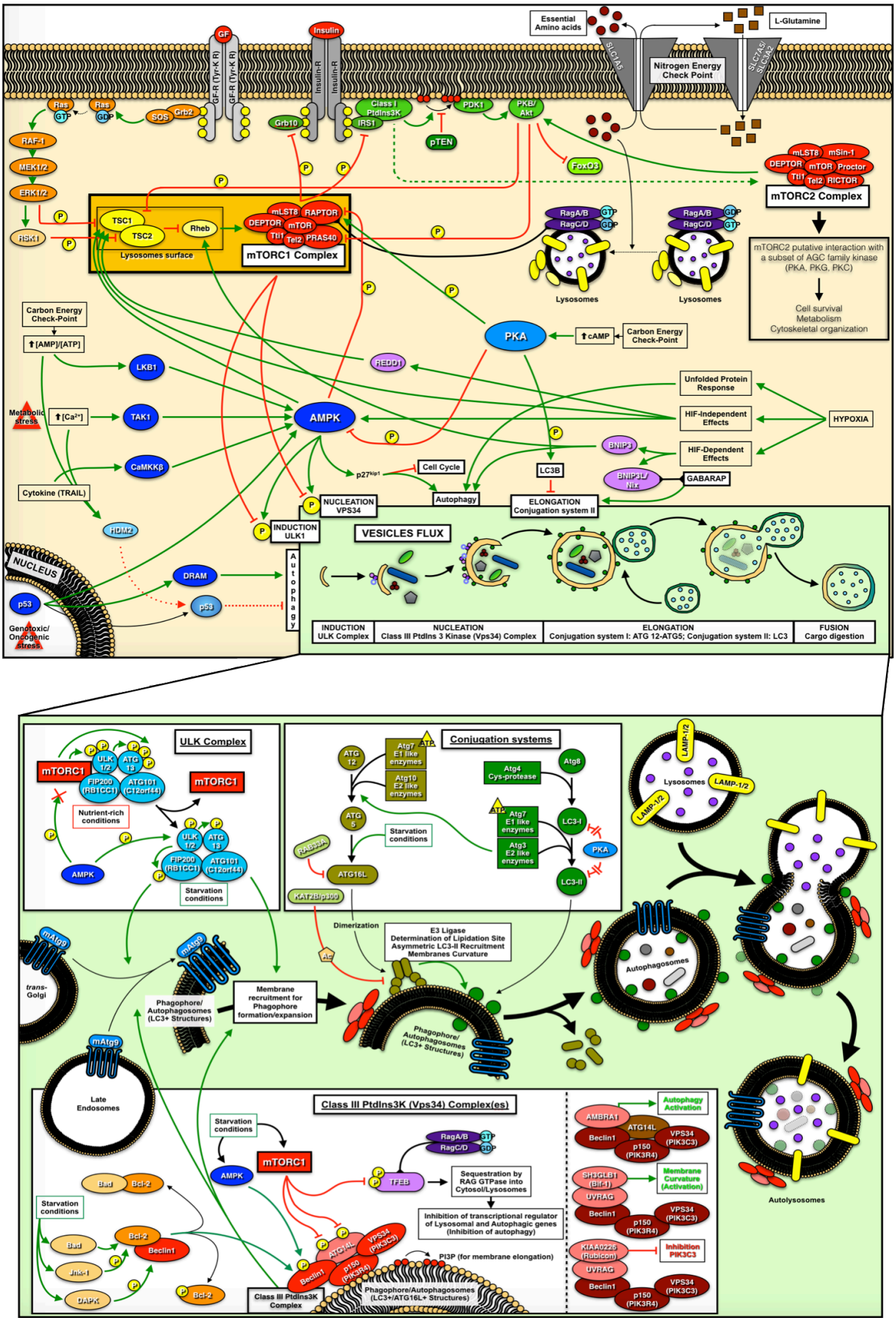

Essential Amino acids
L-Glutamine
Nitrogen Energy Check Point
SLC7A5/ SLC3A2
Insulin
GF
Insulin-R
GF-R (Tyr-K R)
Ras GTP
Ras GDP
SOS
Grb2
Grb10
Class I PtdIns3K
IRS1
PDK1
PKB/ Akt
pTEN
FoxO3
RAF-1
MEK1/2
ERK1/2
RSK1
TSC1
TSC2
Rheb
Lysosomes surface
mTORC1 Complex
mLST8
RAPTOR
DEPTOR
mTOR
Tti1
Tel2
PRAS40
mSin-1
Proctor
RICTOR
mTORC2 Complex
RagA/B
RagC/D
GTP
GDP
Lysosomes
mTORC2 putative interaction with a subset of AGC family kinase (PKA, PKG, PKC)
Cell survival
Metabolism
Cytoskeletal organization
Carbon Energy Check-Point
↑[AMP]/[ATP]
PKA
↑cAMP
LKB1
REDD1
Unfolded Protein Response
Metabolic stress
↑[Ca2+]
TAK1
AMPK
HIF-Independent Effects
HYPOXIA
HIF-Dependent Effects
BNIP3
CaMKKβ
p27kip1
Cell Cycle
LC3B
BNIP3L/ Nix
GABARAP
Cytokine (TRAIL)
Autophagy
NUCLEATION VPS34
ELONGATION Conjugation system II
INDUCTION ULK1
VESICLES FLUX
HDM2
NUCLEUS
DRAM
p53
Genotoxic/ Oncogenic stress
INDUCTION ULK Complex
NUCLEATION Class III PtdIns 3 Kinase (Vps34) Complex
ELONGATION Conjugation system I: ATG 12-ATG5; Conjugation system II: LC3
FUSION Cargo digestion
ULK Complex
mTORC1
ULK 1/2
ATG 13
FIP200 (RB1CC1)
ATG101 (C12orf44)
Nutrient-rich conditions
Starvation conditions
AMPK
Conjugation systems
ATG 12
Atg7 E1 like enzymes
Atg10 E2 like enzymes
Atg4 Cys-protease
Atg8
ATG 5
Atg7 E1 like enzymes
Atg3 E2 like enzymes
LC3-I
LC3-II
PKA
RABGEF1
ATG16L
KAT2B/p300
Dimerization
E3 Ligase
Determination of Lipidation Site
Asymmetric LC3-II Recruitment
Membranes Curvature
Lysosomes
LAMP-1/2
Autophagosomes
mAtg9
trans-Golgi
Late Endosomes
Phagophore/ Autophagosomes (LC3+ Structures)
Membrane recruitment for Phagophore formation/expansion
Autolysosomes
Class III PtdIns3K (Vps34) Complex(es)
Starvation conditions
AMPK
mTORC1
RagA/B
RagC/D
TFEB
Sequestration by RAG GTPase into Cytosol/Lysosomes
Inhibition of transcriptional regulator of Lysosomal and Autophagic genes (Inhibition of autophagy)
PI3P (for membrane elongation)
Bad
Bcl-2
Beclin1
Jnk-1
DAPK
ATG14L
VPS34 (PIK3C3)
p150 (PIK3R4)
Class III PtdIns3K Complex
Phagophore/Autophagosomes (LC3+/ATG16L+ Structures)
Autophagy Activation
AMBRA1
SH3GLB1 (Bif-1)
UVRAG
KIAA0226 (Rubicon)
Membrane Curvature (Activation)
Inhibition PIK3C3

Figure 8. Autophagy regulatory (upper square) and molecular pathways (lower square). Following a brief legend of the colours and the shapes of the elements in the graph is reported. The vesicular structures, molecules and molecular complexes are defined directly in the graph and are always reported with the same shape and colour when the name is not written. In order to help the individuation of the different pathways, all the molecules concerning the same pathways has been colored with the same colour, or with different shades of this. Concerning the connecting lines, the green continuous lines with the final arrows indicate activating events while the red continuous lines with the final bar denote inhibitory events. Dotted lines are normally used to show supposed or not well defined effects. The red dotted lines with the final arrow reported at the left bottom of the upper square (HDM2 - p53 event) show inductor effects to promote protein degradation and subsequent inhibition event. Black lines define different things depending on the format and thickness: dotted thin black lines with final arrow show a change of state, while continuous thin black lines with final arrow indicate physical movement; continuous medium thickness black lines with final triangles on both extremity have been used to show direct contact between molecules while continuous medium black lines with final arrow indicate passage to following step of the molecules activity or the molecular complexes formation; lastly, the thickest continuous black lines indicate the passage to the following step of the pathway. In conclusion, the yellow circles represent phosphate groups and indicate phosphorylation events when are associated to the colored lines, while the blue circles indicate GTP-GDP molecules associated to Ras-like protein.

1.2.2 Autophagy and diseases

Autophagy pathway involves many proteins that in turn modulate other key pathways for the proper functioning of the cells in order to vouch the housekeeping maintenance of physiological homeostasis in virtually all eukaryotic cells (Degenhardt et al. 2006). In particular, basal autophagy ensures an important role in quality control of proteins and organelles by delivering superfluous, aged or damaged cytoplasmic material to the lysosomes (Bravo-San Pedro et al. 2017; Klionsky et al. 2016), driving polyubiquituinated and aggregated proteins to proteasomal degradation (Klionsky & Emr 2000) thus providing also substrates for catabolism to produce energy in stressing conditions (Mizushima et al. 2002) and providing energy during neonatal period in order to counteract prolonged starvation periods and to attend the high rate of proteins and organelles turn-over (Kuma et al. 2004). In addition, autophagy is also involved in the engulfment of apoptotic cells (Qu et al. 2007) and in the elimination of intracellular pathogens (Colombo 2007): peptides derivate from protein degradation can also be exposed and presented to T-cells for activation of immunity process for host defence (Crotzer & Blum 2009; Levine et al. 2011). Lastly, autophagy pathway, or at least molecules involved in its pathway, result to be etiologically involved in regulated cell death (RCD) (Berry & Baehrecke 2007; Denton et al. 2009; Liu et al. 2013; Mariño et al. 2014).

Considering the wide range of cellular functions influenced by autophagy, including its role in innate and adaptive immunity, defects in this pathways can be at the base of the onset of many different kind of disease, including cancer, neurodegeneration, aging, metabolic syndrome, inflammatory disorders and cardiovascular diseases (Arroyo et al. 2014; Bhutia et al. 2013; Bravo-San Pedro et al. 2017; Levine & Kroemer 2008; Mei et al. 2015; Shi et al. 2013). Below, the involvement of autophagy in some of these diseases will be briefly analyzed.

1.2.2.1 Autophagy and cancer

Initially autophagy was thought to be an anti-oncogenic mechanism but this assumption was changed after observation of evidences suggesting that autophagy can also promote tumorigenesis maintaining tumor cells survival (Degenhardt et al. 2006) and at our days it's clear that autophagy results is involved in both tumor-suppressive or tumor-promoting functions (Baehrecke 2005; Høyer-Hansen & Jäättelä 2008; Maiuri et al. 2009). More specifically, autophagy can assume a specific role depending on the stage of cancer in which is involved: it is a tumor-suppressive mechanism during the initial stages of cancer development, by removing oncogenic protein substrates, toxic unfolded protein and damaged organelles (Bhutia et al. 2013; Janku et al. 2011; White 2012), while it acts as a tumor-promoting tool for cancer defence and progression later, providing substrates for increased metabolism also in hypoxia conditions and

maintaining the functional pool of mitochondria (Bhutia et al. 2013; Janku et al. 2011; White 2012) especially for the cells located in the central areas of the tumour mass (Li et al. 2011a; Thorburn et al. 2009), and promoting moreover the development of metastasis in TRAIL-resistant cancer cells (Han et al. 2008; Herrero-Martin et al. 2009).

Many of the ATGs, which encodes the principal proteins involved in the autophagic pathway, are oncogenes (Akt, mTOR, Bcl-2, Beclin-1, FLICE-like inhibitory protein (FLIP)) and tumor suppressor genes products (pTEN, Death-Associated Protein Kinase (DAPK), p53 and LKB-1) which are believed to be essentials for tumor initiation and development (Chen & Debnath 2010; Eisenberg-Lerner & Kimchi 2009; Huang & Klionsky 2007; Kung et al. 2011; Lee et al. 2009; Levine & Kroemer 2008; Liang & Jung 2010; Liang et al. 2008b; Morselli et al. 2009; Turcotte & Giaccia 2010). Following, some of the main oncogenes, that are also autophagy inhibitors, and tumor suppressor proteins, also involved in autophagy activation, will be listed and briefly discussed:

- pTEN is a tumor-suppressor phosphatase that normally activates autophagy through inhibition of Class I PI3K activity. In cancer, PTEN gene is frequently mutated resulting in autophagy suppression (via Akt activation) and contributing in parallel to tumor promotion (Hafner et al. 2007; Horn et al. 2008; LoPiccolo et al. 2008; Maehama 2007; Vivanco & Sawyers 2002; Yin & Shen 2008);
- Tumor suppressor p53 can localize in the nucleus, promoting autophagy via AMPK and TSC1/2 (Tasdemir et al. 2008), or in the cytoplasm, inhibiting autophagy (Notte et al. 2011) and in both cases its mutation(s) cause alteration in autophagy pathway and leads to cancer development (Bhutia et al. 2013). In addition, Damage-Regulated Autophagy Modulator (DRAM) is a positive autophagy-regulator dependent from p53 (Crighton et al. 2006) usually deleted in multiple types of cancer (Bhutia et al. 2013);
- DAPK is a cytoskeleton-associated calmodulin-regulated serine/threonine protein kinase involved in autophagy activation (Moretti at al. 2007) that acts as a tumor-suppressor factor (Bialik & Kimchi 2006; Eisenberg-Lerner & Kimchi 2009) and possess metastasis-suppressor ability, contrasting the formation of metastatic foci in Lewis lung carcinoma in mice (Inbal et al. 2002);
- Tumor-suppressor LKB-1 (STK-1) is a serine/threonine kinase that is upregulated during metabolic stress and induces autophagy by several phosphorylation events, involving the activation of TSC2 downstream AMPK (Shaw et al. 2004a,b) activation, leading to the final inhibition of mTOR through its regulatory subunit RAPTOR (Corradetti et al. 2004). Further, LKB-1 leads in parallel to the activation of p27 (downstream AMPK), a cyclin-dependent kinase inhibitor that induce autophagy and cell cycle arrest in order to conserve energy during autophagy (Liang et al. 2007);
- Not only oncogene Akt but all its upstream and downstream regulators are affected by mutation in most cancers (Shaw & Cantley 2006). For example, activation of mTOR stimulates cell growth and inhibits autophagy, and constitutive activation of Akt leads to autophagy inhibition *in vitro* and *in vivo* in Bax and Bak (pro-apoptotic members of Bcl-2 family) double mutants (Bhutia et al. 2013; Rubinsztein et al. 2007);
- The overexpression of oncogene Bcl-2, simultaneously with monoallelic deletion of the oncogene Beclin-1, leads to strong increment of tumor growth *in vivo* (Degenhardt et al. 2006). In general, Bcl-2 results to be mutated in most of cancers (Levine et al. 2008; Pattingre & Levine 2006) and it has been demonstrated that knockdown or silencing of its gene promote autophagy activation in MCF-7 cells (Akar et al. 2008);
- Beclin-1 is a very important molecule because of its involvement in autophagy activation by acting on Class III PI3K complex and its ability to inhibit tumor development by acting along with its numerous positive and negative interacting molecules (Kang et al. 2011; Liang et al. 2008a). Beclin-1 is an haploinsufficient tumor suppressor containing a deletion in many type of cancer (human breast cancer, ovarian cancers, prostatic cancers, hepatocellular carcinoma and lymphoma) and has represented the first evidence of a link between

autophagy and cancer (Bhutia et al. 2013; Qu et al. 2003; Liang et al. 2006; Marino et al. 2007; Takahashi et al. 2007). Many other evidences regarding mutations of Beclin-1 which affects its roles in tumor suppression and autophagy activation have been reported in several research, involving different cells and cancer types (Liang et al. 1999; Qu et al. 2003; Yue et al. 2003) and also other molecules, as in the case of the accumulation of p62/SQTSM in consequence of allelic loss of Beclin-1 in hepatocellular carcinoma (HCC) (Mathew et al. 2009). Moreover, also Beclin-1-intercating molecules, including UVRAG, Bif-1 and RUBICON, have been investigated for their tumor-suppressive or tumor-supporting properties and the effects of their mutation on cancer onset and progression have been documented (Coppola et al. 2008; Funderburk et al. 2010; Ionov et al. 2004; Kim et al. 2008b; Liang et al. 2006; Matsunaga et al. 2009; Takahashi et al. 2007; Zhong et al. 2009).

Several other ATGs involved in autophagy and which contribute to tumor-suppressor or tumor-induction signalling pathways have been identified. For example, Atg7 is involved in the maintenance of hematopoietic stem cells (HSCs) (Mortensen et al. 2011) and particularly in the correct production of both lymphoid and myeloid progenitors in mice (Bhutia et al. 2013). More, the suppression of Atg5 and Atg16L genes is one of the cause of tissue injury in Paneth cells and can leads to Crohn's death, that is one of the main factor risk for human colorectal cancer (Cadwell et al. 2008) and will be discussed later.

Role in autophagy	**ATGs**	**Effect of mutation**	**References**
Induction	ULK3	Induction of cell senescence	Young et al. 2009;
Nucleation	Beclin-1	Haploinsufficient tumor suppressor mutated in human breast, ovarian and prostate cancers	Liang et al. 1999;
	UVRAG	Haploinsufficient tumor suppressor mutated in human colorectal, breast and gastric cancers	Ionov et al.2004; Kim et al. 2008b;
	Bif-1	Decreased (*Bif-1*$^{-/-}$) in mice cancer prone	Coppola et al. 2008;
	Ambra1	Neural tube defects in mice *Ambra*$^{-/-}$	Garber 2011;
Elongation	Atg12-Atg5	*Atg5* frameshift mutation in gastric cancer	Cadwell et al. 2008;
	Atg4C	Fibrosarcomas developed in *Atg4C*$^{-/-}$ mice after carcinogen treatment	Mariño et al. 2007;
	Atg7	Development of liver cancer in live-specific *Atg7*$^{-/-}$ mice	Takamura et al. 2011;
	Atg16L	Mutation involved in the onset of Crohn'disease	Cadwell et al. 2008;
Maturation	Rab7	Aberrant expression of *Rab7*$^{-/-}$ in human leukemia	Liang and Jung 2010;
Cargo selection	p62	Accumulation in HCC after ROS production and NF-kB pathway inhibition in autophagy-deficient condition	Mathew et al. 2009;

Table 1. Some of the main ATGs mutated in cancers (table adapted from Bhutia et al. 2013).

Further, mice with Atg5 and liver-specific Atg7 deletions have developed liver cancer, and mitochondrial swelling, p62 accumulation, oxidative stress and genomic damage response has been observed in isolated hepatocytes (Takamura et al. 2011). Moreover, Atg4C deficient mice are resulted more susceptible in the development of tumorigenesis induced by carcinogen tissues (Mariño et al 2007). In addition, p62 (normally degraded during autophagy) is resulted to be overexpressed after autophagy inhibition and has been demonstrated that it is an important tumor promoting factor and is linked to NF-kB signalling way deregulation, NF-E2 activation, accumulation of ROS and increment of DNA damage (Arroyo et al. 2014; Mathew et al. 2009). Following the observation of the correlation between senescence and autophagy through a negative feedback in the PI3K-mTOR pathway (Young et al. 2009), a subset of ATGs have been

found to be upregulated during senescence. In this context, an important contribute in tumor suppression activity has been attributed to the induction of autophagy and senescence by the overexpressed ULK3/Atg3, as well as the inhibition of autophagy has been linked to a delay in the onset of the senescence phenotype (Bhutia et al. 2013). Table 2 reports the most important tumor inducing genes mutation and their usual involvement in autophagy.

1.2.2.2 Autophagy and inflammation

Inflammation is an important cellular and tissue process that occurs in case of loss of homeostasis and is very useful for host defence, tissue remodelling and repair, metabolism regulation (Medzhitov & Horng 2009). More specifically, both sterile or microbially-induced inflammation processes consist in the recruitment of phagocytes that remove infectious agents and damaged portions of tissue and then secrete cytokines and chemokines for adaptive immune response activation (Arroyo et al. 2014). Among these, some important examples are tumor necrosis factor (TNF) and Interleukin1 (IL1) (Medzhitov & Horng 2009), or the pro-inflammatory cytokines IL-1β and IL-18, synthetized as precursors and then processed by caspase-1 in specialized platforms known as inflammasomes, assembled after pro-inflammatory stimulation (Stehlik & Dorfleutner 2007), in order to produce their bioactive forms before secretion (Arroyo et al. 2014).

Multiple links between pathways and proteins involved in autophagy and inflammatory signalling pathways have been observed (Deretic et al. 2013; Levine et al. 2011), thus confirming that autophagy contributes to the host defence and to the induction of acquired immunity (Arroyo et al. 2014). Following, some examples of molecules involved in the regulation of both cellular processes have been reported:

- Several Toll Like Receptors (TLRs) are resulted able to induce autophagy. For instance, TLR2 is able to induce autophagic cell death in phagocytes stimulated with peptidoglycan (PGN) from *Staphylococcus aureus* (Arroyo et al. 2014), TLR4 adaptor protein TRAF promotes autophagy and stimulates NF-kB signalling via Beclin-1 ubiquitination (Shi & Kehrl 2010) and TLR9, activated by DNA containing immune complexes, induces secretion of type I interferons (IFNs) form plasmacytoid dendritic cells, through convergence between phagocytic and autophagic pathways (Henault et al. 2012);
- Another link between autophagy and inflammation comes from the observation that ATG16 deficient mice, presenting an impairment in LC3-PE conjugation, produce a very huge amount of IL-1β and IL-18 pro-inflammatory cytokines after stimulation with Lipopolysaccharide (LPS) or other pathogen molecular patterns (PAMPs) (Saitoh et al. 2008). Maturation of IL-1β and IL-18 is stimulated by the inflammasome complex containing NOD-like receptor (NLR) cryopyrin proteins, the adaptor protein ASC and the Caspase-1, which is activated by infection or other stimuli. As the LPS-induced inflammasome stimulation is dependent on K+ efflux and ROS, there's a chance to suppose that the link between inflammation and autophagy involves the inflammasome NLRP3 (Arroyo et al. 2014). Recent studies have revealed that ROS blockade suppresses the NLRP3 activity and that 3-methiladenine (3-MA) dependent autophagy/mitophagy inhibition in THP1 macrophages provokes ROS accumulation that is in turn responsible of the inflammasome activation (Zhou et al. 2011). Moreover, AIM2 or NLRP3 inflammasomes activation in macrophages can induce the autophagosomes formation through small G protein RalB, then requiring p62 to assist their delivery to the autophagosomes (Shi et al. 2012);
- Another class of molecules involved in both autophagy and inflammation are the Damage-Associated Molecular Patterns (DAMPs), which can be secreted (ATP and High Mobility Group protein B1 (HMGB1)),

exposed de novo or enriched in plasma membrane (calreticulin and Heat Shock Protein 90 (HSP90)) (Krysko et al. 2012), or produced as results of degradation processes of cell death (uric acids) (Krysko et al. 2012), in consequence of cells dying, injuries or stresses, working that work as adjuvant or signals to activate innate immune system (Matzinger 1994). Recently, it has been proposed that release and degradation of some DAMPs, such as HMGB1, ATP, IL1β and DNA is regulated by autophagy in several cell types (Zhang et al. 2013). More specifically, it has been observed that autophagy can regulate ROS-dependent release of HMGB1 in fibroblast, macrophages and cancer cells, extracellular trap-mediated HMGB1 release in neutrophils (Tang et al. 2011; Thorburn et al. 2009; Zhang et al. 2013), degradation of intracellular exogenous HMGB1 in macrophages (Li et al. 2011b) and the release of ATP from cancer cells in order to stimulate anticancer immune response (Michaud et al. 2011).

Conversely, some DAMPs can in turn regulate autophagy induction (Tang et al. 2011; Tang et al. 2012; Zhang et al. 2013) and it has been observed that both intracellular and extracellular HMGB1-mediated autophagy leads to chemoresistance in leukemia, colon and pancreatic cancers (Huang et al. 2012; Liversey et al. 2012; Zhang et al. 2013), as well as ATP can induce autophagy in human monocytes/macrophages, thus leading to the rapid elimination of intracellular bacteria (Biswas et al. 2008), and can stimulate the extracellular release of autophagolysosomes form microglia cells (Takenouchi et al. 2009).

Crohn's Disease (CD) is a common inflammatory disorder arising from defects in self-recognition of the intestinal flora and loss of the mucosal barrier function (Cooney et al. 2010). Even if the etiology of CD is still undefined, three genes involved in autophagy have been identified for their susceptibility in CD (Barrett et al. 2008; Franke et al. 2010): ATG16L1, essential during elongation step of autophagy, Immunity-Related GTPase family M (IRGM), that increases clearance of bacterial pathogens by inducing autophagy after the IFN-γ-stimulation (Feng et al. 2008), and NOD2, an intracellular PRR expressed in Paneth cells and few other cell types, which recruits ATG16L1 at the entry sites in order to target bacteria for autophagic degradation (Travassos et al. 2010). In particular, ATG16L1-mutated mice present alteration in CD pathogenesis (Murrow & Debnath 2013) and further, ATG16L1-lacking chimeric mice macrophages show an increment of IL-1β production after LPS stimulation or infection by non-invasive enteric bacteria, and a higher sensitivity to sodium sulphate-induced colitis, thus suggesting a promoting role of pro-inflammatory cytokine in intestinal damage in ATG16L1-dependent CD (Saitoh et al. 2008). Other studies have demonstrated that NOD2 activated by the bacterial ligand muramyldipeptide (MDP) can induce autophagy, requiring autophagy proteins such as PI3K, ATG5, ATG7, and ATG16L (Cooney et al. 2010), and that autophagy is essential for NOD2-mediated antigen presentation in human antigen-presenting dendritic cells (DCs), considering that DCs expressing CD variant of NOD2 (1007fsinsC, R702W or G908R) and ATG16L1 T300A have showed a reduction in the MHC class II exposed molecules and in the induction of antigen-specific CD4+ T cell responses (Brain et al. 2010; Cadwell et al. 2010).

1.2.2.3 Autophagy and infection

Autophagy, working together with innate immune responses, contribute also to the defence against pathogen infections. More specifically, xenophagy is the main responsible of the degradation of a wide board of intracellular pathogens bacteria such as *Streptococcus pyogenes*, *Mycobacterium tuberculosis*, *Shigella flexneri*, *Salmonella enterica*, *Listeria monocytogenes*, and viruses, as *herpes simplex virus type 1* (HSV-1) or protozoan pathogens including *Toxoplasma gondii* (Rubinsztein et al. 2012; Schmid & Munz 2007). However, the exact function of autophagy response depends on

the microorganisms and the infected cell types involved, also because many intracellular pathogens have evolved mechanisms for escaping, inhibiting or controlling autophagy in order to promote their own survival, replication and pathogenesis (Deretic et al. 2013). Several studies have demonstrated links between autophagy and responses to infection:

- Streptococcus A (GAS) is an intracellular pathogen of epithelial cells that is rapidly degraded in an autophagy dependent manner (Nakagawa et al. 2004). Notably, it has been demonstrated that the diameter of the autophagosomes-like vacuoles containing GAS is much larger than normal (10 μm instead of 0.3-1 μm) and that this process involves the autophagy machinery and additional components such as Rab7 (Sakurai et al. 2010: Yamaguchi et al. 2009), Rab9 and Rab23 (Nozawa et al. 2012);
- Another microorganism that infects epithelial cells is *Salmonella enterica* serotype *typhimurium*, which after infection is enclosed in Salmonella-containing vacuoles (SCVs) that promote bacterial survival and replication (Arroyo et al. 2014). However, *S. typhimurium* is labelled at the entry sites by several autophagy adaptors (Johansen & Lamark 2011; Kirkin et al. 2009a; Pankiv et al. 2007; Thurston et al. 2009; von Muhlinen et al. 2010; Wild et al. 2011), all binding ubiquitin, LC3 and GABARAP-1 in order to drive its delivery to autophagosomes (Arroyo et al. 2014). Mechanisms dependent from the autophagy adaptor p62 are responsible of the degradation of Several other pathogens such as *Listeria monocytogenes* (Yoshikawa et al. 2009) and *Shigella flexneri* (Dupont et al. 2009);
- Numerous studies have revealed several host proteins involved in autophagy as well as in the maintenance of intracellular pathogen *Mycobacterium tubercolosis* (Kumar et al. 2010). In the past, investigations were carried out on the attenuate vaccine strain of *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) (Gutierrez et al. 2004a) and it was demonstrated that exogenous stimulation of autophagy was required for LC3-targeting of BCG containing-vacuoles but, however, *M. tubercolosis* failed in own itself replication and in activation of innate responses in macrophages because of the lack of several virulence factors, like the type VII secretion system ESX-1 (Arroyo et al. 2014; Watson et al. 2012; Wong & Jacobs 2011). In a recent study, it has been showed that ubiquitin-mediated targeting of wild-type (WT) *M. tubercolosis* in resting macrophages requires both bacterial and host components, and that its autophagy-dependent delivery to autophagosomes needs to ubiquitin-autophagy receptor p62, NDP52 and the DNA-responsive kinase TBK-1 (Arroyo et al. 2014; Watson et al. 2012). Further, other studies have demonstrated that the autophagic clearance of M. tubercolosis in human myeloid cells is enhanced by IRGM (Singh et al. 2006) and that autophagy can participate in its elimination also producing antimicrobial peptides by a p62-dependent mechanism (Ponpuak et al. 2010; Alonso et al. 2007) which involves two guanylate-binding GTPase proteins, Gbp1 and Gbp7 (Kim et al. 2011b). Surprisingly, *M. tubercolosis* is also able to interfere with the phagosome maturation thus evading its own degradation (Gutierrez et al. 2004a);
- *Toxoplasma gondii* also can use autophagic vesicles for its own replication, even if two mechanisms of macrophage activation for killing the parasite in cultured cells are known. The first mechanism depends on the INFγ induced by LPS stimulus, that is very important in the control of acute infection, while the other is dependent on CD40 ligation (Arroyo et al. 2014). Recent studies have highlighted the importance of Atg5 for *in vivo* resistance to *T. gondii* infection in granulocytes and macrophages and for the recruitment of the INFγ-inducible p47 GTPase IIGP1 (Irga6) to the vacuolar membrane (Liesenfeld et al. 2011; Zhao et al. 2008), suggesting that phagosomes need to be processed for an efficient clearance of the parasites. Moreover, macrophage activation by CD40-CD40L interaction increases Beclin-1 levels and stimulates autophagy-dependent killing of *T. gondii* (Portillo et al. 2010);

- Viruses can influence autophagy during nucleation, elongation and/or maturation steps by targeting autophagic proteins (most of the viruses prefer Beclin-1) and thus producing different effects depending on the virus and the infected host cell types. For instance, infection by Herpes Simplex Virus 1 (HSV-1) can interfere in autophagosome initiation by interaction of the neurovirulence factor ICP34.5 (Orvedahl et al. 2007) or the oncogenic γ-herpes virus-encoded viral Bcl-2 like protein with Beclin-1 (Liang et al. 2008b). Moreover, the factor ICP34.5 can interact with Protein Phosphatase 1α (PP1α) to help elF2α dephosphorylation and allow HSV-1 replication (Li et al. 2011a; Orvedahl et al. 2007), thus promoting the onset of neurophatogenesis (Arroyo et al. 2014). In support of these observations, several studies have shown that functional and intact form of protein ICP34.5 is essential to inhibit starvation-induced autophagy in the breast cancer cell line MCF7 and that a mutant form provoke a reduction of the replication rate in *in vivo* primary neurons (Li et al. 2011a). In addition, it has been observed that the autophagy inhibition due to the mutated form of Bcl-2 encoded by a mouse γ-herpes virus maintains the infection in a latent form (E et al. 2009).

  Also RNA viruses are directly and strictly linked to autophagy. An important example is the Human Immunodeficiency Virus type-1 (HIV) which has been observed to block autophagosomes maturation in infected macrophages thus preventing their own degradation (Kyei et al. 2009) and take advantage form several components of autophagy to carry on its replication (Eekels et al. 2012). Several studies have been published describing mechanisms and molecules, including among the others Gag, LC3B II and Beclin-1, that are involved in both autophagy and HIV infection cycle and that determine their interactions (Blanchet et al. 2010; Harman et al. 2009; Kyei et al. 2009; Zhou & Spector 2008).

1.2.2.4 Autophagy and neurodegeneration

The central nervous system (CNS) is a very particular tissue, characterized by a high sensitivity and a poor regenerative capacity, and for these reasons it can react to several insulting stimuli, such as trauma, infections, toxins and systemic pro-inflammatory cytokines, by inducing a dynamic and acute immunological and inflammatory response guided by the activation of microglia cells in order to fight the threat and limit potential damages caused by inflammatory activity (Arroyo et al. 2014; Franck-Cannon et al. 2007; Galea et al. 2007; Popovich & Longbrake 2008; Rivest et al. 2009). As this is a self-limiting response, the presence of exogenous or endogenous factors or defects in self-limiting mechanism can maintain the inflammatory cycles active thus producing a huge amount of pro-inflammatory cytokines (as IL-1, TNFα and IL-6) and superoxide and nitric oxide, eventually leading to a pathological condition (Glass et al. 2010; Hanisch et al. 2002).

The role of autophagy in CNS is still not completely clear but is well known that autophagosomes accumulation is a features of several brain disorders (Nixon et al. 2008; Rosello et al. 2012; Winslow & Rubensztein 2008) and that autophagy process is a very important tool for maintenance of neuronal homeostasis, plasticity and quality control of neuronal proteins (Wang et al. 2006; Wang et al. 2009). In addition to neurons, recent in vivo and in vitro studies have revealed that PNG or other TLR2 ligands are able to activate microglia cells before and to induce their autophagic cell death in autophagy-dependent manner later (Arroyo et al. 2014). TLR2, a PRR already cited for its involvement in inflammatory responses and infections (Arroyo et al. 2014), has also a role in host defence and neurodegeneration processes (Arroyo et al. 2014), and it can be used by pathogens in order to bypass innate immunity mechanisms of the host by virtue of its ability in controlling microglia cell population (Arroyo et al. 2014).

Activated microglia cells acquire several functions including phagocytosis, through which it guarantees the brain

homeostasis (Lucin & Wyss-Coray 2009; Lucin et al. 2013). It has been demonstrated that microglial receptor-mediated phagocytosis involves several molecules typical of autophagic pathway, such as Beclin-1 and Vps34, which play a role in the delivery to the lysosomes or in recycling of cellular components, and that the Beclin-1 decrement is involved in the reduction of phagocytosis of β-amyloid peptides (aβ), that are a typical feature of the onset of Alzheimer's Disease (AD) (Lucin et al. 2013). In effect, a role of mononuclear phagocytosis in the context of innate host defence against several pathological conditions of the CNS, as AD, Parkinson's Disease (PD) and Multiple Sclerosis (MS) has been clearly demonstrated (Arroyo et al. 2014), showing that diseases progression is directly linked to the microglia and astrocytes inflammatory responses in the CNS, which is determined by the balance between pro- and anti-inflammatory signals (Glass et al. 2010).

Neurodegeneration is a pathology of the nervous system and autophagy helps neurons in fighting the onset of this pathological condition by preventing the accumulation of protein aggregates and damaged mitochondria, which otherwise can lead to progressive neuron loss (Wong & Cuervo 2010). Neurodegenerative diseases are usually thought to be a direct consequence of aging and the decreased expression of Beclin-1 with age in human brain, as well as the subsequent decrement of the autophagy rate, supports the observation in favour of the link between age advancement and increasing incidence of neurodegenerative diseases (Shibata et al. 2006).

Alzheimer's disease (AD) is neurodegenerative disorder which typically occurs in advanced age and is characterized by extracellular deposition of Aβ plaques and progressive intracellular accumulation of neurofibrillary tangle composed by hyperphosphorylated tau protein (Murrow & Debnath 2013). Identification of AD features were performed firstly in well-conserved neocortex biopsy of AD patients, and observation of various type of autophagic vesicles in intermediate stages (Nixon et al. 2005) suggested that a general feature to identify AD could be the defects in autophagic vesicles maturation (Arroyo et al. 2014). Several investigations have revealed that autophagy in AD can be affected on both autophagosomes formation (Lee et al. 2010b) or degradation stages (Pickford et al. 2008) and have led to the identification of the mutations causing the familiar forms of AD, which involve amyloid precursor protein (APP) and Presenilin (PS) 1 and 2 (Price et al. 1998; Sherrington et al. 1995). For instance, mutations of PS1, a transmembrane protein with a critical role in lysosomes acidification and activation of lysosomal proteases during autophagy (Lee et al. 2010b), are responsible of the most common early-onset familiar AD (Arroyo et al. 2014). Other evidences have revealed that the amount of Beclin-1 in AD patients is reduced with respect to the normal conditions, suggesting that autophagy could be impaired at autophagosomes formation step (Pickford et al. 2008): about this, several studies have reported that Beclin-1 mutated mice have shown an increment of APP, Aβ and tau proteins aggregation accompanied by a higher neurodegenerative and toxicity rates (Berger et al. 2006; Pickford et al. 2008; Wang et al. 2009). However, all these evidence are still to be verified because several controversial results have been published (Wang et al. 2010) and further studies are necessary in order to determinate if autophagy has a cytoprotective or cytotoxic role in AD.

Another late-onset neurodegenerative disorder is Parkinson's Disease (PD), characterized by the presence of intracellular inclusions containing α-synuclein and ubiquitin (Lewis bodies) and accumulation of autophagic vesicles and damaged mitochondria, that lead to degeneration of dopaminergic neurons and occurs most frequently in "sporadic" form, even if exists also in familiar forms (Cheung et al. 2011). Autosomal recessive PD has been associated with mutations in two genes: PINK1, responsible of the ubiquitination of outer mitochondrial membrane proteins, AP recruitment and mitophagic degradation, and PARK2, which produce Parkin (Lesage et al. 2009; Youle & Narendra 2011), a protein selectively recruited by PINK1. Autophagy is involved in degradation of both wild-type and mutant A53T α-synuclein, and the mutant form results to be overexpressed in neurons that are destined to PD onset (Arroyo et

al. 2014). In addition, it has been demonstrated that neurons expressing mutant A53T induce mitophagy, together with depletion of cellular ATP and cell death (Choubey et al. 2011), and the action of 3-MA or the knockdown of Parkin or Beclin-1 can protect at least in part against A53T-mediated cytotoxicity (Arroyo et al. 2014). Further, familiar PD can be induced by duplications of α-synuclein only (Singleton et al. 2003), and it has been demonstrated that an excess of intracellular level of this protein negatively affects autophagy through inhibition of small GTPase Rab-1A (Winslow et al. 2010), thus increasing cytosolic levels of the proteins aggregations and reducing autophagic rate, and then causing a subsequent impairment of the clearance of dysfunctional mitochondria and an increment of neuronal susceptibility to pro-apoptotic insults (Winslow et al. 2010).

1.3 NPs investigation methods scenery: the High-Content Screening (HCS)

Despite the numerous difficulties in the development of an effective investigation approach (reported in the paragraph 1.1.2), NPs are still among the protagonists in the drug discovery landscape (as introduced in the paragraph 1.1.3). The reasons of this important role, in addition to the unsatisfying results coming from the chemical synthesis (Newman & Cragg 2012), are attributable to the new evidences emerged from the important recent findings, comprising the new knowledges derived from the end of the human genome project, the very wide and fast evolution of the investigation methods and techniques, mostly High Throughput and High Content Screening (HTS and HCS) approaches, systems biology and metabolomic (Carnero 2006; Zang et al. 2012) as well as the development of fully automated instrumentation, as for example microscopes (Bickle 2010). Such new knowledges have revolutionized the drugs discovery scenery, also by shifting the attention from the "magic bullet" paradigm, in which researchers considered a single compound directed against a single target enough for the treatment of a given disease, basing on the point of view of the Nobel Prize in 1908 Paul Ehrlich, to the network pharmacology approach, based on the paradigm defined as "magic shotguns", which considers multi-target therapeutics essential to fight a complex and interrelated system such as a disease (Isgut et al. 2017).

HTS is a drug discovery approach developed and widely diffused since '90s which has allowed to increase the speed for testing bioactivity of samples contained in libraries ("drug-like" chemical compounds), mostly by automatization of the classical investigation methods (Baker et al. 2007), thus raising the number of assays carried out per day from 1000 up to more than 200000 (David et al. 2015) and allowing to meet the increasing demand for successful results in natural product programs (Baltz 2006). Currently, HTS represents one of the most used approaches for drug discovery in pharmaceutical industry and also in academic research (Szymański et al. 2012). When HTS is accompanied by an automated multiparametric quantitative image and data analysis, mostly in the fields of microscopy techniques, is referred as part of a High Content Screening (HCS) (Mattiazzi Usaj et al. 2016). HCS is a multiplexed functional screening based on the imaging of multiple fluorescent targets inside cell kept in physiologic conditions in order to extract a large amount of data with a limited rate of false-positive and false-negative results (Kozak et al. 2009). The subsequent analysis of these data allows to produce multidimensional profiles of the images of cells in order to identify aberrant phenotypes produced by a perturbation such as addition of treatments or genetic mutations (Mattiazzi Usaj et al. 2016). HCS assays are applied mostly in cell based assays, which allow to investigate drugs effects on a generalized pathway or more specific targets because they are models very close to the human body (Monks et al. 1991; Zang et al. 2012) and can provide more interesting results by observing phenotypes change in colorimetric, luminescent or mostly fluorescence assays (Zang et al. 2012).

In recent times, it has been started to use HCS also in a preliminary phase of the drug discovery and, despite provides less quantitative information than traditional biological assays, it can leads to detect very interesting treatments producing phenotypes on specific cellular models in an early stage of the drug discovery process: this new paradigm allows to incorporate secondary into the primary screens leading to an early and very efficiently selection of positive results ("hits"), reducing costs and timelines of the drug discovery process (Bickle 2010). More specifically, HCS allows to establish the activity of the tested samples without needing to find the specific molecular target in advance (Bickle 2010), thus improving the problems regarding the attrition rate by limiting false-positive and false-negative results (Durr et al. 2007) and more, providing information concerning the minimal requirements of ADMET (Adsorption, Distribution, Metabolism, Excretion, Toxicity) for tested samples, such as cell penetration and toxicity effects (Barabasz et al. 2006; Bickle 2010; Loo et al. 2007; Perlman et al. 2004; Young et al. 2008).

Several factors are essential for the success of an HCS project, mostly the availability of technologies and instrumentation for automatic images acquisition, like automated confocal fluorescence microscopes, and algorithms to perform data mining, presently gathered in both open source or paid software, by which is possible to automatically, quickly and reliably extract quantitative information concerning cellular features form large amount of images (Bickle 2010; Kozak et al. 2009; Mattiazzi Usaj et al. 2016). All these quantitative measurements can be further analyzed in order to produce univocal results which define the phenotypes produced by the treatment: for this purpose, a large number of approaches, consisting in data normalization, reduction of data dimension and data analysis through supervised and unsupervised methods, are currently available (Alon et al. 1999; Kozak et al. 2009; Manetta et al. 1992; Mattiazzi Usaj et al. 2016; Meyer & Cook 2000; Nasir & Jolley 1999; Owicki 2000; Tavazoie et al. 1999;).

Below, some examples of the most modern instrumentations, techniques and software for data analysis will be briefly discussed.

1.3.1 Cell Voyager: automated confocal microscope and image acquisition strategy

The Cell Voyager CV6000 (Yokogawa©, Meters & Instruments Corporation, Japan) is an automated confocal fluorescence microscope which allows to analyze cells cultured and simultaneously stained using different antibodies directly in multi-well microplates. The CV6000 uses a high-resolution real confocal method based on a pinhole disk, an original optical system to obtain clear images and technologies to reduce the background noise to one-third. On a technical level, the light path of a 100-W halogen light for bright-field illumination can be diverted around a Yokogawa spinning disk with four solid-state lasers, in order to provide wide-field as well as confocal imaging. Moreover, this microscope is equipped with three Electron-Multiplying Charge Couple Device (EMCCD) cameras for parallel acquisition of more than three channels. The Cell Voyager 6000 is the first instrument to have EMCCD cameras and offers one of the industry's highest resolution and clear images (Bickle 2010).

In addition to the classic functions of a confocal microscope, the CV6000 is equipped with some devices which allow to analyze cells also in living conditions. The Yokogawa's CSU confocal scanner unit, which utilizes a multi-beam scanning method exciting with a weak laser repeatedly, enabling continuous observation of live cells by minimizing cellular damage: working in test mode, it is possible to arrange in advance the assay configuration in order to determine the best conditions to get high resolved and well-focused images from samples, avoiding to damage samples and create artefacts. Further, an integrated incubator keeps the plates in culturing conditions (37°C; 5% CO2; controlled humidity rate) during storage while waiting for the analysis and the possibility to reproduce these conditions also inside the microscope during the imaging allows to avoid artefacts creation caused by the possible stressing conditions for the

long duration of the image acquisition time. All these parameters and conditions are handled by the operator through the Yokogawa “CV 6000 Measurement” software which controls the microscope, the injection platform and the incubator (Bickle 2010).
In a typical experiment, the first step consists in the specification of the plate type (brand, material and number of well) and the different lasers (channels) which were to be used to perform the analysis (405 nm, 448 nm, 531 nm, 635 nm lasers), basing on the cells and the chromophore used in the assay. It was also possible to decide if the acquisition of the images on different channels will be performed by activating the lasers simultaneously or consecutively, basing on the type of samples and the goodness of their preparation: in the first case the imaging duration will be shorter but the resolution of each channels could be affected by the possible cross-talk effects caused by the overlapping of the excitation/emission spectra of the chromophore; in the second case, conversely, the possible cross-talk effects will be avoided, or at least reduced to the minimum, but the duration of the imaging will be very longer. Moreover, it was possible to optimize the images acquisition also working on parameters like the "exposure time" and the "emission gain" for each single channel, in order to limit possible high-background and overexposure problem and optimize the duration of the acquisition for each single field and channel in each well.
Other important parameters which is possible to optimize are the layout of the acquisition fields for each wells, in order to limit empty images or artifacts caused by their position thus increasing the final yield, and the selection of the best focal plane for the images acquisition, established by working on the Z-Stacks (focal planes) number and position in test mode, in order to set the best focus for the images acquisition to detect specific cell types, thus getting the highest quality final images, consisting in the maximum intensity projection of the different Z-stacks.
Taken together, all the described parameters allow to design the best conditions for each single assays, improving significantly the resolution and the goodness of the final output images.

Basing on all the described features, the CV6000 microscope is considered one of the most modern and powerful drug discovery High Content Analysis (HCA) systems, which can automatically and at high speed takes pictures of treated cells to evaluate the biological effects of the administrated substances by analysing the produced phenotypes after treatments.

1.3.2 Cell Profiler: a free image analysis software

Cell Profiler is a versatile, open-source software tool designed for modular and flexible analysis for quantifying data from biological images by measuring size, shape, intensity, and texture of every cell (or other object) in every image, particularly in high-throughput experiments (Kamentsky et al. 2011; Lamprecht et al. 2007). Thanks to the point-and-click graphical user interface (GUI) Cell Profiler allows to build very easily a "pipeline", a series of modules performing different image processing functions such as illumination correction, object identification (segmentation), and object measurement in order to identify and quantify different cellular phenotypes: a typical good pipeline consists in few modules which identify the main cellular elements, conventionally named “objects”, in a process known as “cell segmentation” (Carpenter et al. 2006). Even if the software has been originally designed for the analysis High Throughput images, it is a valid tool to analyze images from Low Throughput assays form automated analysis in the context of High Content Screening.
From a technical point of view, it is firstly necessary to load the images on the software and to define them. Cell Profiler allows to select the images and define, based on the image name, their content (what channel each image is

referred). Moreover, it is also possible to use images metadata in order to define a “regular expression” that allows to sort images in groups (such as the different treatments), making the final data interpretation easier and faster. After, several classes of modules (“Object Processing”, “Image Processing”, “Measurement” and “Data Tools” modules) can be used to correct the noise or artifacts or to define the Regions Of Interest (ROIs) like cell or subcellular elements into the images: all these modules work mostly through bianrization or thresholding algorithms, such as Otsu’s method, combined with the watershed algorithm (Beucher 1991; Otsu 1979) and the most important of them will be briefly discussed below.

“Identify Primary Objects” is a module that allows to identify subcellular compartments defined as “Primary Objects”, because they can be found in an image without needing the assistance of any other cellular features as a reference. Several information have to be provided to the module in order to get the better definition of the objects: the minimum and maximum possible diameter of the objects (in number of pixels), both the best thresholding strategy and method to define objects and distinguish them to the background, the methods to distinguish clumped objects (based on the shape or the intensity values) and to use some automatic strategy for objects declumping and how and where to fill possible hole detected inside the identified objects, possibly caused by the detection and identification strategies (Padmanabhan et al. 2010; Sezgin & Sankur 2004). It is very important to use the best combination choosing between different thresholding strategy and thresholding methods (Malpica et al. 1997).

“Identify Secondary Objects” is the module for identification of the so-called named “Secondary Objects”, that needs of another reference object (primary object) for guiding their detection (Jones et al. 2005). In this module it is possible to decide the input images and primary objects and more importantly the methods for the identification of the secondary objects. Moreover, this module allows also to discard all the objects that touch or are cut off by the image edges, in order to exclude the possibility to consider data coming from incomplete secondary objects and to introduce artefacts (Vincent & Soille 1991).

Several modules are available in order to help the identification of particular features or to adjust images with technical inaccuracies and have been used when necessary in the pipeline development. Among these, “Apply Threshold” is a module which allows to increase the contrast between foreground and background through the application of a pre-selected or automatically calculated threshold value, in order to reduce the noise in the images by setting pixel intensities below or above a certain threshold to zero, depending on the images (Padmanabhan et al. 2010; Sezgin & Sankur 2004). Another important module is “Enhance Or Suppress Features”, which allows to enhance or suppress the intensity of certain pixels in the images in order to magnify particular features and to improve their identification.

Other important modules allow to measure a lot of different features of previously identified objects, such as size, shape, intensity and texture. For example, “Measure Object Intensity” module allows to measure a great number of quantitative features relative to the intensity of the identified objects by working on the features of the pixels contained inside such objects: among all the outgoing feature measurements, the most important are Mean and the Median Intensity, the Maximal and Minimal Intensity and the Upper and Lower Quartile Intensity. Similarly, “Measure Object Size and Shape” module allows to measure al lot of quantitative features relative to the size and the shape of the identified objects, the most important of them are Area, Perimeter and Form Factor (Rocha et al. 2002).

Among the most important modules, a citation is deserved by “Filter Objects” module, which allows to select or eliminate objects working on particular features like size, shape or intensity values previously measured (Sezgin & Sankur 2004). Very important modules to get a good images segmentation are also “Mask Objects” and "Related Objects”. The first one allows to mask objects covered by particular area in the images, selecting only the objects that are inside/outside the covered area and allowing to make a more accurate analysis and excluding possible errors. The second module, instead, allows to consider each masked objects like “children” of specific bigger “parent” object used

to apply the mask, as for example in the case of organelles or protein and cells, thus excluding all the signals coming from not interesting areas which represent possible artefacts.
Normally, all pipelines are interrupted at the end using some modules for data export, such as "Export To Database" or "Export To Spreadsheet" (CSV format), in order to take out the numerical values of the measurements and to store them in the most comfortable format. The possibility to store the outcomes data in a database is very useful in order to further investigate them using another open source software named Cell Profiler Analyst, that contains useful tools (such as Image Viewer, Plate Viewer, Scatter Plot, Histogram, Heat Map, Density Plot, Box Plot) to quickly explore and analyze multidimensional data coming from high-throughput and high-content image-based experiments analyzed by Cell Profiler (Jones et al. 2008). This software is very important because allows to check for the goodness of the analysis immediately, avoiding wasting time due to errors or artefacts that will only show up later. For example, Plate Viewer is a tool for exploring data according to the spatial layout of the experiment and four different formats are supported: 96 (8 rows x 12 columns), 384 (16 rows x 24 columns), 1536 (32 rows x 48 columns), 5600 well-plate (40 rows x 140 columns). Data from each well are displayed in a colored scale after aggregation in a single number which can be the mean, sum, median, standard deviation, cv% (coefficient of variation), minimum and maximum of all the values measured for a specific well. This tool is very useful to check for possible artefacts, as the shifting of the effects observed in different rows on the plate caused by a too long duration of the microscope reading timings. Another very useful tool is the "Scatter Plot" which allows to display numerical data in a plane defined by two parameters (x- and y-axis, assigned by the user) and allows to check for possible data drift caused by artefacts and, again, for the possible shifting of the effects caused by the too long reading timings.

1.3.3 Multivariate analysis

A good data mining approach is fundamental to get a good interpretation and investigations of the data coming from image analysis. For this reason, before to start to project and develop a bioinformatics analysis pipeline, is very important to get a good understanding of both the biology involved and analytical techniques to use for the analysis, rather than having the right software packages (Kozak et al. 2009).
An HCS approach applied to a cell-based assay is conceived to simultaneously analyze the more relevant information coming from different features directly in one experiment through multivariate analysis methods, rather than in the classical way analyses characterized by information regarding single features read in a series of sequential experiments (Giuliano et al. 2003; Johnston & Johnston 2002; Monk 2005). A good HCS analysis requires both quality control and accurate measurements, in order to reduce systematic errors caused by, for example, liquid dispensing, signal intensity or artefacts, which can affect the results equally or depending to the well-location. These purposes are reaches by comprising references controls within the assay plates in order to check for possible variability plate-to-plate or within the same plate and for assay background levels. Although these strategies and the availability of modern automation can help in the minimization of these bias thereby providing more reproducible results, malfunctions of the automatic systems can introduce systematic errors that need to be corrected during the data mining stages (Kozak et al. 2009).
The first step of a typical data mining process consists in the interpretation of the experimental data. Frequently, data needs to be modified in order to allow their "out of the box" comparison, which means the possibility to analyze data coming from different type of measurements (different features) or from the same measurements repeated in different times (technical or biological replicates) all together. This operation allows to remove all non-biological variation contained inside the measurement results and is normally realized through several possible methods, gathered under the

name “Data Normalization”, and consisting in self-consistency methods like global normalization, linear regression and LOWESS (Locally Weighted Linear Regression), or by using quality elements such as self-normalization and controls (Kozak et al. 2009). One of the most commonly used data normalization method is the so-called Mean or Median Centering, which allows to evaluate the systematic deviations from the expected spatial or timely behavior of experimental parameters after quantification of the spatial-trend structure of an assay plate. Recently, another important developed strategy consists in performing screens in which controls are independent from treatments by using one reference plate containing only positive and negative controls to evaluate and normalize data coming from the analysis of plates containing only technical and biological replicates (Kozak et al. 2009).

After data normalization, quality control issues need to be taken into consideration. Normally, HCS samples are contained in microliter volumes and arranged in two-dimensional 96-well (12x8) or 384-well (24x16) plates: in this contest, a good quality control for measurements, mainly performed by automatic routines, allows to limit systematic errors occurrence thus improving objectivity, reproducibility and ease of the comparison across different screens. However, some almost unavoidable source of systematic errors exist and are following reported: ageing and reagent evaporation or decay of cells, which can be recognized by observing a smooth trend in the plate’s means/medians; localized deviation of the expected values are caused by liquid handling errors or malfunction of pipettes; drift in measures from different well/plates or other reader effects can occurs due to variation on incubation time and can be recognized as smooth attenuations of measurement over an assay. Examples of systematic signal variations occurring in all plates of any assay have been demonstrated by Brideau et al. (2003) and Heuer et al., cited in Kevorkov & Makarenkov 2005, which describes also a systematic error caused by the positional effect of detector. Several published papers report various quality controls methods (Brideau et al. 2003; Gunter et al. 2003; Heuer et al., cited in Kevorkov & Makarenkov 2005; Heyse 2002). Data quality controls is required at different levels in order to limit the occurrence of these systematic errors during the assay, starting since from the optimization phases by evaluating for example signal window (Z-factor) (Zhang et al. 1999), stability and sensitivity (evaluated by comparison with control compounds) (Cox et al. 2000; Lutz & Kenakin 2000), in order to reduce the false-positive/negative results and increase the effectiveness rate of the HSC assays to identify the "Hits".

Another important step of the data mining process consists in the application of “Statistical Deconvolution” methods to identify common patterns of groups of plates, that provide a quick overview of the gradients/patterns in an assay, which help the researcher to decide if certain experimental plates are to be repeated or just to be corrected (Kozak et al. 2009).

A very important step is the “Dimension Reduction” which is required to reduce the number of variables of the HCS assay: normally, the starting experimental variables are represented by a matrix described by n x m dimensions, but needs to be reduced until two or three in order to allow their graphical representation (Kozak et al. 2009). Many different methods are available in order to reduce assay dimensions, as Multidimensional Scaling (MDS) (Cox and Cox, 2000), Artificial Neural Network (ANN) and Self-Organizing Map (SOM) (Bernard et al. 1998; Kohonen et al. 1996; Zupan & Gasteiger 1993).

Particularly important step in data mining approaches is the multidimensional analysis of data set coming from image processing (n x m dimensional matrix). Currently, the most common techniques exploit pattern recognition algorithms which classify measurements following particular criteria, and are grouped in two major categories: supervised approaches, which work by determining data fitting with a predetermined pattern, and unsupervised approaches, to analyze data without any a priori input or knowledge. Many of the pattern recognition existing algorithms are currently available already packed into various for free or for fee software, such as for example KNIME or Orange, and all of them work by calculating the distance between any two observations, known as dissimilarity measure, commonly used to build clustering relationships: typical examples of dissimilarity measures are Euclidean distance and Pearson

Correlation Coefficient (Kozak et al. 2009). Following, some of the most used supervised and unsupervised approaches has been listed.

Supervised methods are generally used to individuate significant difference between groups of samples, and significance is evaluated in many different ways, including among the others parametric and non-parametric tests as well as analysis of variance. Examples of these methods are Nearest neighbours, which allows to find samples that best matches with a designed query pattern like control, and Support Vector Machines, which helps in finding combinations of samples that better split sets of biological data (Kozak et al. 2009; Tarca et al. 2007).

Differently, unsupervised methods allow to determinate internal structure or relationship in a data set characterized by uncertain or unknown phenotype classes (Mattiazzi Usaj et al. 2016), instead of trying to individuate samples that best matches a predicted pattern (Kozak et al. 2009). Many different algorithms and relative techniques are available and they are sorted in three different classes: 1) features determination, without looking for a particular pattern, as the Principal-Component Analysis (PCA), 2) cluster determination, to identify groups of similar phenotypes, and 3) network determination, to determinate complex sample-sample or sample-phenotype interaction using Boolean networks (Butte & Kohane 1999; Butte & Kohane 2000; Friedman et al. 1998; Liang et al. 1998a; Szallasi & Liang 1998; Wuensche 1998). One of the most used strategy is Hierarchical Clustering, which work by gathering similar data in the same group, and has been largely and successfully used in order to group proteins by their subcellular patterns (Chen & Murphy 2005) and drugs by their effects (Perlman et al. 2004). Many different Hierarchical Clustering algorithms (Sokal & Sneath, 1963) can be used depending on the methods by which distances between the growing clusters and the free members of dataset are calculated. These algorithms include Single-Linkage Clustering, which considers the distance between two clusters as the minimum distance between two members of such clusters (also known as the minimum or the nearest neighbour method); Complete-Linkage Clustering, that calculate the distance between two clusters as the greatest distance between members of the relevant clusters (also known as the maximum or the furthest neighbour method); Average-Linkage Clustering, that calculates clusters distance using average values of the members and is also known as Unweighted Pair-Group Method Average (UPGMA); Weighted Pair-Group Average; Within-Groups Clustering; Ward's Method, about which cluster members are assigned depending on the total sum of squared deviations from the mean of a cluster and joining cluster in order to produce the smallest possible increment in the sum of squared errors (Ward 1963). Independently to the algorithm used, clustering is lastly visualized like a Dendogram, in which all leaves represent a samples and the length of the branches as well as their relative position determinate the distance between the other samples (Kozak et al. 2009).

One important method that deserve to be described is the already mentioned Principal-Component Analysis (PCA). PCA is a non-parametric method which performs a mathematical decomposition of the analyzed dataset to reduce its dimensions to a small number of data and principal components (PCs) without needing any explicit background model (Ong et al. 2012) and is a particularly powerful tool to analyze biological issues related to the highest variance allowing to visualize the similarities between biological samples and to filter possible noise in datasets (Yao et al. 2012). More technically, the PCA allows to transform a number of potentially correlated measurements into a number of relatively independent variables organized depending on their contribution for explaining the variance contained in the whole data set: this approach allows to discard components with minor contribution, indirectly reducing the dimension of the dataset and avoiding to lose too much information. In this contest, principal components are conceived as a set of completely new vectors that capture data variance in a decreasingly fashion, with most of the variance comprised inside the first component, the largest part of the remaining variance in the second component and so on (Kozak et al. 2009). These new vectors, also named factors, eigenvectors, singular vectors or loadings in addition to principal components depending on the contest, are uncorrelated and orthogonal to the original variable vectors, and individuate a new space

in which data are distributed and identified by a score corresponding to their projection on the components, allowing also to represent data with graphs by plotting the projections of the data onto the components (Abdi 2003; Yao et al. 2012). As the importance of each component is represented by the variance of its projections and by the proportion of the variance explained by each component, PCA can be thought as an orthogonal decomposition of the variance (also called inertia) of data (Abdi 2003).

Obviously, it is recommended to represent the highest percentage of the variance with the minimum possible number of components in order to avoid too much difficulties in the final data interpretation: representing most of the variance with two or three principal components will allow to visualize the total dataset in a two- or three-dimensional graph like Scatter Plot, thus simplifying the final data interpretation, which remains the most difficult step of the data mining process.

2. The aim of the work

The present work consisted in the analysis of the effects produced by 47 crude extracts on the autophagy process in order to search for compounds possibly able to interfere in such cellular pathway. Being the autophagy a physiological cellular process commonly involved in the onset of many diseases, like cancer, inflammatory and neurodegenerative diseases, the determination of the these "autophagy-responsive" extracts could provide a starting point to find out new natural products potentially interesting as active principles for pharmaceuticals. Commonly, such kind of investigations were and is performed by following target-driven approaches based on single-target biochemical assays for primary screening (Mhadhebi et al. 2011; Spavieri et al. 2010; Suzgec-Selcuk et al. 2011), consisting in "in vitro" assays providing highly productive results, but that have often led to the isolation of candidates which produced very poor effects when tested "in vivo", or worse, that failed depending on toxicity problems. Starting from these evidences, and also considering that a great number of drugs present on the market have been discovered on the base of the phenotypic observations of the pharmacology (Feng et al. 2009), the present investigation of the extract effects on autophagy has been carried out by developing a strategy based on phenotypic approaches empowered by modern screening technologies (Feng et al. 2009) and in particular by an imaging-based High-Content Screening (HCS), a modern, emerging and very powerful analytical tool for primary screening which allows to get information from both single molecules as well as biological extracts (Bray et al. 2016; Caicedo et al. 2016; Korn & Krausz 2007; Kremb et al. 2017; Schulze et al. 2013; Young et al. 2008).
The screening of the phenotypes produced by each extract has been carried out following an "agnostic approach", which means that the extracts were analyzed without knowing anything about their potential activities and focussing the efforts only on the evaluation of their potential activity related to the autophagy pathway: from a technical point of view, the screening consisted in the simultaneous evaluation of multiple parameters regarding lysosomes and autophagosomes, considered as cellular markers descriptive of the autophagic activity, which were quantitatively measured from the images of cells treated with the extracts as well as on completely untreated samples, used as control reference samples. Moreover, due to the dynamic nature of the autophagic flux, the analyses have been carried out observing the effects produced on the autophagic activity after 2 different timing check points, one shorter and the other one longer, in order to evaluate them over time, as made in many experimental works on autophagy and also suggested in different reviews reporting the guidelines to investigate autophagy (Conte et al. 2017; Dowaidar et al. 2017; Klionsky et al. 2008; Klionsky et al. 2016; Mithener et al. 1976; Warnes 2015; Webb et al. 2003; Yu et al. 2010). The results were interpreted considering that such extracts, or molecular phytocomplexes and/or single compounds contained inside, can affect the pathway positively or negatively, thus increasing or decreasing its activity. Furthermore, in order to limit possible failure due to the potential toxic activity produced by some of the treatments, they were also selected on the basis of their cytotoxic effects, evaluated considering the mortality rate produced in relation to the control samples.

Depending on the effects produced on the cells the analysis has allowed to divide treatments in different categories. Treatments producing a high toxicity rates were discarded from this analysis and considered interesting for their potential cytotoxic effects. Treatments considered not toxic, instead, were further divided in different groups depending on their activity on the autophagic pathway: treatments producing an autophagy-inhibitory activity, treatments producing an autophagy-inducing activity and treatments having other kind of effects. As the investigations have been realized after both shorter and longer time durations, it has been possible to compare groups of treatments previously defined, sorting out them depending on their activity over time and thus addressing them towards different subsequent

deeper investigations. Treatments considered as not toxic were grouped basing on the following criteria: treatments having the same effects (inducing, inhibiting or undefined effects) on autophagy after both short and long incubation time, treatments revealing the activity only after longer incubation times, treatments changing their activity over time and those presenting any interesting effects over time. Treatments which hold their effects just for short time durations were discarded because considered as not interesting for possible further involvement in pharmacological investigations.

Hence, the present work has allowed to address natural crude extracts towards specific investigations without information about their effects in advance, with a deeper interest for substances possibly able to perturb the physiological status of the autophagy pathway as well as for those producing cytotoxic effects. Moreover, a theoretical strategy has been developed and then put into practice thus assessing a High Content Screening (HCS) approach to carry on this kind of analysis, characterized also by the possibility of being further developed and improved in order to obtain more interesting and detailed results. Lastly, different cell types have been analyzed by using this approach thus allowing to evaluate the applicability of such method to the analysis carried out using different cell models as well as to analyze the phenotypes produced by the administration of extracts.

Concluding, this work joins together some of the most interesting issues that presently occupy the scientific landscape, such as natural products, autophagy and the new investigation method based on the analysis of very huge amount of data by using the modern screening technologies coupled with the old investigation approaches. The obtained results constitute a good starting point for further and deeper investigations of the extracts here selected as interesting and for a further development of the analytical method and improvement of its potential.

3. Materials and Methods

3.1 Cell Model

HeLa CCL-2™ are adherent epithelial cells of human adenocarcinoma (B.S.L. 2) derived from cervix tissue of a 31 y.o. black woman. The culturing medium used to grow HeLa cells is ATCC-formulated Eagle's Minimum Essential Medium supplemented with 1% L-Glutamine 200 mM (#25030081, Thermo Fisher Scientific), 1% PenStrep (#15070063, Thermo Fisher Scientific) and 10% Fetal Bovine Serum (#26140079, Thermo Fisher Scientific). Common culturing conditions for HeLa cells are 95% air, 5% CO_2 and 37°C and their doubling time is approximately 24 hours (ATCC, Virginia, USA). It is recommended to use SH-SY5Y cells not later than 40-50 passages (ATCC n.d. a and references therein).
SH-SY5Y cells have been chosen because they are a very good model to carry out fluorescence and immunofluorescence investigations.
HeLa LC3B-GFP are HeLa cells wild type transfected in order to bind Green Fluorescent Protein to LC3B protein, provided by the DZNE in Bonn (Germany). Transfected HeLa cells allow to monitor LC3B immediately in living cells, avoiding to use antibody after fixation.
Further information on HeLa (ATCC® CCL-2™) cell line are available on the ATCC website.

SH-SY5Y CRL-2266™ are mixed adherent and suspension epithelial cells of human neuroblastoma (B.S.L. 1) derived from bone marrow of a 4 y.o. woman. SH-SY5Y is a thrice cloned (SK-N-SH → SH-SY → SH-SY5 → SH-SY5Y) subline of neuroblastoma cell line SK-N-SH which was established as a metastatic cone tumor in 1970. The culturing medium used to grow SH-SY5Y cells is composed by 1:1 mixture of ATCC-formulated Eagle's Minimum Essential Medium and F-12 medium supplemented with 1% Sodium Pyruvate (#11360070, Thermo Fisher Scientific), 1% GlutaMAX™ (#35050061, Thermo Fisher Scientific), 1% PenStrep (#15070063, Thermo Fisher Scientific) and 10% Fetal Bovine Serum (#26140079, Thermo Fisher Scientific). Common culturing conditions for SH-SY5Y cells are 95% air, 5% CO_2 and 37°C. The doubling time of SH-SY5Y cells is approximately 48 hours and is strongly dependent to the density of the cells (ATCC, Virginia, USA). SH-SY5Y cell have a reported saturation density greater than 1X 10^6 cells/m^2, and they exhibit moderate levels of dopamine beta hydrolase activity. It is recommended to use SH-SY5Y cells not later than 20 passages, because after they start to change and lose their neuronal morphology (ATCC n.d. b and references therein).
SH-SY5Y cells have been chosen because they are a very good neuronal cell model to study the autophagy pathway.
Further information on SH-SY5Y (ATCC® CRL-2266™) cell line are available on the ATCC website.

3.2 Cell culturing conditions

HeLa cells were normally cultured in 75 cm^2 flasks using 12 ml medium volume and renewing it 2-3 times per week. For splitting, cells were rinsed with 1X PBS and then left in 2-3 ml of 0.25% (w/v) Trypsin-0,53 mM EDTA for 5 minutes at 37°C until the cells are completely detached. It is preferable to avoid to shake the cells by hitting or shacking the flask in order to limit clumping and to use an inverted microscope to check the cells detachment. After detachment, a double volume of complete medium is added in the flask to inactivate enzymatic activity of the trypsin and the cells were collected and centrifuged at 1000 rpm for 5 minutes at Room Temperature (RT). After centrifugation, exhausted

medium was removed, cells were resuspended in fresh media and seeded at the right density, mostly depending to the vessel/plate and to their destination (culture or experiments). For cryopreservation, HeLa cells were collected in concentration $1,5x10^6$ cell in 1 ml of freezing medium, consisting in complete medium supplemented with 5% DMSO (v/v) and stored in cryotubes in liquid nitrogen vapour phase (ATCC n.d. a and references therein).
Further information on culturing conditions of HeLa (ATCC® CCL-2™) are available on the ATCC website.

SH-SY5Y cells were normally cultured in 75 cm^2 flasks using 12 ml medium volume and renewing it every 4-7 days. SH-SY5Y cells are a mixture of floating and adherent neuroblastic cells which present multiple shorts and fine processes called neutites, and tend to aggregate in clumps and float thus growing in cluster.
During splitting procedure, SH-SY5Y adherent cells were selected and the floating component discarded. After rinsing with 1X PBS, cells were detached adding 1-2 ml of 0.25% (w/v) Trypsin-0,53 mM EDTA and left for 5 minutes at 37°C. After complete detachment, the enzymatic activity was inhibited by adding a double volume of complete medium and the cells were collected and centrifuged at 1000 rpm for 5 minutes at RT. SH-SY5Y cells were then diluted in fresh complete medium and seeded in an appropriate density for keeping in culture or using them in an experiment. SH-SY5Y cells were cryopreserved by diluting $2,5x10^6$ cell in 1 ml of freezing medium consisting in complete medium supplemented with 5% DMSO (v/v) and then stored in cryotubes in liquid nitrogen vapour phase (ATCC n.d. b and references therein).
Further information on culturing conditions of SH-SY5Y (ATCC® CRL-2266™) are available on the ATCC website.

3.3 Extracts sources and extraction

Forty-seven crude extracts coming from three researchers working in different Countries were available to test their effects on cells.
Twenty-nine dried crude extracts have been received from the Dr. Kamal Kucherbaev, working at the South Kazakhstan State Pharmaceutical Academy (SKSPh), based in Al-Farabi sq., 1, 160019, Shimkent, Kazakhstan. All the extracts were obtained in 20% water-80% ethanol extraction solvent and then dried.
Thirteen dried crude extracts have been received from Dr. Nilufar Mamadalieva, working at the Institute of the Chemistry of Plant Substances AS RUz, Tashkent 100170, Mirzo Ulugbek Str 77, Uzbekistan. All the extracts were obtained in 100% methanol extraction solvent and then dried.
The last five dried crude extracts have been received from the Dr. Cesar Donoso Fierro, working at the Universidad Católica del Norte, Depto. De Agricoltura, Facultad de Ciencias del Mar, Larrondo 1281, based in Coquimbo, Chile. The marine samples *Ulva sp.*, *Cryptomenia sp.* and *Ciona intestinalis* were collected by apnea dive from the culture lines of the submarine facilities of the Universidad Católica del Norte, The Herradura Bay, IV Region, Province of Elqui, Coquimbo, Chile (29° 57'S–71°21'W). The samples were washed in a solution consisting of sterile sea water and ethanol 30% in order to remove all any associated microflora, and then washed again with fresh water to remove surface salts and sand particles. *Heliotropium sp.* samples were collected in the Coastal mountains of Coquimbo (20m.a.s.l.) (29° 59'S–71°21'W). All samples were allowed to dry in the shady and aerated place and keeping the weight constant. Subsequently, extract dilutions were performed following a specific protocol: 50 g of each dried sample was accurately weighed, chipped and macerated in 150 mL of ethanol 99,98% to exhaustion at 40 °C. The samples were then filtered and they were concentrated under vacuum at 40 °C. After, further extractions were performed sorting several solvents with increasing polarity: hexane, chloroform and ethyl acetate (4x100mL). The

Plant Extracts List				
#	**Source organism**	**Extraction solvent**	**State**	**Country origin**
1	EAGRC	80% Ethanol (EtOH)	Semi-solid	Kazakhstan
2	ERAAlop	80% Ethanol (EtOH)	Semi-solid	
3	EAGPS	80% Ethanol (EtOH)	Semi-solid	
4	EAGAAsia	80% Ethanol (EtOH)	Semi-solid	
5	EAGAAlop	80% Ethanol (EtOH)	Semi-solid	
6	EAGMA	80% Ethanol (EtOH)	Semi-solid	
7	EAGMD	80% Ethanol (EtOH)	Semi-solid	
8	EAGKL	80% Ethanol (EtOH)	Semi-solid	
9	EAGAS	80% Ethanol (EtOH)	Semi-solid	
10	EAGAT	80% Ethanol (EtOH)	Semi-solid	
11	EAGAU	80% Ethanol (EtOH)	Solid	
12	ERPS	80% Ethanol (EtOH)	Solid	
13	EFIAAlop	80% Ethanol (EtOH)	Semi-solid	
14	ERHA	80% Ethanol (EtOH)	Semi-solid	
15	EAGFA	80% Ethanol (EtOH)	Semi-solid	
16	EAGAA	80% Ethanol (EtOH)	Semi-solid	
17	EAGFO	80% Ethanol (EtOH)	Semi-solid	
18	EAGPD	80% Ethanol (EtOH)	Semi-solid	
19	EAGTM	80% Ethanol (EtOH)	Semi-solid	
20	EAGC	80% Ethanol (EtOH)	Solid	
21	EFHA	80% Ethanol (EtOH)	Semi-solid	
22	EAGSS	80% Ethanol (EtOH)	Semi-solid	
23	EFIPS	80% Ethanol (EtOH)	Semi-solid	
24	EAGCB	80% Ethanol (EtOH)	Semi-solid	
25	EAGCT	80% Ethanol (EtOH)	Semi-solid	
26	EAGOT	80% Ethanol (EtOH)	Semi-solid	
27	ERKL	80% Ethanol (EtOH)	Solid	
28	ERKS	80% Ethanol (EtOH)	Semi-solid	
29	ESHA	80% Ethanol (EtOH)	Semi-solid	
1	*Verbascum blattaria*	100% Methanol (MetOH)	Solid	Uzbekistan
2	*Stachys hissarica*	100% Methanol (MetOH)	Solid	
3	*Verbascum songoricum*	100% Methanol (MetOH)	Solid	
4	*Stachys betoniciflora*	100% Methanol (MetOH)	Semi-solid	
5	*Phlomis sewertzovii*	100% Methanol (MetOH)	Semi-solid	
6	*Phlomis salicifolia*	100% Methanol (MetOH)	Semi-solid	
7	*Silene oreina*	100% Methanol (MetOH)	Semi-solid	
8	*Phlomoides tadschikistanica*	100% Methanol (MetOH)	Solid	
9	*Cousina umbrosa*	100% Methanol (MetOH)	Semi-solid	
10	*Nepeta olgae*	100% Methanol (MetOH)	Semi-solid	
11	*Scutellaria scharistanica*	100% Methanol (MetOH)	Semi-solid	
12	*Schrophullaria sp.*	100% Methanol (MetOH)	Semi-solid	
13	*Leonurus panzeroides*	100% Methanol (MetOH)	Semi-solid	
1	*Ulva sp.* (U-2C)	99% Ethanol (EtOH) at 40°C	Semi-solid	Chile
2	*Cryptomenia sp.* (C-3B)	99% Ethanol (EtOH) at 40°C	Semi-solid	
3	*Ciona intestinalis* (C-4B)	99% Ethanol (EtOH) at 40°C	Semi-solid	
4	*Heliotropium sp.* (T-1B)	99% Ethanol (EtOH) at 40°C	Semi-solid	
5	*Heliotropium sp.* (T-1C)	99% Ethanol (EtOH) at 40°C	Semi-solid	

Table 2. List of the extracts investigated.

resulting fractions were concentrated and then stored at -40 ° C until use.
All the available extracts are reported in Table 2, along with the solvent used for its extraction, its physical state and the Country of origin.

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3.4 Treatments preparations

To produce the best assay conditions, preparation and administration protocols have been specifically determined for the present experiments. Extracts were diluted and administrated on HeLa and SH-SY5Y cells to evaluate their effects.
For administration to HeLa cells, dried extracts were weighed and resuspended in 100% Dimethyl sulfoxide (#D5879, Sigma Aldrich) at the concentration 200 mg/ml; dimethyl sulfoxide was chosen as solvent because able to almost completely dissolve all the extracts. After the addition of the solvent, solutions were kept in shaking for 1 hour on vortex to promote the dissolution of the sample and then left at 4°C (in the fridge) over-night, in order to inhibit the growth of possible contaminant microorganisms and to allow sterile conditions by exploiting the cytotoxicity power of the solvent. The day after the extracts were diluted in complete culturing medium and in completely serum-deprived culturing medium at the final concentration of 0,5 mg/ml in 0,25% Dimethyl sulfoxide.
For the experiments carried out on HeLa cells, the obtained solutions were diluted at four serial concentrations, from 0,5 mg/ml down to 0,06 mg/ml, directly in the respective culturing media and then administrated to the cells in 96-well plates in a specific arrangement (reported later in Figure 10), maintaining the treatment for 2 or 20 hours in culturing conditions.

To investigate the effects of the extracts on SH-SY5Y cells, the protocol described above has been followed with some differences. After weighting, dried extracts were resuspended in 100% Dimethyl sulfoxide (#D5879, Sigma Aldrich) at the final concentration of 100 mg/ml, kept on vortex for 1 hour and the obtained solutions stored at 4°C (in the fridge) over-night. The day after, extract solutions were diluted in both complete medium and partially serum-deprived medium (1%FBS) at the final concentration of 0,1 mg/ml in 0,1% Dimethyl sulfoxide. The percentage of FBS in the starvation-inducing medium has been established after investigations carried out to check the effects of media with different percentage of serum (1-5%) on the vitality and the adherence strength of stressed cells. All the investigations on SH-SY5Y cells were performed by using extracts directly diluted in the respective culturing media at four different concentrations, ranging from 0,1 mg/ml to 0,01 mg/ml, and keeping the treatments for 2 or 20 hours depending on the experiment (Klionsky et al. 2016). Since the analysis were performed in 384-well plate, a strategy that involves 96-well plates has been developed to make their administration faster: treatment dilutions were prepared in advance in four 96-well plates which have been considered as part of one 384-well plate (as reported in figure 9) and then stored at -20°C until the administration, that consists in the transfer of the solution from the 96-well plates to 384-well plate in a precise scheme (defined later and showed in Figure 12), in order to make this step faster and to perform it by using automatization.

The dilutions used to test the effects of the samples on autophagy pathway using both SH-SY5Y and HeLa cells have been chosen on the base of their cytotoxic effects, after a series of MTT assays, whose results have not been reported, have been performed in order to test different range of serial dilutions and choosing the ranges showing the best assay conditions for each cell model.

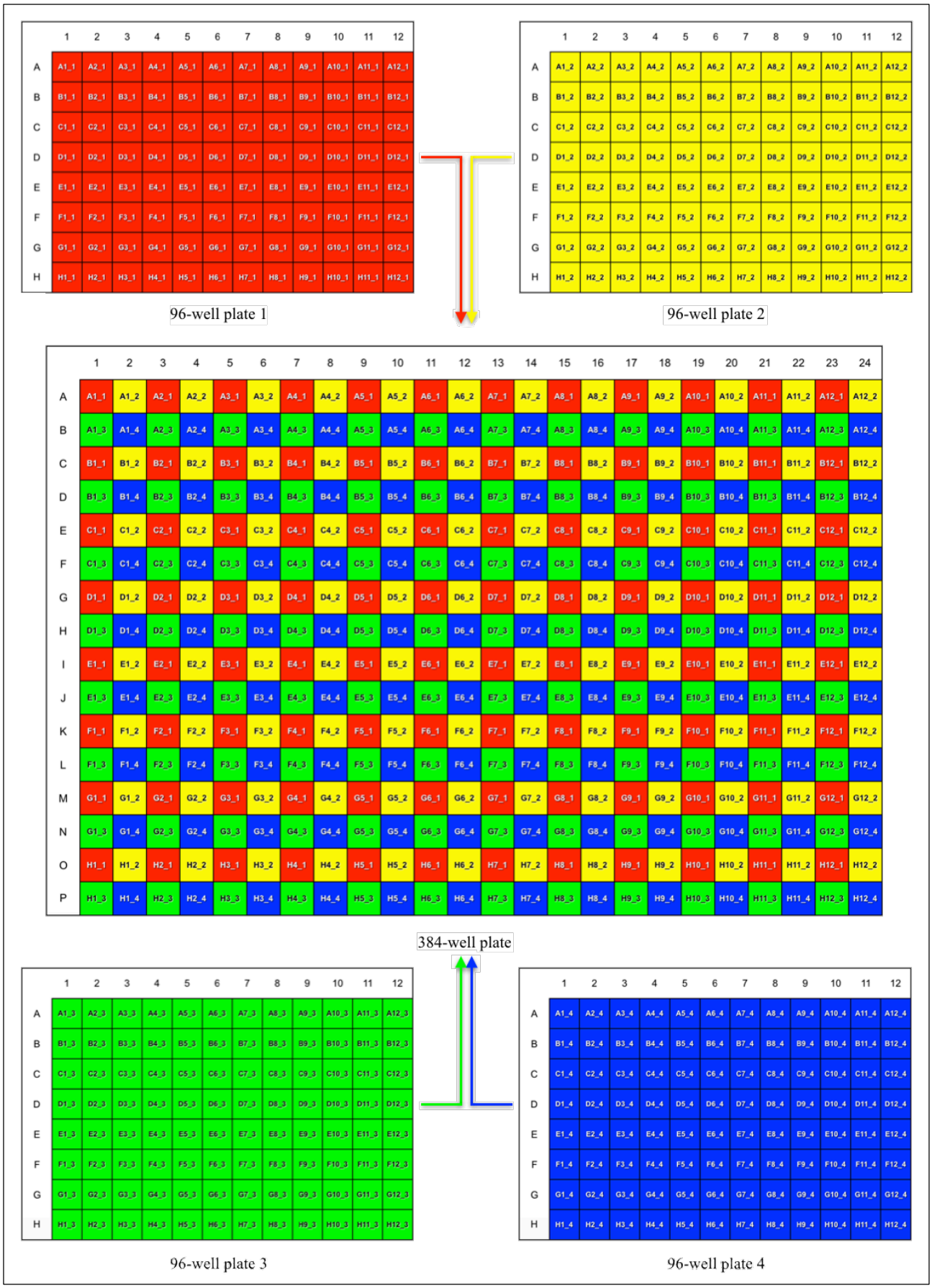


Figure 9. Scheme for 96 to 384-well plate conversion.

3.5 MTT assays

MTT assay is a colorimetric test for the evaluation of the actively growing cells basing the measurement on their ability to convert the yellow compound bromide-3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) in formazan, a blue / purple salt, by the redox activity of living cells (Abe and Matsuki, 2000). This conversion occurs mainly in functional mitochondria through the cutting of ring tetrazolium MTT made by succinct dehydrogenase, an enzyme active only in living cells. Investigations were performed by adapting the original protocol of MTT assay, firstly described by Mosmann (1983). Assays conditions may vary depending on the cell type.

Using HeLa, cells were seeded in 96-well plate at the concentration $1x10^4$ cells/well while for investigation on SH-SY5Y cells were seeded at $8x10^4$ cell/well. In both cases, cells are kept in culturing conditions (reported in paragraph 3.2) for 15-20 hours, and then media were replaced with the respective four serial dilutions of treatments reported in paragraph 3.4. After 2 and 20 hours (depending on the assay treatment duration) a solution of 5 mg/ml MTT (#M2128, Sigma-Aldrich) in PBS 1X, directly diluted in the respective culturing media at the final concentration 0,5 mg/ml, was used to replace treatments and left for 3 hours in culturing conditions. During the incubation time functional mitochondria digest the MTT salt and become blue/purple colored. Then MTT solutions were carefully removed and Dimethyl sulfoxide (#D5879, Sigma-Aldrich) added for 30 minutes to dissolve MTT crystals. Finally, the optical density for each well has been detected by a microplate reader Sunrise (TECAN) using a 595 nm filter.

The assays were repeated 4 times, always working in double, and the resulting cell viability or, more specifically, the mitochondria functionality was displayed as histogram. Results were confirmed with optical microscopy observation and, sometimes, also with cell counts.

3.6 Fluorescence and immunofluorescence assays on fixed cells performed by Fluorescent Microscope

HeLa and LC3B-HeLa cells were prepared to perform preliminary immunofluorescence assays by using a computer-assisted image analysis system which includes an Axiophot Microscope (Zeiss) equipped with a colour video camera (AxioCam MRC, Zeiss) and a software package AxioVision (Zeiss). These assays were carried on following, and partially adapting, a protocol commonly applied in our laboratory. More specifically, fluorescence assays were performed on fixed LC3B-HeLa cells while untransfected HeLa cells were used after fixation to perform immunofluorescent assays. In order to allow the adhesion of the cells on the slides, both cell lines were cultured and treated on 6-well plates in which each well was previously filled with a cover-slide before the seeding.

Technically, LC3B-HeLa cells were seeded at concentration of $12x10^4$ cell/well, in a volume of 2 ml per well, while HeLa were seeded at $20x10^4$ cell/well, in a volume of 2 ml per well, and both cell lines were kept in culture for 15-20 hours before to administrate the treatments, consisting in a control sample cultured in complete medium and samples cultured in starvation conditions for different time durations (1, 2, 4, 6 and 8 hours). Then, cover-slides were removed and the cells fixed by keeping the cover-slides on a drop of solution consisting in 4% paraformaldehyde (PFA) in 1X PBS for 15 minutes. After fixation, the two cell types were prepared following different protocols.

In LC3B-HeLa cells nuclei were stained, after several washing cycle, by placing the cover-slides on a drop of solution of 2 μg/ml of Hoechst 33258 (#H3569, Molecular Probes) for 5 minutes. Since the LC3B proteins were constitutively stained by the transfection with the Green Fluorescent Protein (GFP). Cover-slides were then put on microscope slides and, after the adhesion helped by propylgallate, they were ready for the observation at the microscope.

HeLa cells, were permeabilized by keeping the cover-slides on a drop of a solution of 0,1% Triton X-100 for 5 minutes and then the protein linkage-sites were saturated by using a blocking solution containing 1% BSA for 30 minutes with the drop methods previously described. After that, cells were treated with a series of solutions for staining of LC3B protein and nuclei. The Rabbit anti-LC3B polyclonal antibody (#L7543, Sigma-Aldrich) was diluted in blocking buffer at the final concentration of 10 µg/ml and then the cover-slides were put on a drop of primary antibody solution for 1 hour. Further, after several washings, cover-slides were kept on a drop of a solution prepared by diluting a Goat anti-Rabbit IgG (H+L) Secondary Antibody Alexa Fluor® 488 conjugate (Invitrogen # A11008) in blocking buffer at the final concentration of 2,5 µg/ml and then washed repeatedly. As a final step, nuclei were stained by a drop of a solution 2 µg/ml of Hoechst 33258 (#H3569, Molecular Probes) for 5 minutes; after adding propylgallate, the microscope glasses were assembled and then were ready for microscopy investigations.

3.7 Fluorescence assays on living cells performed by automated confocal microscope

Fluorescent assays on HeLa cells were performed in 96-well plates and six extracts in total were investigated for evaluating their effects on such cell line. HeLa cells were seeded in concentration $2{,}5x10^4$ cells/well, in a volume of 100µl per well, and then kept for 15-20 hours in culturing conditions before to replace exhaust media with the four treatment dilutions as reported in paragraph 3.4. Treatments were added on the plate by using a particular layout (showed in Figure 10), in order to follow the reading pathway of the microscope (reported with a green line) and trying to limit eventual artefacts caused by reading wells with the same treatments at different timing: in this manner possible artifact measurements should be easily detected by observing results related to the single pairs of rows before to consider all the rows with the same treatments together (rows B, D, and F for treatments in complete medium and rows C, E, and G for treatments in serum-deprived medium). Fresh staining solutions were prepared immediately before the administration by diluting dyes in the respective culturing media and using double concentrations with respect to the use-concentrations: 200 nM Lysotracker green (#L-7526, TermoFisher Scientific) has been added in order to stain lysosomes and 300 nM Hoechst 33342 (#B2261, Sigma-Aldrich) for the staining of nuclei in living conditions. Then, the staining solutions were added directly on the cells during the last 30 minutes of treatment, thus diluting each dying in order to respect the correct dosage (100 nM Lysotracker green and 150 nM Hoechst 33342). This protocol has been established specifically for this assay to reduce the cells stress and limit the aspiration of stressed and not perfectly adherent cells, especially in the wells with the highest extract concentrations. After 30 minutes in incubator (37°C, 5% CO_2), the staining solutions were replaced with the respective fresh media and the cells immediately scanned by automated confocal microscope “Cell Voyager 6000” (Yokogawa), obtaining images in 9 different fields for each well.
Cell Voyager 6000 microscope, described more in detail in paragraph 1.3.1, is supported by a measurement software which allows to plan the experiment setup before to start the analysis. Among the other, a very important tool allows to choose the number and the position of the acquisition fields in each well in order to assess the best assays conditions. Several preliminary investigations were performed to determine the best positions for the acquisition fields by checking the cell density, mostly depending on the area in which the cycle of addition/aspiration of solutions and staining solution are performed, named *working area* in this work.
Figure 11 shows the well layout used to perform the analysis of the extracts effects on HeLa cells in 96-well plates: nine squared fields were chosen in order to cover most of the area of the well and to avoid to analyze areas near to the edges of the well. Such places are sometimes interested by artefacts and present a lower number of cells because of the high rate of stress caused by the addition/aspiration of the solutions performed on the wall of the wells.

Fluorescence assays on SH-SY5Y cells were performed in 384-well plates and several extracts, ranging from 10 to 13 depending on the experiment, were analyzed in each plate. SH-SY5Y cells were seeded in concentration $8x10^3$ cells/well, in a volume of 40 µl per well and left in incubation for 15-20 hours before to remove exhausted media and add treatments as explained above (paragraph 3.4).

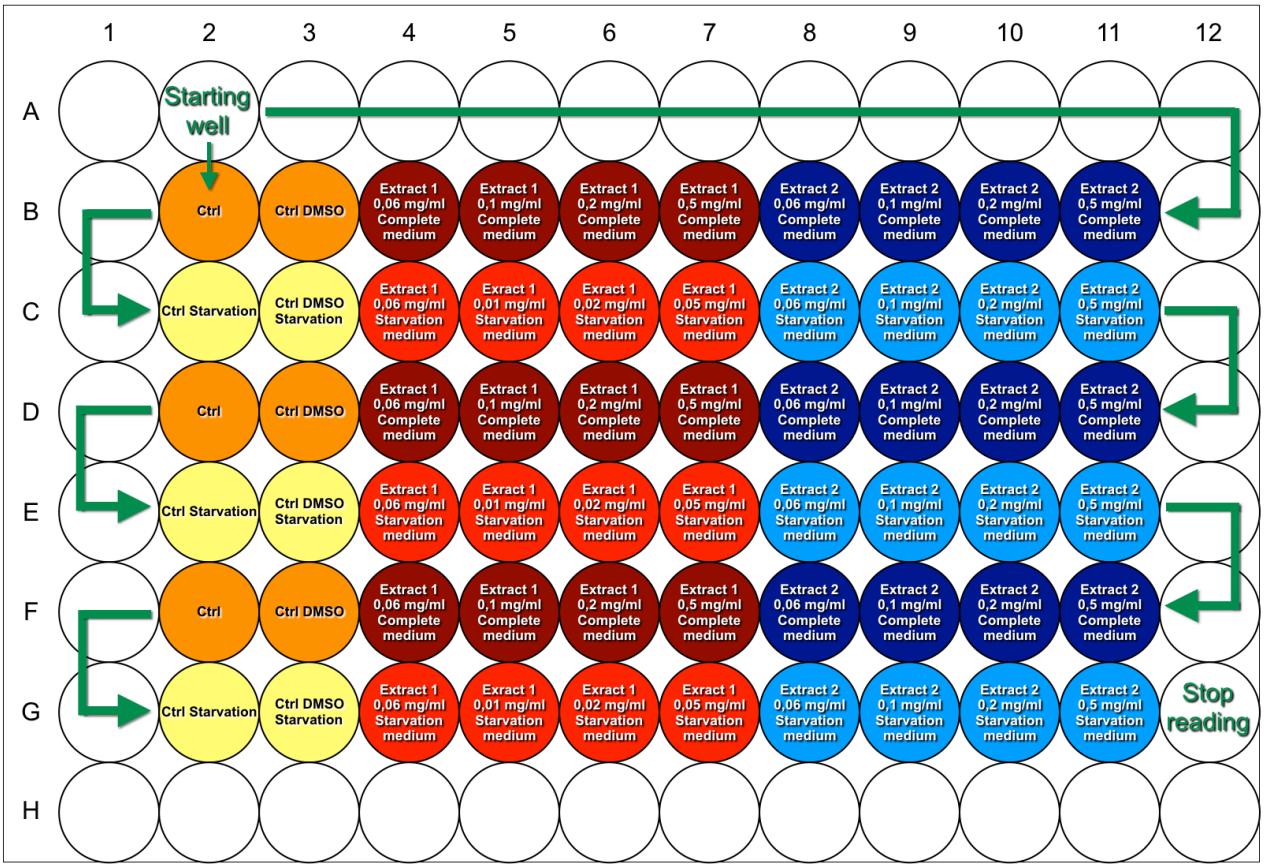


Figure 2. 96-well plate organization for investigation of extracts effects on HeLa cells. The edges of the plate have been left free because of a technical requirement of the microscope, necessary to avoid artefacts caused by the edge effects when the screening is carried out on 96-well plate. Each plate was used to analyze the effects of 2 extracts. Orange wells contain controls, in particular completely untreated (Column 2) and 0,25% DMSO treated (column 3) cells cultured in complete medium while yellow wells contain the same controls for cells cultured in serum-deprived medium. Following, bordeaux and red wells contain cells treated with the four serial dilutions of the extracts 1, from the lower to the higher concentration (columns 4-7), and cultured in normal (rows B, D and F) and starvation conditions (rows C, E and G) respectively. Lastly, blue and light blue wells contain cells treated with the four serial dilutions of the extracts 2, from the lower to the higher concentration (columns 8-11), and cultured in normal (rows B, D and F) and starvation conditions (rows C, E and G) respectively.

The specific layout used to perform the analysis on 384-well plate is showed in Figure 12. As for the 96-well plates, such organization has been chosen on the basis of the reading pathway of the microscope (reported with a green line) in order to limit eventual artefacts, as the shifting of the effects observed in the different rows due to a too long duration of the microscope reading timings: for instance, artifacts can occur because the control samples located in the upper side of the plate (rows A and B) are scanned by the microscope at early time if compared with the samples placed on the rows in the lower side (rows M, N, O or P), which suffer the effects of the treatments for longer time before being read.

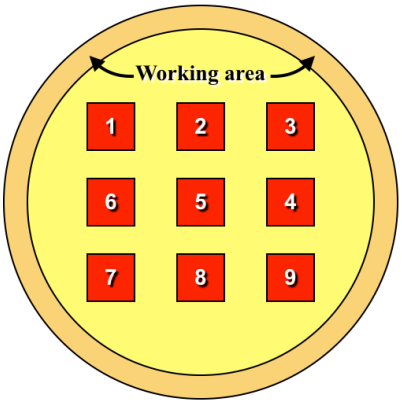


Figure 11. Layout of the disposition of the image acquisition field for each well in 96-well plates.

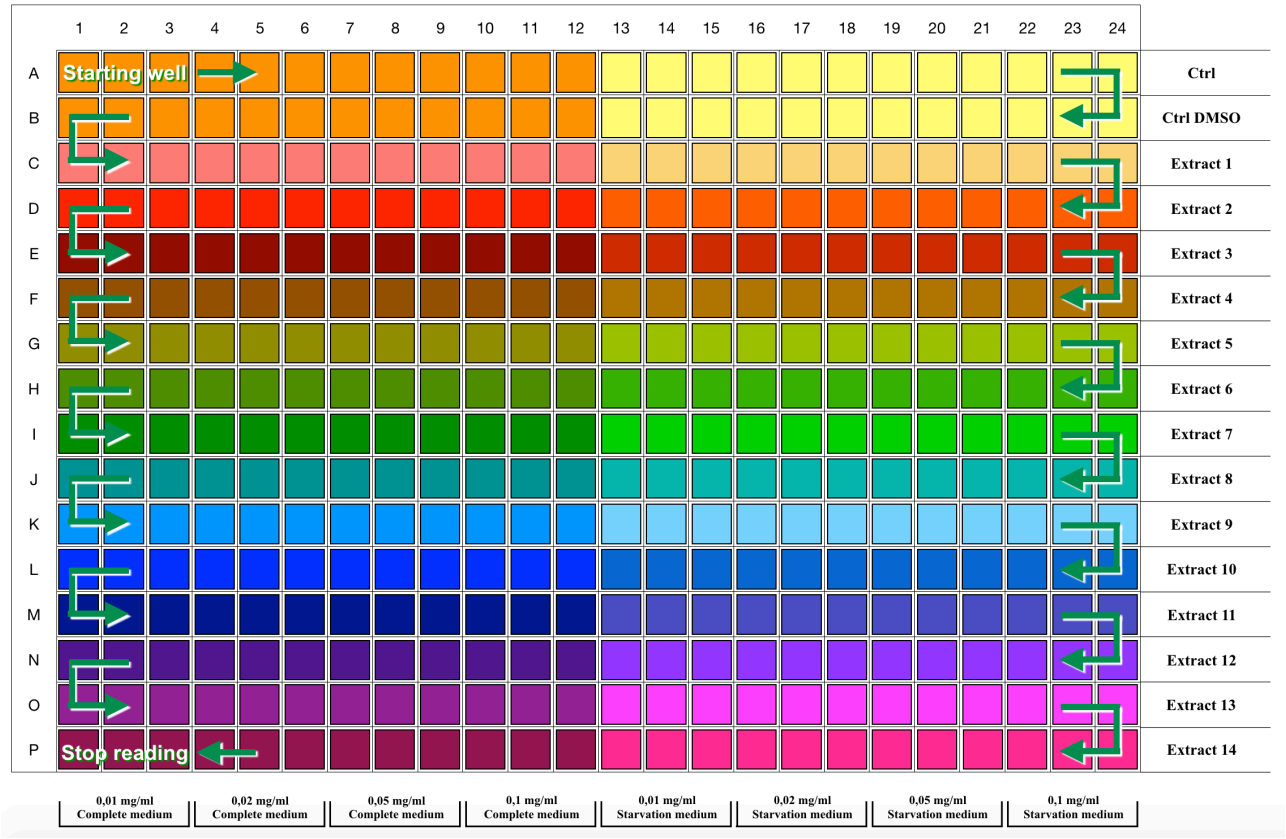


Figure 12. 384-well plate organization for investigation of extracts effect on SH-SY5Y cells. Each plate was used to analyze the effects of several extracts (10, 12 or 13 depending on the experiment). Orange wells contain controls, in particular completely untreated (Row 1) and 0,25% DMSO treated (Row 2) cells cultured in complete medium while yellow wells contain the same controls for cells cultured in serum-deprived medium. Following, the four dilutions of each treatment have been administrated in one row, occupying three wells for each dilution, in complete medium in the left side of the plate (Columns 1-12) and in serum-deprived medium in the right side of the plate (Columns 13-24). Empty wells were filled with complete medium in order to avoid artefacts caused by the edge effects.

Several investigations have been carried out in order to optimize the microscope setup to perform the analysis in the best conditions: the first attention was focused on the establishment of the number of the extracts tested in each plate, correspondent to the number of the used rows; other important assessment assays have allowed to establish the best conditions in preparing the staining solutions in order to reduce the exposure time for each channel and thus the duration of the imaging of the whole plate.

As in case of the staining of HeLa cells, also the staining solution used for the SH-SY5Y living staining is prepared fresh and double concentrated by diluting dyes directly in the respective culturing media. However, differences concerning staining substances and their concentration need to be mentioned: 200 nM Lysotracker Red (#L-7526, TermoFisher Scientific) has been used to stain lysosomes while the living staining of the nuclei were performed by adding 200 nM Hoechst 33342 (#B2261, Sigma-Aldrich). The staining solutions were added directly on the cells and thus diluted in order to reach the correct use concentration during the last 30 minutes of treatment, (100 nM Lysotracker green and 150 nM Hoechst 33342), thus limiting the cells detachment during the aspiration step (as already explained). Thirty minutes later the solutions were aspirated from the wells, fresh media were added and the plates immediately scanned by the automated confocal microscope “Cell Voyager 6000” (Yokogawa), obtaining images in 9

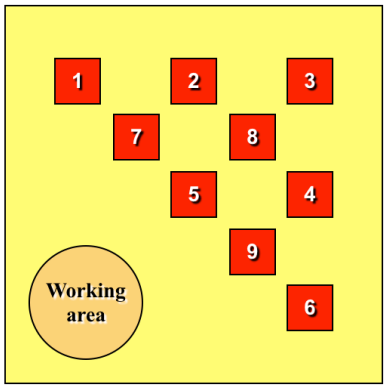


Figure 13. Layout of the disposition of the image acquisition field for each well in 384-well plates.

fields for each well with a particular layout reported in figure 13. Wells in 384-well plates are squared and smaller than the correspondent 96-well plate and all the aspiration/addition operations were performed in the corner at the left bottom for each well. Considering this technical ploy, acquisition field were chosen in order to avoid the left and lower area of the well, as well as areas near the edges.

After imaging, both cell lines were immediately fixed by adding a solution 8% PFA (#10010023, Gibco) in 1X PBS directly to the media contained in each well, thus reaching the final use concentration (4% PFA). PFA solution was kept on the cells for 15 minutes in the dark at Room Temperature (RT) and then quickly replaced with 1X PBS. This protocol has been adopted after making several tests in order to confirm that any artefact occurred by diluting PFA with media. In this manner it has been possible to optimize the fixation of the cells and, further, to improve the antibodies signal yield in the next steps. Fixed cells were then stored at 4°C (in the fridge) for few days (not more than 4-5 days), until testing by immunofluorescence assays.

3.8 Immunofluorescence assays on fixed cells performed by automated confocal microscope

Despite immunostaining protocols are similar, some important issues are different depending on the cell line, mainly concerning the substances employed in each step that should be always prepared fresh immediately before use.
Fixed HeLa cells were permeabilized by replacing 1X PBS with a solution containing 0,1% Triton X-100 in PBS 1X, kept on the cells for 15 minutes in the dark at RT. After removal of the permeabilization buffer, blocking buffer solution consisting in 1% BSA in 1X PBS was added to the cells and kept for 30 minutes in the dark at RT. During this incubation step, fresh primary antibody solution has been prepared by diluting anti-LC3B polyclonal antibody developed in rabbit (#L7543, Sigma-Aldrich) in blocking buffer at the final concentration of 10 µg/ml. Cells were then kept in incubation with primary antibody solution at 4°C over-night in a dark room and the following day a fresh solution consisting in 2,5 µg/ml Goat anti-Rabbit IgG (H+L) Secondary Antibody Alexa Fluor® 488 conjugated (Invitrogen # A11008) diluted in blocking buffer has been added to the cells and kept in incubation at room temperature for 1 hour. Lastly, secondary antibody solution was replaced with PBS 1X solution and plates were immediately scanned by automated confocal microscope “Cell Voyager 6000” (Yokogawa), obtaining images from 9 different acquisition fields for each well following the same scheme previously described for the fluorescence assays (Figure 11).
SH-SY5Y cells were permeabilized using a solution containing 0,1% Digitonin (#D141, Sigma-Aldrich) in PBS 1X for 5 minutes (absolutely avoiding to exceed this time duration) in the dark at RT. After permeabilization the blocking buffer solution consisting in 5% BSA in PBS 1X was added to the cells for 30 minutes in the dark at RT and then replaced with a solution consisting in 2,5 µg/ml of an anti-LC3B polyclonal antibody developed in rabbit (#L7543, Sigma-Aldrich) diluted in blocking buffer, that was left on the cells for 45 minutes in the dark at RT. Subsequently, primary antibody solution was removed and cells were left in incubation with a solution containing 2 µg/ml Goat anti-Rabbit IgG (H+L) Secondary Antibody Alexa Fluor® 488 conjugated (Invitrogen # A11008) diluted in Blocking buffer for 50 minutes at RT. At the end, secondary antibody solution was replaced with PBS 1X and plates were immediately scanned by the automated confocal microscope “Cell Voyager 6000” (Yokogawa), exploiting the same layout established for the fluorescence assays (previously described and reported in Figure 13) to obtain images from 9 different fields for each well.

3.9 Automated confocal microscopy analysis and image acquisition strategy

All the conditions described below were optimized and standardized by several repetitions of test modality for each assays.

Living HeLa cells were assayed by performing the treatments into BD #353219 96-well plate while SH-SY5Y cells were treated into PerkinElmer #6007430 384-well plate. Signals from chromophores used for the features investigations were stimulated by lasers with different wavelengths: the laser at 405 nm has been used on both cell lines for the imaging of living and fixed cells for the stimulation of Hoechst 33342 in order to detect chromatin signals for nuclei identification; the laser at 488 nm has been used to stimulate Lysotracker Green in living HeLa cells to identify lysosomes and the Alexa Fluor® 488-conjugate GAR and GAM Secondary Antibodies used on both fixed HeLa and SH-SY5Y cells to detect LC3B protein; lastly, the laser at 531 nm has been activated on living SH-SY5Y cells in order to stimulate Lysotracker Red for identification of lysosomes. Differently from the other chromophores, the Hoechst 33342 has been used for detection of nuclei in both living and fixed cells because it is able to penetrate in living cells and is not degraded by the used fixation agent (PFA 4%).

Concerning the lasers activation modalities, images from different channels were detected consecutively for experiments performed on HeLa cells in 96-well plates in order to limit possible cross-talk effects due to the overlapping of the Hoechst 33342 emission spectra with the Lysotracker Green excitation spectra. On the contrary, the acquisition of the different channels for experiments performed on SH-SY5Y cells in 384-well plate was simultaneous, thus significantly reducing the long time required to scan the whole plate.

Another important parameter established in advance concerns the 96- and 384-well plates wells layout, that have been previously described in paragraph 3.7 (Figures 11 and 13).

Lastly, for all the experiment the best focal plane for image acquisition were established in test modality by acquiring images every 2 μm in order to centre the focal plane to get the best focus for the maximum intensity final images, mostly depending on the different plate and cells type.

3.10 Image analysis

All the image analyses were carried out by using the software “Cell Profiler” (Version 2.1.1 - Broad Institute, Inc. Copyright © 2009-2014). Two different pipelines have been built for the analysis of the phenotypes produced by the treatments on HeLa and SH-SY5Y cells according to and readapting the general information provided by Carpenter et al. (2006), in order to define several objects and some relative interesting features: nuclei were identified and assigned as belonging to living or dead cells depending on their intensity values; cells were identified starting using nuclei as references objects; lysosomes or LC3B spots were identified and assigned as child object of their own parent cells; mean and median intensity, size and shape of each lysosomes or LC3B spot were measured and then used to interpret the produced phenotypes. All the results are exported in CSV format for further investigations.

3.11 Data interpretation and multivariate analysis

Multivariate analysis has been carried on in order to analyze all together the seven variables contained in the dataset and chosen to evaluate the phenotypes: a) percentage of living cells detected per each sample after administration of each

treatments, measured considering only images coming from living cells; b) percentage of cells in which the acidic or the autophagic compartment results to be activated by the administrated treatment, named “active cells”, and measured as cells in which at least one lysosome or LC3B spot has been detected; c) the number of lysosomes or LC3B spots per cells measured for each samples; d) the size of the lysosomes or LC3B spots measured for each samples by considering the number of pixels covering the objects and named "area" of the objects.
The multivariate analysis has been carried out by applying in series the techniques reported below.

3.11.1 Data Normalization

Data coming from image analysis of the phenotype produced by treatments on SH-SY5Y cells were firstly selected and normalized by applying Z-score or Standard Score to the median values of the measured features:

$$Z\ Score\ values = \frac{x_i - X}{\sigma}$$

where x_i can represent every single median value for each feature measurements outcoming from the image analysis while X and σ are respectively the mean and the standard deviation values of the whole dataset, representing the population. The reason why median values have been used for data normalization is that these numbers represent the population limiting the effects of possible outlier values, which can be due to possible artifacts or other imperfections occurred during the staining of the samples.

3.11.2 Dataset dimensions’ reduction: Principal Component Analysis (PCA)

Principal Component Analysis (PCA) has been applied to the measurements resulting from the analysis of the phenotypes produced by the treatments administration on the SH-SY5Y cells to reduce the dimensions of the dataset. PCA is a statistical procedure which allows to orthogonally convert a dataset of possibly correlated variables into a dataset of linearly uncorrelated variables called principal components (PCs). The analysis produces a number of PCs which is always less than or equal to the number of original variables. After this transformation the first principal component contains the largest possible variance, meaning that it expresses the most possible variability contained into the dataset, and each following component in turn contains the largest possible amount of the remaining variance with the only obligation that it is orthogonal to the previous components. Most importantly, PCA is directly correlated to the relative scaling of the original variable and hence reproduces the variance of the original dataset.
In the present work the PCA is performed by decomposing a data matrix after mean centring for each attribute by using Z-scores (Abdi & Williams 2010) and it outputs either the transformed values contained into the dataset as components scores, sometimes called factor scores (Shaw 2003).

3.11.3 Computation of the distances between samples

Distance among phenotype produced by the treatments on SH-SY5Y has been computed by considering Euclidean distance as distance metrics. Euclidean distance is the ordinary straight-line distance between two points in a Euclidean space defined by n-dimensions, and is calculated through the formula:

$$d(p,q) = \sqrt{(p_1 - q_1)^2 + (p_2 - q_2)^2 + \cdots + (p_i - q_i)^2 + \cdots + (p_n - q_n)^2}$$

where p and q are two points in the space ($p = (p_1, p_2)$, $q = (q_1, q_2)$), and n represents the dimensions of the space.
The Euclidean distances among the different samples have been calculated considering the factor scores associated to each sample as the coordinates p and q.

3.11.4 Hierarchical Cluster Analysis (HCA)

Hierarchical Cluster Analysis (HCA) has been used to analyze and join together all the treatments producing a similar phenotype in different groups named as clusters. Final identified clusters have been represented in a dendogram in which all leaves (treatments) were linked considering the "Ward Linkage" method, which is a general agglomerative hierarchical clustering procedure that groups all the analyzed samples starting form *n* clusters each containing just 1 sample, where *n* is equal to the total leaves analyzed, in different clusters based on the Euclidean distances among the data identifying the samples as well as including the minimum variance in each produced cluster (Ward, 1963). The Ward method, also known as the Ward's minimum variance criterion method, is the most appropriate for the analysis of quantitative variables and not recommended for binary values. While the Euclidean distances have been calculated as explained in the previous paragraph, the minimum variance criterion has been applied by considering the Error Sum of Squares (ESS), the Total Sum of Squares (TSS) and the R-Squares (r^2). If X_{ijk} is a value for variable k in observation j belonging to cluster i, the parameters ESS, TSS and r^2 are defined by the formulas:

- Error Sum of Squares (ESS):

$$ESS = \sum_i \sum_j \sum_k |X_{ijk} - \bar{x}_{i\cdot k}|^2$$

according to which all the individual observation for each variable was compared with the cluster means for such variable, and all the variables and units within each cluster were summed over. Obviously, small ESS values mean that data are close enough to the cluster means and is thus possible to include the units in the same cluster.

- Total Sum of Squares (ESS):

$$TSS = \sum_i \sum_j \sum_k |X_{ijk} - \bar{x}_{\cdot\cdot k}|^2$$

that describe the total sum of squares, defined in the classical way, and compare the individual observation for each variable against the grand mean for that variable.

- R-Square (r^2):

$$r^2 = \frac{TSS - ESS}{TSS}$$

which is interpreted as the proportion of variance explained by a particular clustering of the observation.

According to the algorithm that defines the minimum variance criterion, in the first step n – 1 clusters are created, of which just one cluster of size 2 (containing 2 samples) and all the others of size 1, and the ESS and r^2 have been subsequently calculated: the pair of samples units having the smallest ESS, or equivalently the greater r^2 value, are included in the same cluster. After, in the second step a set of n – 2 clusters is created, with the sample units contained in 2 clusters of size 2 or in a single cluster of size 3 including the two items clustered in step 1, and the values of the ESS are then minimized (or the r^2 value is maximized). The analysis is then completed continuing to apply these simply roles. After the whole dendogram has been built, it is then cut at a specific level of its total height (expressed in %), depending on the number of clusters needed to include the different sets of control samples (Control and Control DMSO; Control Starvation and Control DMSO Starvation) in different groups. Further, the spatial distribution with respect to the 2 considered principal components has been showed in a two-dimensional scatter plot reporting also the class density areas for each cluster, in order to help the interpretation of the results, as well as the analysis of the samples included in each cluster and how the clusters are distributed one relatively to the others and occupy the analyzed area.

All the operations described in paragraphs 3.11.2, 3.11.3 and 3.11.4 have been realized using the software "Orange - Data Mining Fruitful & Fun" (Version 3.3.8 - University of Ljubljana - Copyright © 2013-2017), a workflow system consisting in data visualization and analysis, components for machine learning, add-ons for bioinformatics and text mining, features for data analytics, used in the present work for data mining through visual programming (Demšar et al. 2013; Mattiazzi Usaj et al. 2016).

3.12 Statistical analysis

Results about the actively growing cells rate coming from MTT assays were expressed as mean values and relative error while results about the cytotoxic effects, the number of spots per cell and size of spots for both lysosomes and LC3B proteins coming from image analysis were expressed as median values and relative error. For all data, anyway, the statistical analyses have been carried out by applying one-way ANOVA test through Prism7 software (Version 7 - GraphPad Software, Inc. - Copyright © 1994-2017), with the confidence interval set at 95% in order to evaluate the p-values relative to the entire data sets and to data describing each samples, alone and with respect to the data describing the control sample.

4. Results

4.1 Preliminary tests

Preliminary tests were carried out in order to choose the cell models and to assess the best conditions for investigations of the affection of the autophagy pathway by the 47 crude extracts.

4.1.1 Choice of the best HeLa cell models

During the experiment design, three different cell lines have been tested as model for investigation of the effects of the crude extracts on the autophagy pathway: HeLa cells, chosen for their relatively big dimensions that make them an excellent cellular model for investigations based on fluorescence and immunofluorescence assays, beside their wide usage as model for investigations on autophagy pathway (Bjørkøy et al. 2005; Conte et al. 2017; Dowaidar et al. 2017; Jiang & Mizushima 2015; Klionsky et al. 2007; Mitchener et al. 1976; Parganlija et al. 2014; Tanida et al. 2005; Yu et al. 2010); LC3-GFP HeLa cells, which add the possibility to easier and faster investigate LC3 protein in addition to the advantage brought by the non-transfected HeLa cells; SH-SY5Y cells, which are largely used as model for investigations concerning autophagy as well as Alzheimer's and Parkinson's diseases, mostly for the impaired dopamine homeostasis in their metabolism (Alberio et al. 2012; Arsikin et al. 2012; Jang et al. 2014; Kim et al. 2014; Li et al. 2017; Long et al. 2014; Parganlija et al. 2014; Park et al. 2013; Plowey et al. 2009; Song et al. 2015; Wang et al. 2010).

The best HeLa cell line was established between the two available by fluorescence and immunofluorescence assays carried out on both transfected and wild-type HeLa cell lines kept in normal and starvation conditions for several time durations (1, 2, 4, 6 and 8 hours). Cells were then fixed and stained as explained in paragraph 3.6 in order to evaluate the autophagy induction level and the goodness of the signal detection by using a fluorescence microscope Axiophot 2 equipped with a colour camera (AxioCam MRC) coupled with the software AxioVision (Zeiss, Germany). The goal of this preliminary assay was the confirmation of previously published data regarding the highest rate of autophagy, which has been observed approximately after keeping cells in starvation culturing conditions for few hours (most of the time 2-6 hours, depending on the cell model) (Klionsky et al. 2016; Yu et al. 2010).Obtained results are shown below in figures 14 and 15 (following page): figures 14A, B, C, D, E and F report images concerning phenotypes of untreated (Control) and starved LC3-GFP HeLa cells, while figures 15A, B, C, D, E and F images shown phenotypes concerning untreated (Control) and starved wild-type HeLa cells. From the images, it is evident that the evaluation of the variation in the number of LC3 spots per cell after 1, 2, 4 and 6 hours of starvation in LC3-GFP HeLa cells resulted very difficult with respect to the HeLa cells, that showed a high and increasing number of spots per cell after 1 and 2 hours of starvation and a subsequent constant decrement after longer incubation periods, as reported in literature (Klionsky et al. 2016; Mizushima & Yoshimori 2007; Mizushima et al. 2010). Moreover, the observation of all the images obtained from LC3-GFP HeLa cells (results not shown) have clarified the difficulties to logically interpret the distribution of the LC3 spots from the images of cells kept in starvation conditions for different periods, leading to suppose that the results can be affected by possible artefacts. Differently, the observation of all the images obtained from wild-type HeLa cells (results not shown) confirmed wild-type HeLa as the best model between the two screened cell lines for further investigations of effects of extracts on the autophagy pathway.

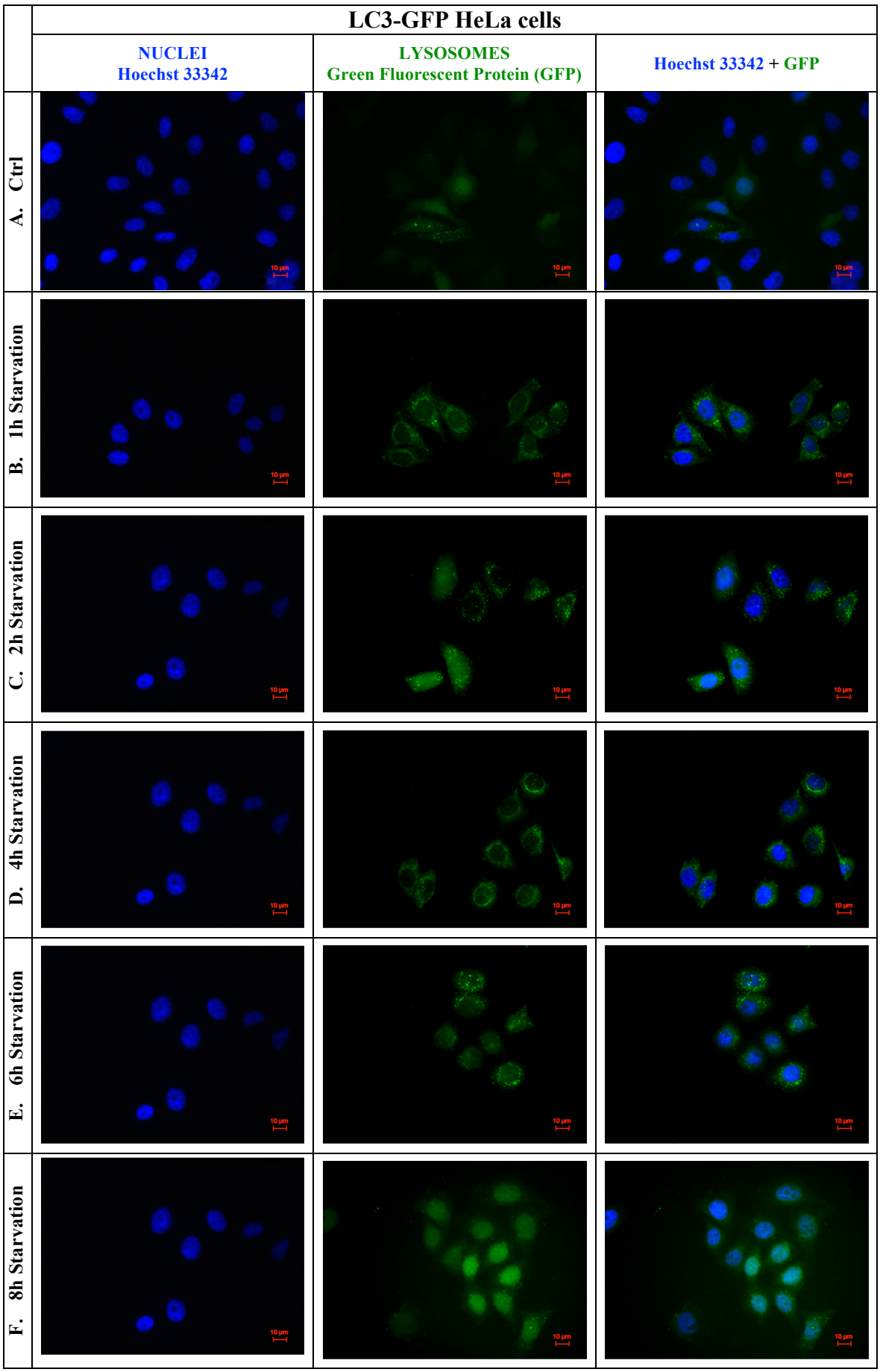


Figure 14. Images regarding LC3 in LC3-GFP HeLa cells untreated (Ctrl) and after 1, 2, 4, 6 and 8 (A-F) hours of starvation.

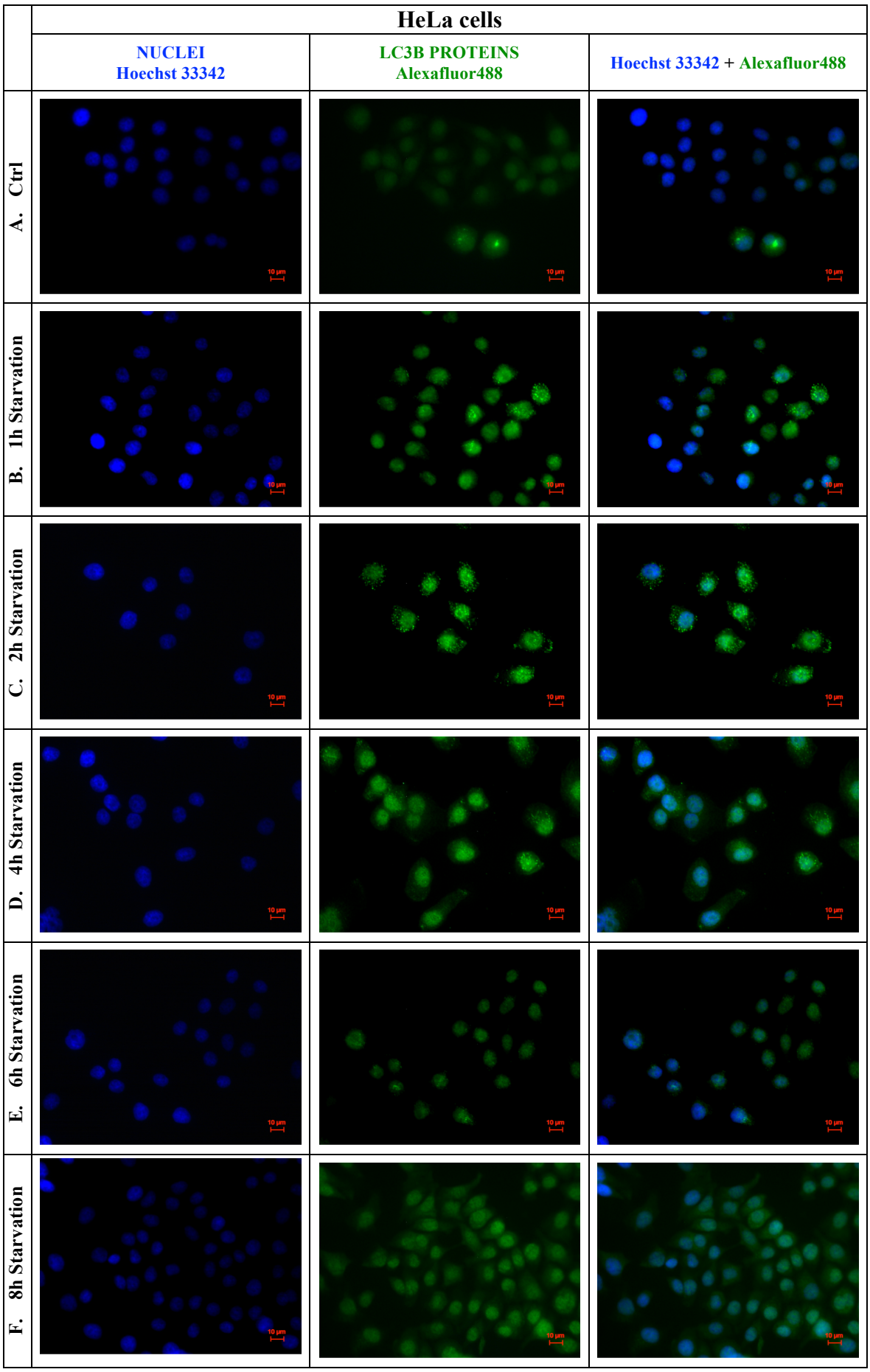
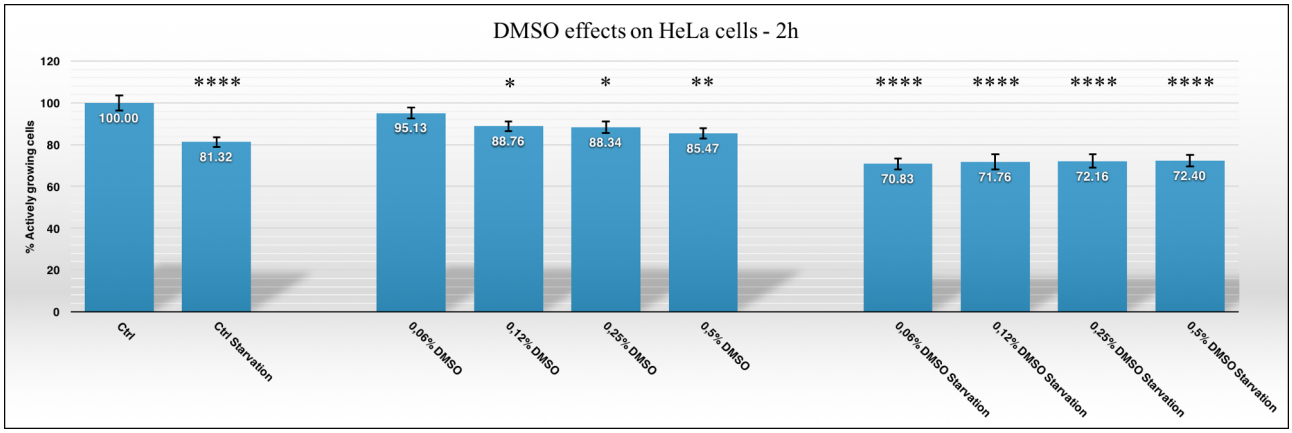


Figure 15. Images regarding LC3 in wild-type HeLa cells untreated (Ctrl) and after 1, 2, 4, 6 and 8 (A-F) hours of starvation.

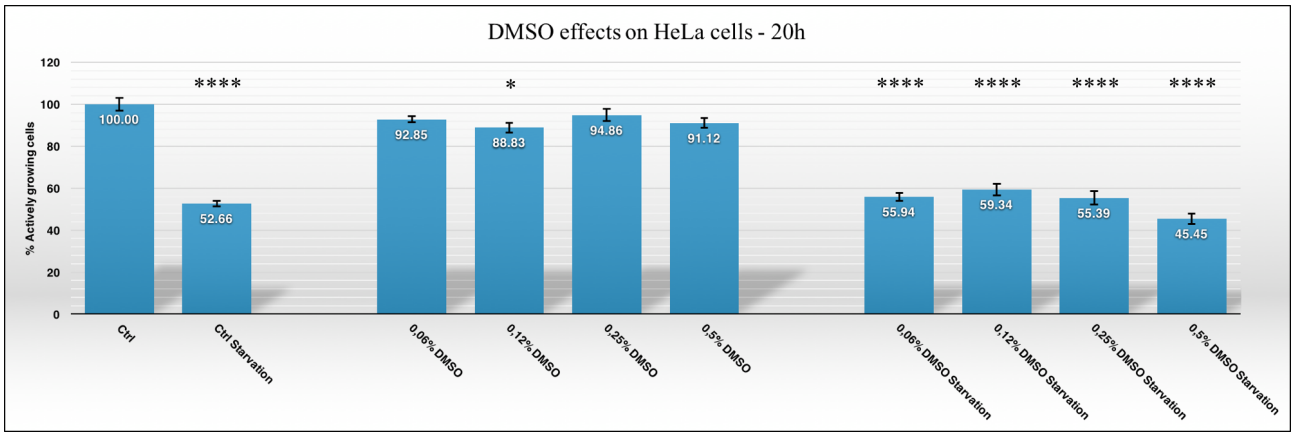
4.1.2 Evaluation of the effects of DMSO treatments on both HeLa and SH-SY5Y cell lines

After discarding the LC3-GFP HeLa cell line, the effect of DMSO on cell viability has been evaluated. This very important step has been performed in order to establish the effects of the highest percentage of DMSO to use to dilute crude extracts without affecting either wild-type Hela or SH-SY5Y cell lines, and to get a better interpretation of the final results. For this purpose, MTT assays were carried out as explained in paragraph 3.5 in order to evaluate cell viability by observing the percentage of actively growing cells after 2 and 20 hours of treatment with increasing percentages of DMSO (0,6%, 0,12%, 0,25% and 0,5% v/v) diluted in normal and autophagy-inducing culturing media on both cell lines. Results concerning DMSO effects on HeLa cells after 2 and 20 hours are reported in figures 16 and 17, respectively, while figures 18 and 19 report effects resulting from treatments of SH-SY5Y cells: graphs report bar plots showing the percentages of the actively growing cells and the standard error for each treatment and are supported by tables reporting the numerical values of the data displayed in the graphs.



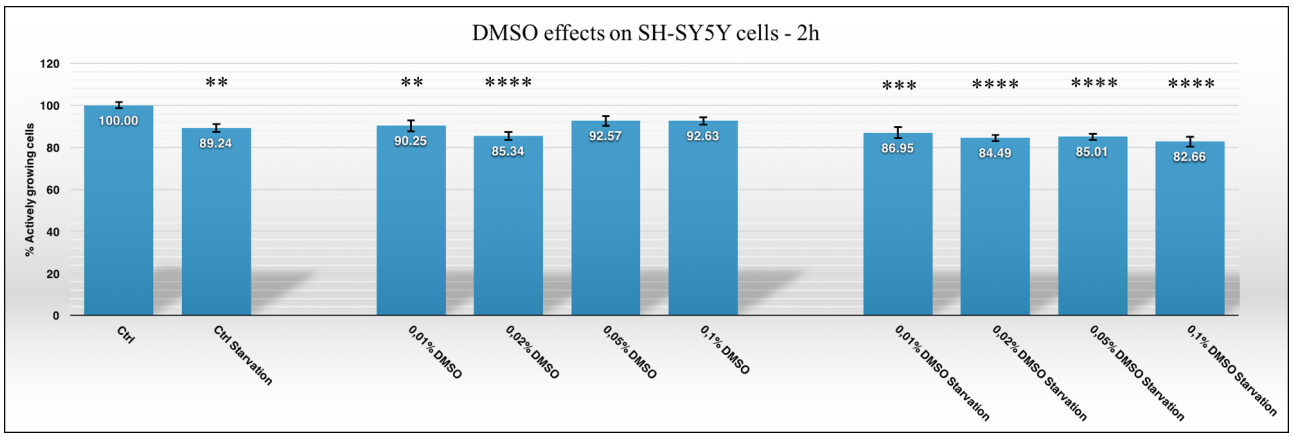
	Ctrl	Ctrl S		0,06%	0,12%	0,25%	0,5%		0,06% S	0,12% S	0,25% S	0,5% S
%	100	81.318		95.134	88.758	88.339	85.471		70.829	71.762	72.164	72.396
% Std Err	3.686	2.345		2.629	2.194	2.662	2.475		2.606	3.525	3.105	2.729

Figure 16. Effects of serial dilutions of DMSO on HeLa cells after 2 hours of incubation. The table at the bottom of the bar plot reports the results in a more detailed form.



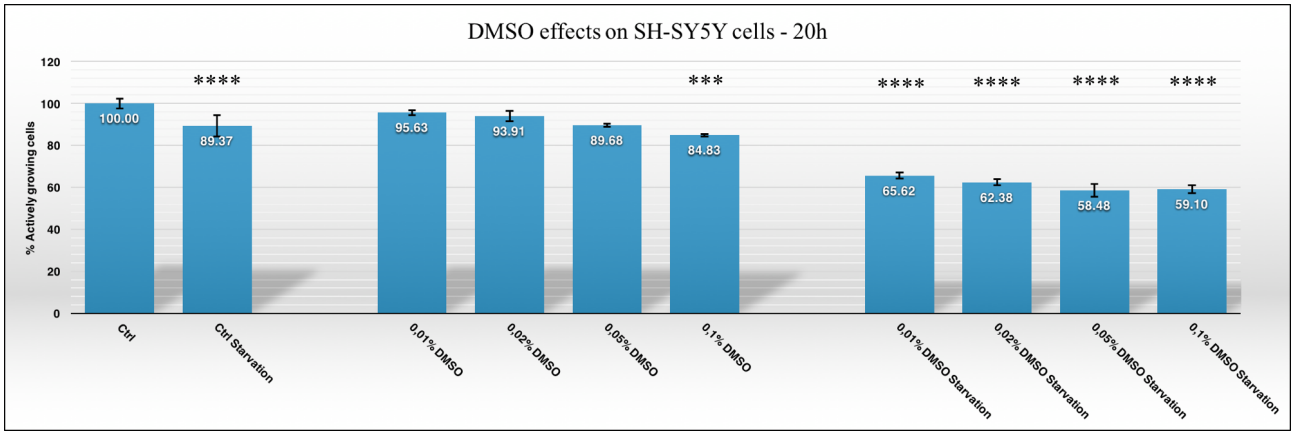
	Ctrl	Ctrl S		0,06%	0,12%	0,25%	0,5%		0,06% S	0,12% S	0,25% S	0,5% S
%	100	52.66		92.849	88.831	94.858	91.124		55.938	59.337	55.389	45.452
% Std Err	2.924	1.415		1.535	2.36	2.869	2.217		1.824	2.705	3.121	2.514

Figure 17. Effects of serial dilutions of DMSO on HeLa cells after 20 hours of incubation. The table at the bottom of the bar plot reports the results in a more detailed form.



	Ctrl	Ctrl S		0,06%	0,12%	0,25%	0,5%		0,06% S	0,12% S	0,25% S	0,5% S
%	100	89.241		90.251	85.336	92.572	92.627		86.947	84.489	85.008	82.660
% Std Err	1.501	1.922		2.628	1.932	2.359	1.728		2.538	1.475	1.515	2.39

Figure 18. Effects of serial dilutions of DMSO on SH-SY5Y cells after 2 hours of incubation. The table at the bottom of the bar plot reports the results in a more detailed form.



	Ctrl	Ctrl S		0,06%	0,12%	0,25%	0,5%		0,06% S	0,12% S	0,25% S	0,5% S
%	100	89.373		95.625	93.912	89.683	84.834		65.622	62.377	58.476	59.096
% Std Err	2.385	5.019		1.168	2.539	0.683	0.538		1.532	1.431	3.094	1.949

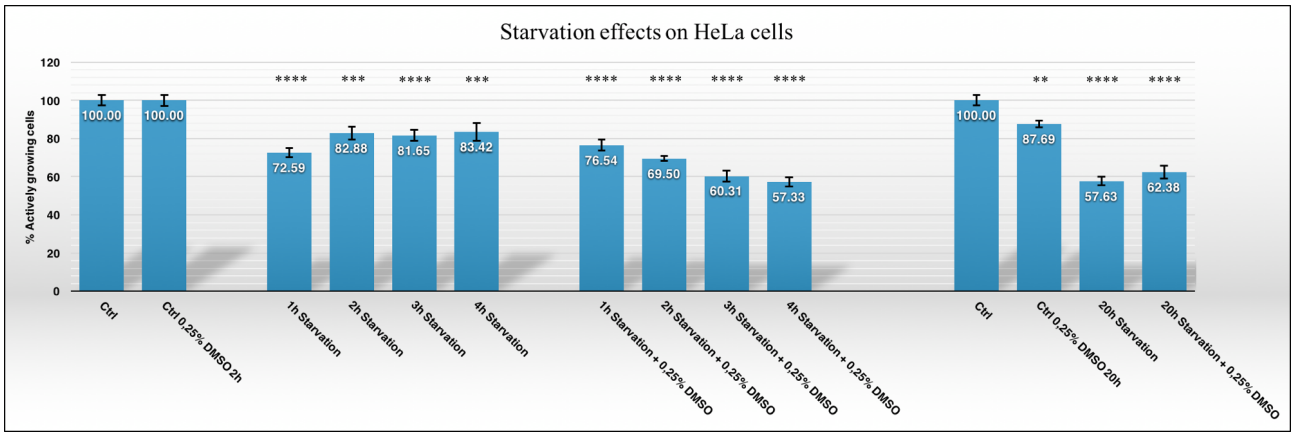
Figure 19. Effects of serial dilutions of DMSO on SH-SY5Y cells after 2 hours of incubation. The table at the bottom of the bar plot reports the results in a more detailed form.

Evaluating the results, it is evident that the percentages of actively growing cells, used as parameter of cell viability, are mostly affected by DMSO percentages diluted in serum-deprived medium and administrated for 20 hours, showing a decrement of approximately 40-50% in both cell lines (figures 17 and 19). However, the different percentages of DMSO administrated for both 2 and 20 hours, seem do not produce different effects on SH-SY5Y cells while show a very slight decrement of actively growing HeLa cells for increasing DMSO percentages, especially after 20 hours since treatment administration. Interestingly, HeLa cells cultured for 20 hours in starvation conditions have been highly influenced also independently from the DMSO (look the control starvation bar in figure 17). Due to this observation, several experiments have been performed to verify the effects caused by prolonged starvation periods, necessary to induce autophagy, on cells viability for both cell types.

For all the analysis, the statistical significance of the analysis has been reported on each bar using asterisks, the number of which reflects the level of statistical significance for each sample.

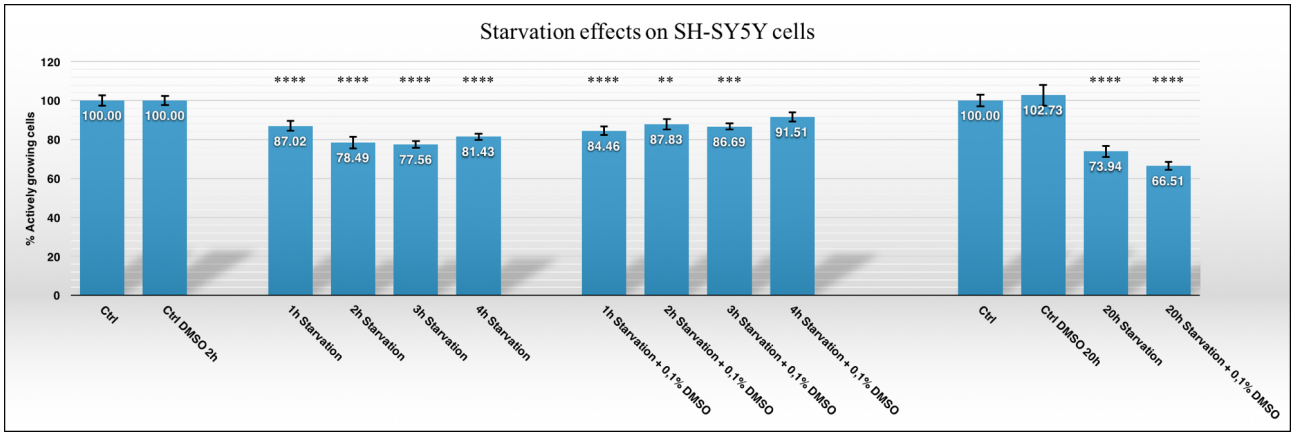
4.1.3 Evaluation of the effects of starvation on both HeLa and SH-SY5Y cells lines

MTT assays have been used as reported in paragraph 3.5 also to investigate the effect caused by prolonged starvation periods on HeLa and SH-SY5Y cells. These experiments have been carried out in presence and absence of the maximus percentages of DMSO chosen to dilute the extracts (0,25% and 0,1% v/v for administration on HeLa and SH-SY5Y cells, respectively), in order to distinguish and evaluate the effects on cell viability caused by starvation or produced by the presence of DMSO. Therefore, both cell lines were cultured in starvation-inducing medium with and without adding DMSO (0,25% and 0,1% v/v) for periods of increasing duration, like 1, 2, 3, 4 and 20 hours, in order to evaluate the effects produced on cell viability by measuring the percentage of actively growing cells for each treatment.
Results are reported in figures 20 (HeLa cells) and 21 (SH-SY5Y cells) as bar plots and standard error, supported by tables below the graph, as explained in the previous paragraph. Looking at the bar plots it is clear that the effect of



	Ctrl	Ctrl 2h DMSO		1h S	2h S	3h S	4h S		1h S DMSO	2h S DMSO	3h S DMSO	4h S DMSO		Ctrl	Ctrl 20h DMSO	20h S	20h S DMSO
%	100	100		72.585	82.884	81.65	83.42		76.535	69.496	60.306	57.334		100	87.688	57.628	62.384
% Std Err	2.695	2.81		2.423	3.297	2.872	4.572		2.864	1.306	2.935	2.334		2.695	1.672	2.254	3.483

Figure 20. Effects produced by 1, 2, 3, 4 and 20 hours of starvation on HeLa cells viability. The table at the bottom of the bar plot reports the results in a more detailed form.



	Ctrl	Ctrl 2h DMSO		1h S	2h S	3h S	4h S		1h S DMSO	2h S DMSO	3h S DMSO	4h S DMSO		Ctrl	Ctrl 20h DMSO	20h S	20h S DMSO
%	100	100		87.016	78.488	77.558	81.434		84.457	87.829	86.686	91.512		100	102.733	73.939	66.512
% Std Err	2.687	2.358		2.564	2.895	1.747	1.593		2.131	2.797	1.646	2.397		2.93	5.196	2.762	1.987

Figure 21. Effects produced by 1, 2, 3, 4 and 20 hours of starvation on SH-SY5Y cells viability. The table at the bottom of the bar plot reports the results in a more detailed form.

starvation on cell viability results to be higher in HeLa cells than in SH-SY5Y cells, presenting a maximum difference greater than 30% after 4 hours in starvation-inducing medium for both cell lines. Moreover, it is also clear that chosen DMSO percentages per se don't produce appreciable effects on both cell lines but affect cell viability of both cell lines if administrated in starvation conditions: viability of HeLa cells results to be affected already after the shorter treatments duration (2, 3 and 4 hours) while after 20 hours of incubation with DMSO in starvation conditions the viability of both cell lines, especially HeLa cells, appears to be affected.

The obtained results suggest that SH-SY5Y is the best cell line to investigate autophagy and most (but not all) of the following experiments concerning the evaluation of the effects produced by treatment with crude extracts will be performed by using such cell line and applying the chosen assay conditions to represent the best compromise to get a complete picture of the effects. As already reported, extracts have been diluted in two different maximum DMSO percentages depending on what cell line will be then used for administration: extracts tested on HeLa cells have been diluted in normal and autophagy-inducing media containing 0,25% v/v DMSO, while for administration on SH-SY5Y cells extracts have been diluted in both media added with 0,1% DMSO v/v; concerning starvation period durations it has been established to investigate the effects of extracts on both cell lines after 1, 2, 3, 4 and 20 hours since treatment administration, in order to investigate the effects produced in shorter and longer time durations.
The statistical significance of the analyses has been reported on each bar using asterisks, the number of which reflect the level of statistical significance for each sample.

4.2 Image analysis approach: features detection and phenotypes interpretation

After preliminary tests, high resolution images of lysosomes and LC3B proteins in SH-SY5Y cells treated for 2 and 20 hours with crude extracts were acquired by an automated confocal microscope "Cell Voyager 6000" (Yokogawa) and then segmented and analyzed by Cell Profiler. Cellular elements, conventionally defined as "objects", were defined by setting several parameters and identified by applying appropriated algorithms to the images, mostly working on the intensity values assigned by the microscope to every pixel: objects like nuclei, lysosomes and LC3 protein spots have been directly identified while cells have been identified indirectly as secondary objects by using previously defined nuclei as reference guiding objects. After the identification some features for each object were measured and quantitative values regarding the most interesting measurements were exported and subsequently used to define the autophagic phenotypes produced by each treatment administered on cells. Features measured in the present work were:

- Nuclei Intensity: the median intensity values for each nucleus detected in images of living cells have been calculated from the intensity values assigned to each pixel contained into the objects. Such values have been directly used during the image analysis process in order to discard nuclei supposed to belong to cells dead or going to die, by applying the upper quartile as a threshold value. The percentage of living cells calculated sample by sample has been used along with the other features for phenotypes interpretation, while numbers of both total and filtered nuclei have been extracted and the mortality rate produced by each treatment and referred to the controls has been indirectly calculated and then used to evaluate if the observed autophagic phenotypes were possibly caused also by toxicity effects.
- Lysosomes and LC3 Number: the software allows to calculate the number of primary objects, like lysosomes or LC3B proteins which were called "children" objects, contained in each secondary object, like cells called "parent" objects, for each images; by virtue of this, the median number of "children"

lysosomes/LC3 proteins for each “parent” cells in every image has been measured and exported for further analysis and/or interpretations.

- Lysosomes and LC3 Area: the median values concerning the area of each lysosomes/LC3 proteins, consisting in the number of pixels contained in each object, have been measured and exported for further analysis and/or interpretations.
- Number of "active cell": this feature has been measured indirectly after the image analysis by exploiting data regarding "children" lysosome and LC3B protein spots assigned to each "parent" cell in the context of both acidic and autophagic compartments. Cells effectively containing at least one lysosome or LC3B spot after treatments administration have been considered as “active cells” and the percentage of such active cells with respect to the total cells detected for each samples has been measured and exported for further analysis and/or interpretations.

All the objects are displayed in figures 22 and 23 (following pages) in which images show examples of lysosomes and LC3B proteins phenotypes produced on SH-SY5Y cells cultured in normal and autophagy-inducing conditions without any other treatment. Two series of three images for each sample have been reported: for each treatment condition, in the upper rows there are “clean” images (A, B, C and G, H, I) while images labelled by arrows pointing out identified objects are reported in the lower rows (D, E, F and J, K, L); moreover, every column from the left to the right reports images regarding different objects, with nuclei (images A, D, G and J), lysosomes (images B, E, H and K) and the composite images (images C, F, I and L).

As previously said, data regarding nuclei measured only from images of living cells have been directly used during the image analysis to distinguish live and dead cells. Further, the measurements of total detected and the filtered nuclei have been extracted and used after the image analysis: the percentages of nuclei belonging to living cells in each samples has been considered as one of the features describing phenotypes, while the percentages of living cells relative to the control samples have been calculated and used to evaluate the effects produced by each treatment on cell viability, considering that such condition can possibly influences the autophagic phenotypes. The measurements of number and area of lysosomes and/or LC3 spots, instead, as well as the number of cells containing at least one “children” spots per each images, have been exported and then considered together with the percentages of living cells for each sample in order to define autophagic phenotypes produced by the treatments. The median values extracted from the datasets of each features, rather than their mean values, have been considered in order to limit drift effects caused by possible outlier values and to better represent the distribution of the whole population. As all measurements regarding the different features were obtained basing on the calculation of different parameters, the resulting numerical values had a very different scales and hence such numbers cannot be compared or analyzed together but need to be modified in a format which make them similar to each other. For this purpose, they have been normalized by calculating the Z-score value for each single measurement as described in paragraph 3.11.1: Z-scores are numerical values distributed around the “0”, whose absolute value depends on the original measured numbers, that help to transform the numbers coming from measurements conceived with different criteria in a new equivalent format which allows their comparison. Table A1 and A2 (contained in the section supplementary data) report all the values concerning the features (white columns), the relative Z-score values (grey columns) and the percentage of living cells with respect to the controls measured respectively after 2h and 20h treatment (in red in the first columns of each table).

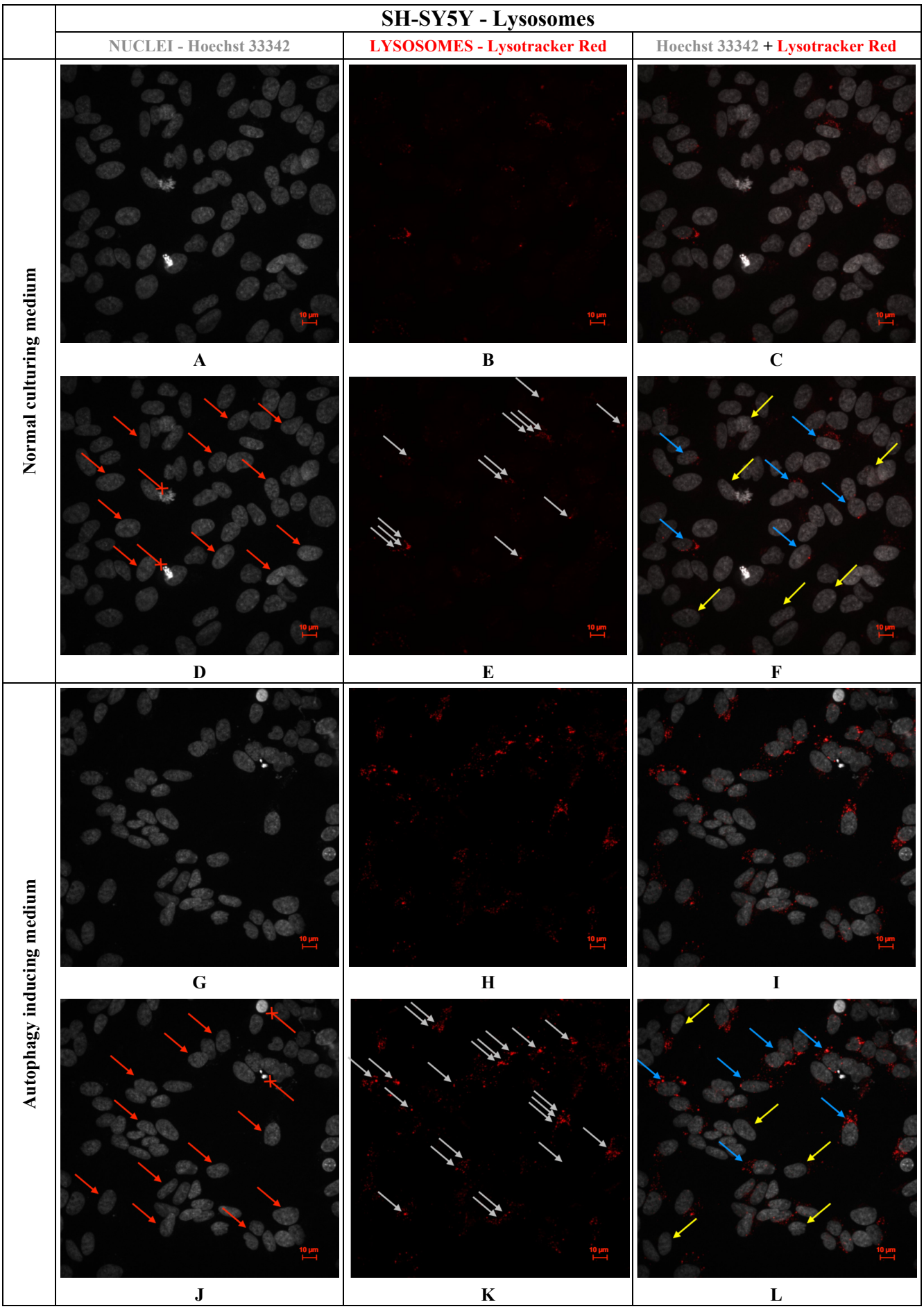


Figure 22. The legend is reported in the following page.

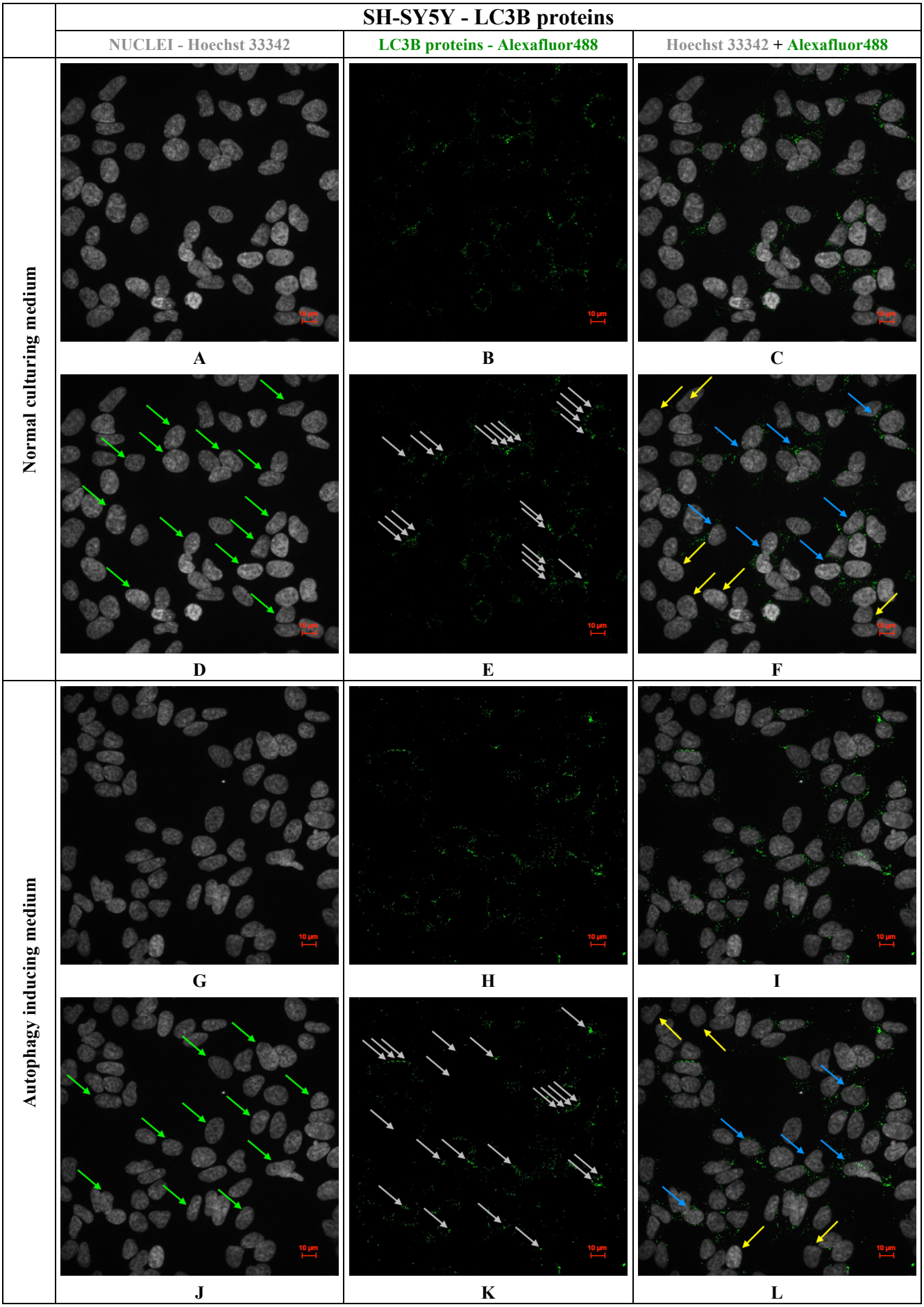


Figure 23. The legend is reported in the following page.

Figure 22. Examples of features extracted by images regarding phenotypes produced on the acidic compartment, detected by analysing lysosomes in cells cultured in normal and autophagy inducing media. Images A and B contain respectively nuclei and lysosomes detected from a sample cultured in normal medium, while image C reports images A and B merged. Images D, E and F are the same images showed in A, B and C reporting in addition arrows which specify detected objects: red arrows in image D indicate nuclei and lines that ended with crosses nuclei considered belonging to dead cells; grey arrows in figure E point out small red spots corresponding to detected lysosomes; in figure F, blue arrows indicate cells supposed to be "active" while yellow arrows point out "not active" cells. Figures G, H, I, J, K and L, which correspond to the previously described images A, B, C, D, E, and F, report images regarding nuclei, lysosomes and the two images merged, with and without arrows indicating the detected features, detected form a sample cultured in starvation conditions.

Figure 23. Examples of features extracted by images regarding phenotypes produced on the autophagic compartment, detected by analysing LC3B proteins in cells cultured in normal and autophagy inducing media. Images A and B contain respectively nuclei and LC3B proteins detected from a sample cultured in normal medium, while image C reports images A and B merged. Images D, E and F are the same images showed in A, B and C, reporting in addition arrows which specify detected objects: red arrows in image D indicate nuclei and lines that ended with crosses nuclei considered belonging to dead cells; grey arrows in figure E point out small red spots corresponding to detected LC3B proteins; in figure F, blue arrows indicate cells supposed to be "active" while yellow arrows point out "not active" cells. Figures G, H, I, J, K and L, which correspond to the previously described images A, B, C, D, E, and F, report images regarding nuclei, LC3B proteins and the two images merged, with and without arrows indicating the detected features, detected form a sample cultured in starvation conditions.

4.3 Multivariate analysis

Multivariate analysis has been performed by developing an approach consisting in a sequence of several investigation methods to analyze all together multiple variables coming from the previous analyses, as described reported below.

4.3.1 Principal Component Analysis (PCA)

The Principal Component Analysis has been carried out to reduce dimensions of datasets containing measurements of features regarding respectively 2 and 20 hours' treatments, thus allowing to analyze them all together. After normalization, the Z-score values of each of the seven chosen features were used to perform the PCA, reducing the dimensions of both datasets to 2 Principal Components (PC1 and PC2) which define 2 factor scores for each treatment. Such new elements have been reported respectively in table A3 and A4 contained in "section supplementary data" at the end of the manuscript. Figure 24 and 25 display screen-diagrams of the PCA regarding respectively 2h and 20 hour treatments datasets, built by using the number of the Principal Components (PC) which is possible to consider (x-axis) and the associated Proportion of Variance defined as percentage (y-axis). Such diagrams allow to establish the percentage of variance that is still contained in the datasets after the reduction of the dimensions depending on the chosen number of considered PCs, representing the most important portion of the total variance. More specifically, in each diagram the percentage of variance associated to each component is reported through the red line (lower line) while the green line (upper line) shows the cumulative percentage of variance covered by the components, as well explained in the manual included into the orange software (University of Ljublijana 2013): such two parameters allow to choose the best compromise between the number of PCs and the appropriate amount of considered variance, letting to optimizing the analysis conditions in order to obtain the most complete information considering just the data most contributing to the variance. Concerning the results, a very high percentage of variance is still contained into the datasets after considering just 2 PCs for both analyses: the variance contained within the first 2 PCs of 2 hours treatments dataset is 63% of total variance, with 36% represented by the PC1 and 27% by the PC2; the first 2 PCs describing dataset of 20 hours treatments contain 68,5% of total variance, of which 54,1% is represented by the PC1 and the remaining variance (almost 14,4%) described into the PC2.

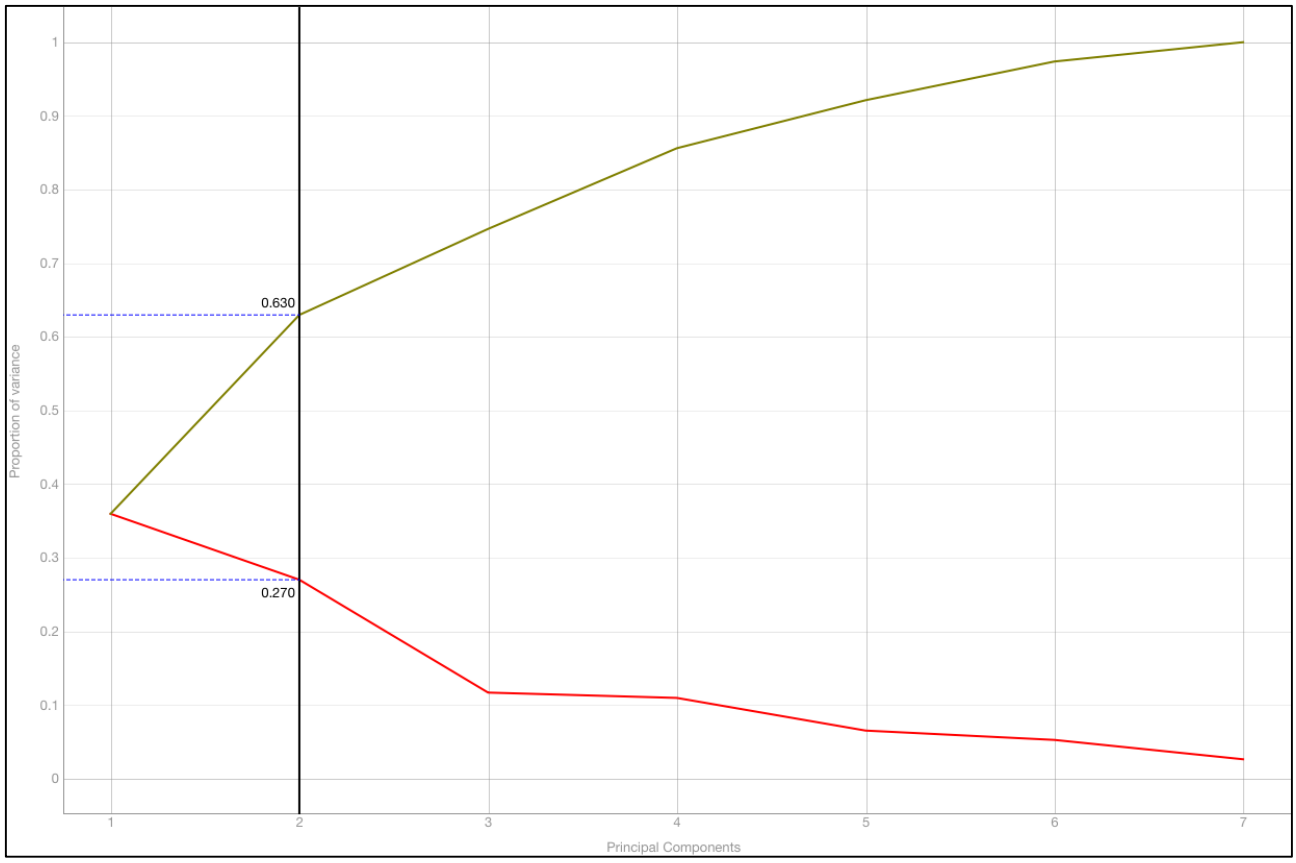


Figure 24. Screen-diagram of the Principal Component Analysis regarding dataset containing features measured form samples treated for 2 hours with the tested extracts under analysis.

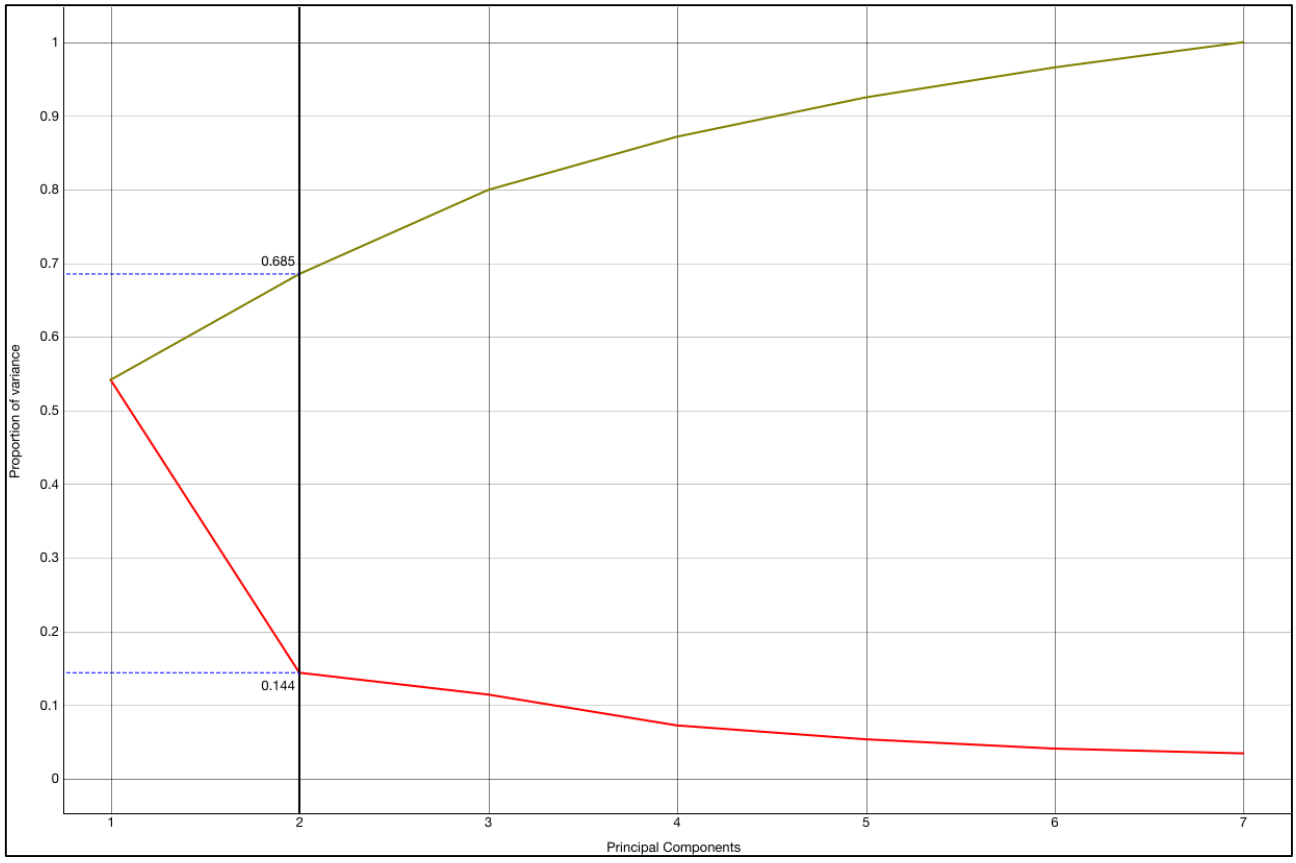


Figure 25. Screen-diagram of the Principal Component Analysis regarding dataset containing features measured form samples treated for 20 hours with the tested extracts under analysis.

4.3.2 Distances evaluation and Hierarchical Cluster Analysis (HCA)

Factor scores associated to PC1 and PC2 of each sample have been used to evaluate the different phenotypes produced by extracts administrated to the cells. Euclidean distance of each sample from all the others has been determined and then used to compare the effects produced by the different treatments under investigation on the autophagic pathway. Samples were organized in different groups having a hierarchical organization basing on the "Ward Linkage" method, by which the clusters are built so that they will include the smallest possible amount of variance, and the different samples are included in the same cluster depending on their Euclidean distance. Results about the ordering of extracts after 2 and 20 hours of treatment in different clusters and their hierarchical organization have been reported as dendograms in figures 26 and 28 and, further, their spatial distribution has been showed in scatter plots reported in figure 27 and 29. Dendograms in the upper side of the figures 26 and 28 represent every sample as a leaf, linked with the other leaves by branches, the length of which describes their distances. In order to separate normal and starvation control samples in different clusters, thus distinguishing treatments producing phenotypes similar to the different reference samples, clusters have been defined by cutting dendograms at a specific level of their whole height: the dendogram which sorts treatments depending on the effects produced after 2 hours has been cut at the 54,7% while dendogram regarding effects produced after 20 hours at the 20,5% of their total height. Further, a list of the samples contained in each cluster, ordered following the scheme presented in table 2, have been reported in the lower side of both figures, in tables defined using the same colour of the represented cluster in order to make their interpretation easier. In scatter plots, instead, each sample has been reported by a circle, colored depending on the membership cluster and positioned in a plane defined by PC1 (x-axis) and PC2 (y-axis) basing on their associated factor scores. Also the free area covered by each cluster has been stained with the specific colour in order to better establish the boundaries of each cluster and allow a more precise interpretation of the clusters distribution. In order to avoid confusion caused by the names of the treatments written on the plot, only the control samples have been labelled, thus preventing the possibility to assign the specific position in the plot for every single sample and allowing, at the same time, the evaluation of the spatial distribution of all the samples contained in each cluster with respect to the control samples.

More specifically, figure 26 reports clusters defined by the effects produced by extracts after 2 hours of treatment: clusters 1 and 4 (light blue and orange clusters, respectively) contain all the samples resulting different from both the normal and the starvation control samples; cluster 2 (faint red cluster) contains all the samples comparable to the normal controls (cells treated in complete culturing medium with/without 0,1% DMSO); cluster 3 (green cluster) contains instead all the samples resulted comparable to the starvation controls (cells treated in serum deprived culturing medium with/without 0,1% DMSO). As previously explained, all the samples contained in each cluster have been reported in the lower side of the image, following the specific order used in table 2. In addition, looking at the scatter plot reported in figure 27 control samples results to be located very close to each other in the left-lower area of the plot and most of the treatments are distributed around and very close to those ones. Most of the analyzed space is contained in cluster 3 (green area), comprising all the starvation control-like samples (green circles) in a small area defined by factor scores located between -0,5; +2 for PC1 and -1; +1 for PC2. Normal control-like samples (faint red circles) contained in cluster 2 (faint red area) occupy instead the left-upper area of the plot and are defined by factor scores comprised between -1; +2 for PC1 and 0; +3 for PC2. Cluster 1 and cluster 4 (respectively light blue and orange clusters), lastly, occupy very little areas respectively in the extreme right-high corner and in the left-lower side of the scatter plot: samples contained in cluster 4 (orange circles) occupy a small area comprised between factor scores 0; +5 for PC1 and -3; -0,5 for PC2, while the only sample contained in cluster 1 (light blue circle) is located in the right upper corner of the scatter plot (PC1: 14,78; PC2: 9,39).

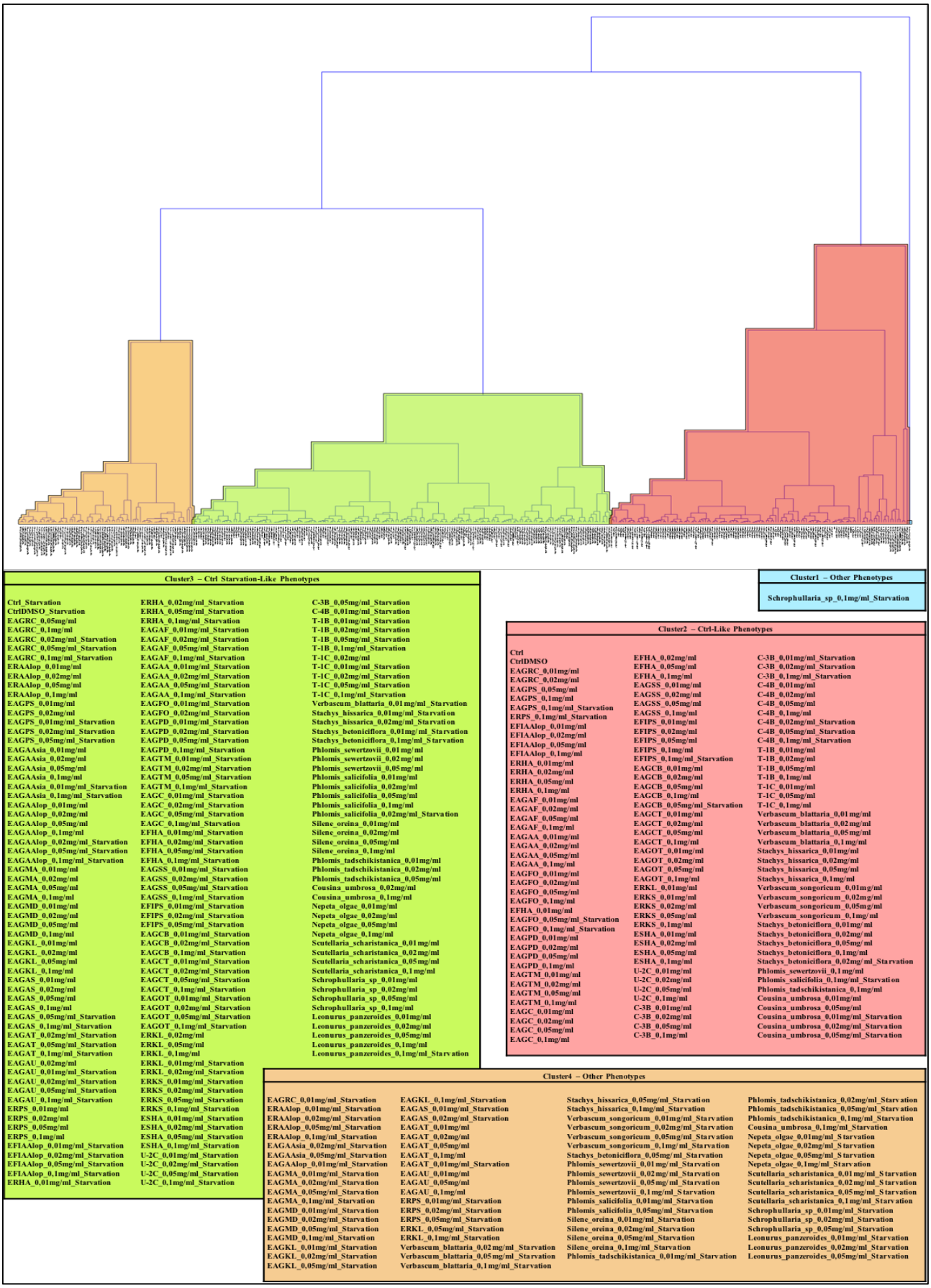


Figure 26. Hierarchical clustering analysis of the data regarding phenotypes produced after 2 hours of treatment with extracts: dendogram in the upper side reports the distribution of the different samples, represented by every single leaf, grouped basing on their Euclidean distances by using the Ward linkage strategy, while tables in the lower side report treatments contained in each obtained cluster.

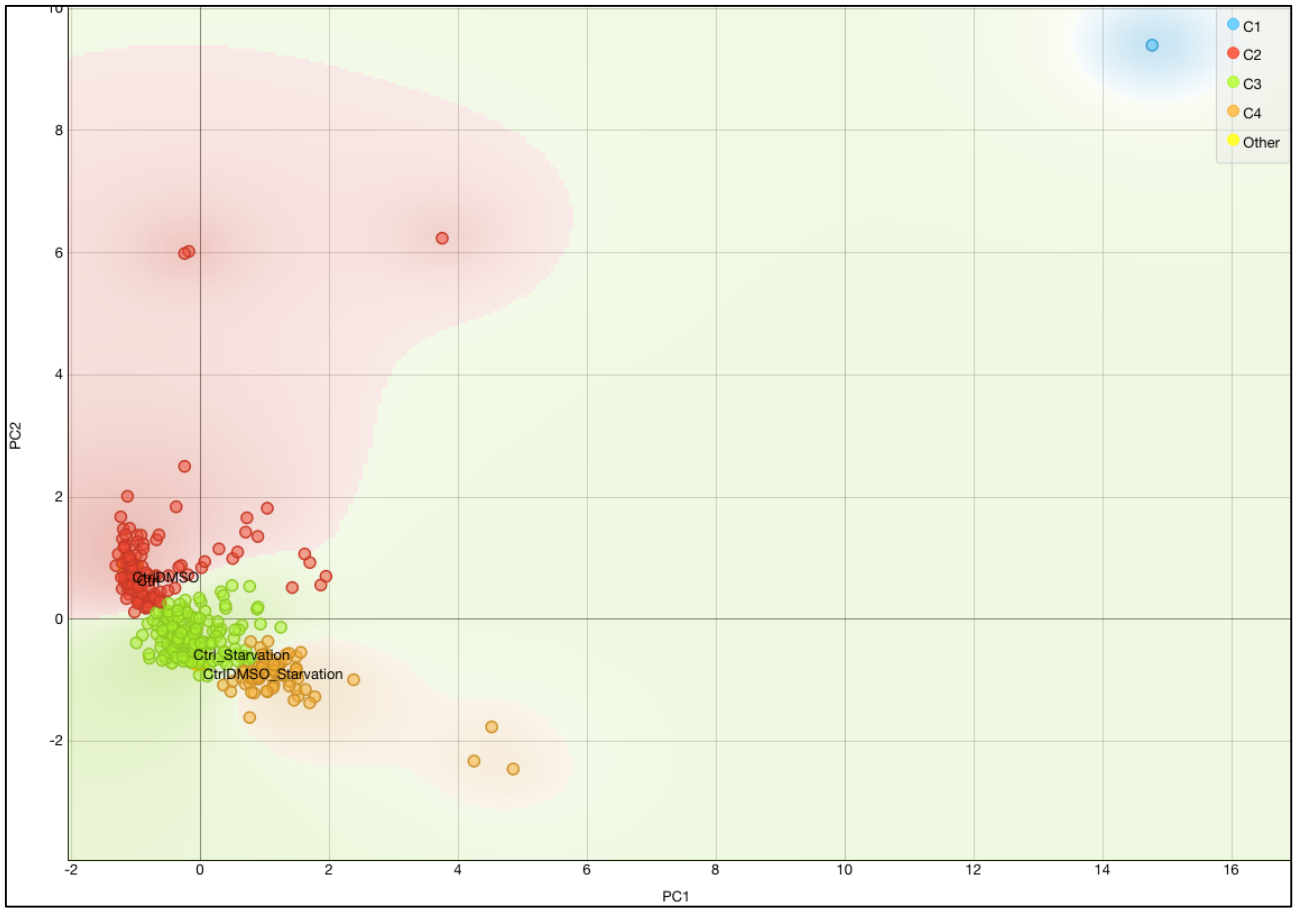


Figure 27. Scatter-plot of the analyzed samples regarding 2 hour treatments. The distribution of the phenotypes is reported on a 2-dimensional plane defined by the 2 Principal Components: the PC1 and PC2 are reported on the x-axis and the y-axis, respectively.

The dendogram that defines clusters determined after analysis of the effects produced by the extract administrated for 20 hours is reported in figure 28: samples resulting to be different from both controls samples are divided in clusters 1 (light blue cluster) and 3 (green cluster), samples similar to normal controls are contained in cluster 2 (faint red cluster) and starvation control-like samples are grouped in cluster 4 (orange cluster). Looking at the scatter plot reporting the spatial distribution of the clusters and the contained samples (figure 29), it is evident that the clusters dimensions are relatively more balanced, with three different cluster occupying most of the analyzed space, and control samples are located very close to each other in the middle-left side of the scatter plot. More specifically, cluster 2 (faint red area) occupies the right-upper side of the scatter plot, and the relative control-like samples (faint red circles) are contained in an area which boundaries are delimited by factor scores comprised between -1; +1,5 for PC1 and 0; +2 for PC2 approximately. Cluster 4 (orange area) occupies an area following the diagonal form the left-lower to the right upper corner of the plot (orange area) and the starvation control-like samples (orange circles) are comprised between factor scores -1,5; +0,5 for PC1 and -1,5; +0,5 for PC2. Lastly, samples regarding treatments which cause different effects from both control samples are contained in cluster 1 and cluster 3: cluster 1 covers almost the whole right side of the plot (light blue area) and the contained samples (light blue circles) are comprised between factor scores greater then +3,5 for PC1 and between -3; +4,5 for PC2, while cluster 2 occupies a little area in the left and middle-lower side of the graph, totally immersed in the orange area (green area) with the contained samples comprised between factor scores -0,5; -2 for PC1 and -3 and -1 for PC2.

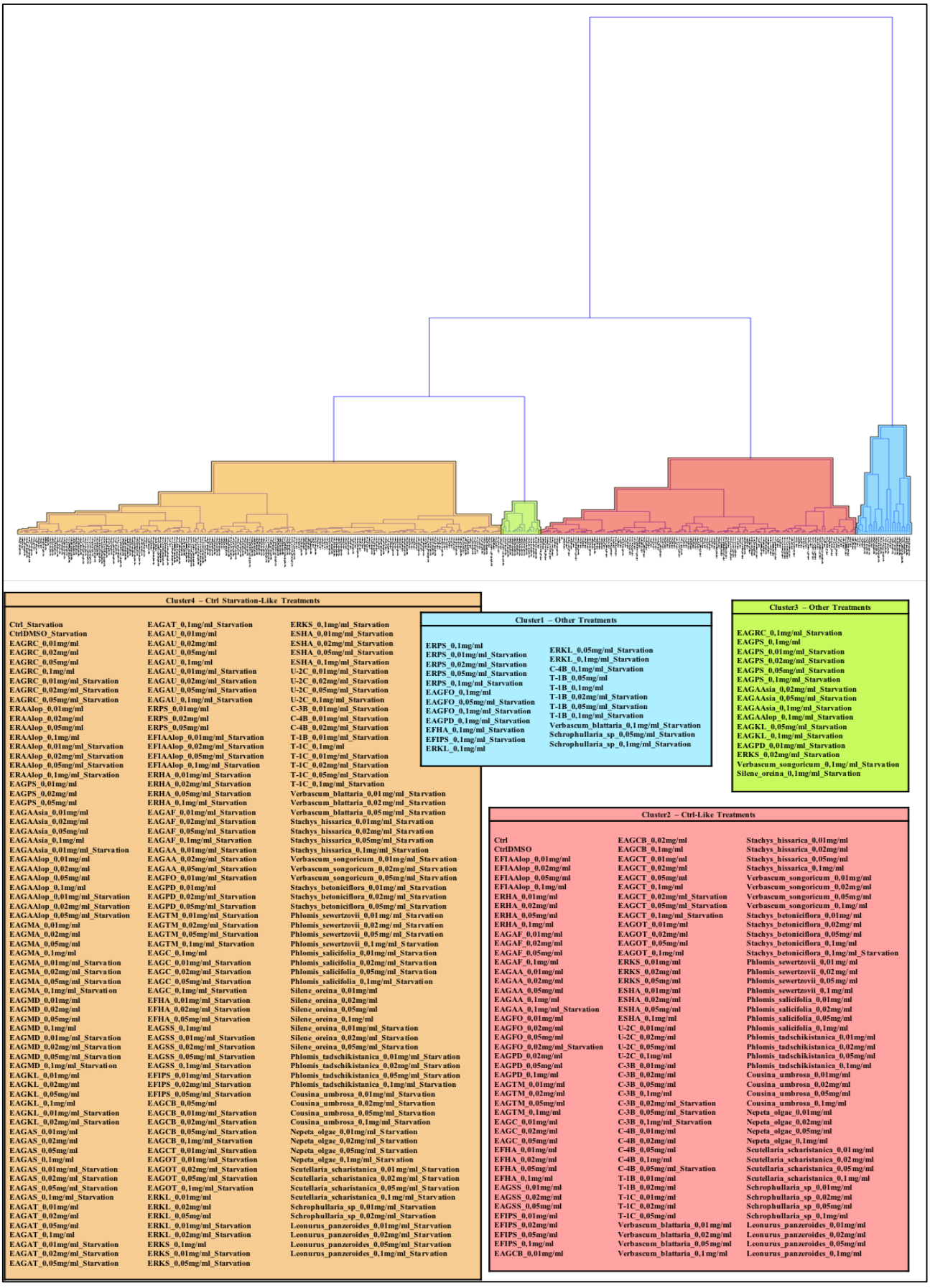


Figure 28. Hierarchical clustering analysis of the data regarding phenotypes produced after 20 hours of treatment with extracts: dendogram in the upper side reports the distribution of the different samples, represented by every single leaf, grouped basing on their Euclidean distances by using the Ward linkage strategy, while tables in the lower side report treatments contained in each obtained cluster.

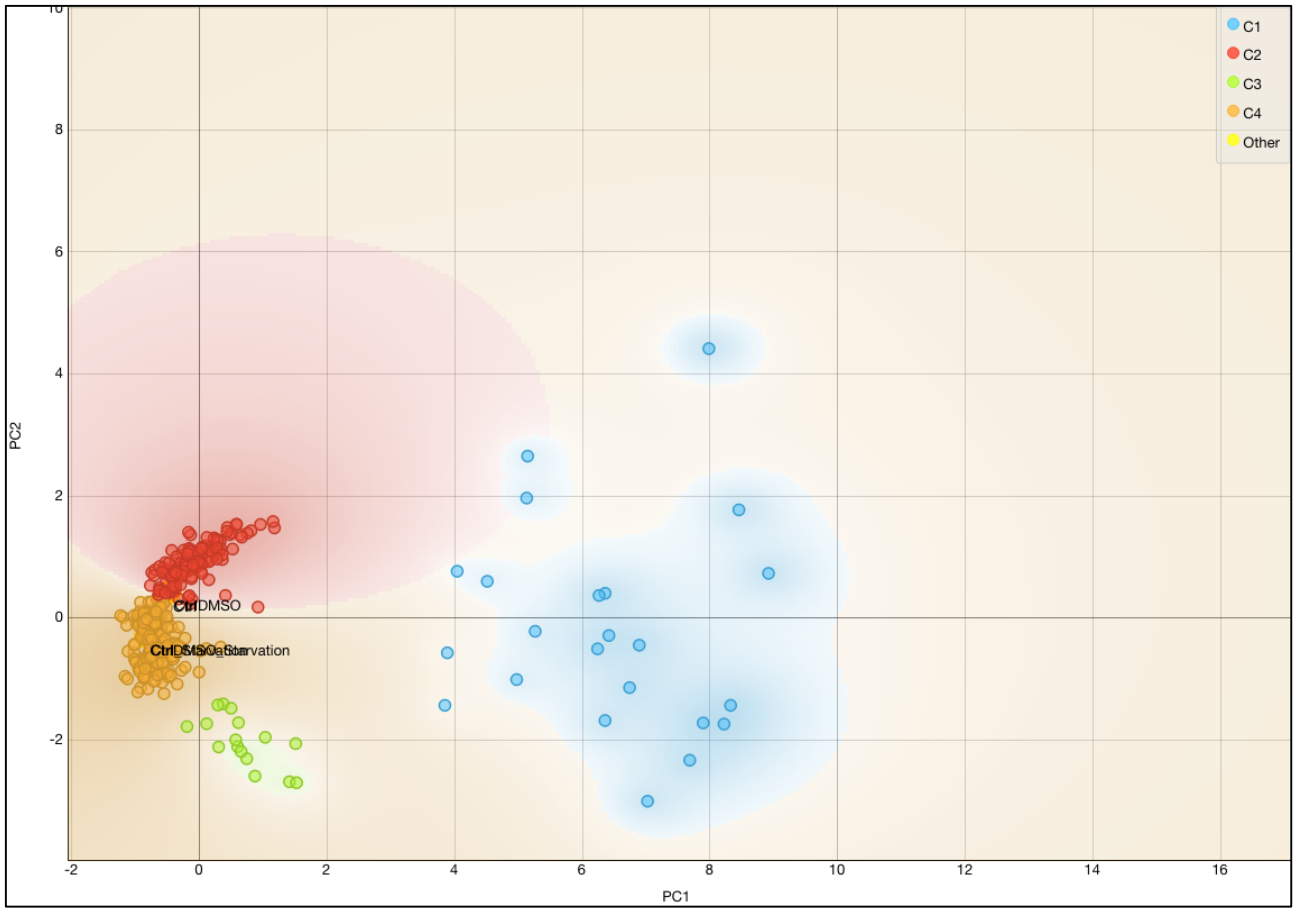
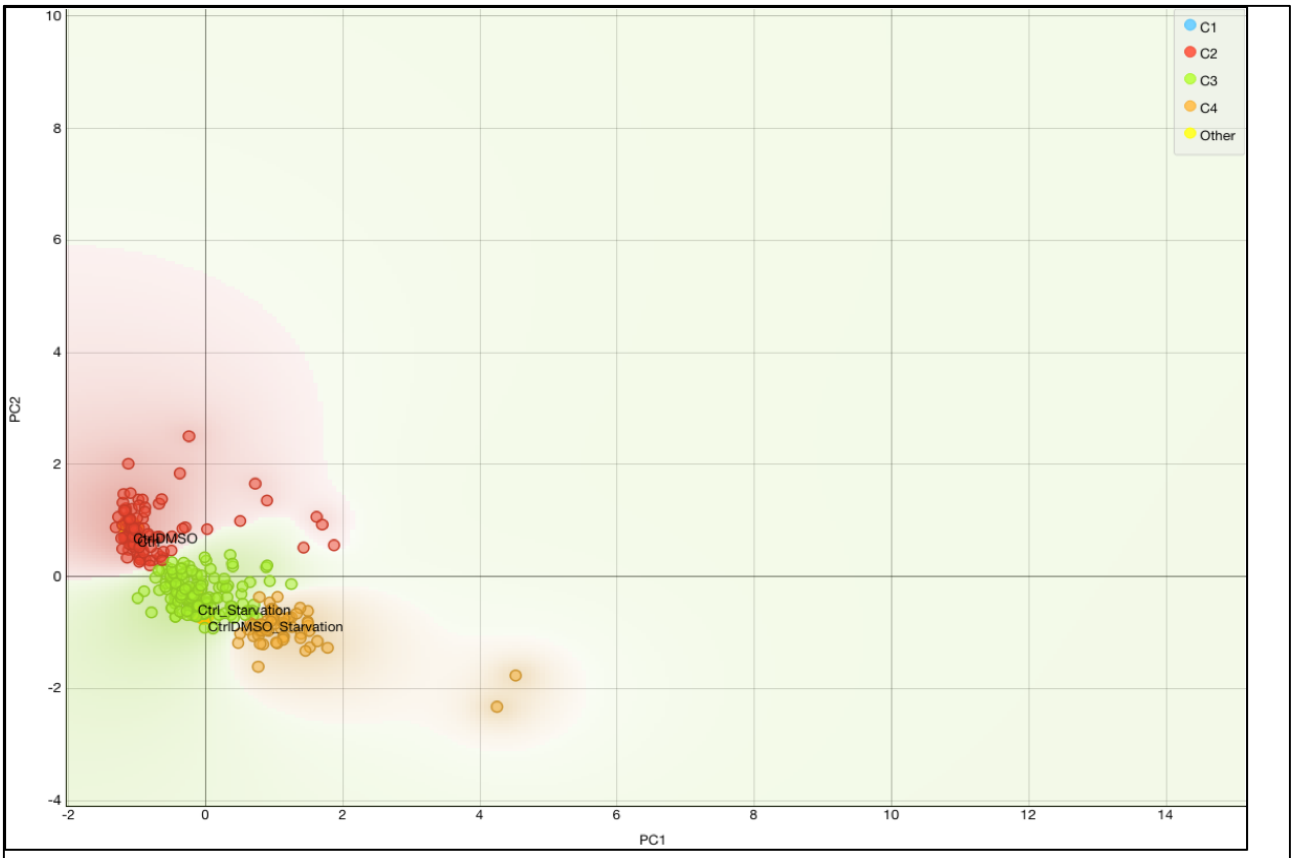


Figure 29. Scatter-plot of the analyzed samples regarding 2 hour treatments. The distribution of the phenotypes is reported on a 2-dimensional plane defined by the 2 Principal Components: the PC1 and PC2 are reported on the x-axis and the y-axis, respectively.

4.3.2.1 Selection of the treatments considered as not toxic for each cluster

After the sorting of all the treatments in different cluster depending on their Euclidean distances, the analyses have been sharpened by excluding all the samples associated with a mortality rate greater then 50%. Such improved results concerning effects caused by treatments after 2 and 20 hours have been respectively reported below in figure 30 and 31 showing in the upper side the new scatter plots displaying the spatial distribution of the selected treatments, with just the control samples highlighted, and in the lower side the lists of the treatments considered as not toxic reported name by name and divided per cluster, using a representation method similar to that used to describe the content of each clusters after the Hierarchical Cluster Analysis (HCA) in figure 26 and 28.

In particular, as evident from both the figures, treatments administrated for 2 as well as 20 hours inducing a mortality rate greater than 50% are mainly those distributed in the farthest area of the plot with respect to the control samples; as a consequence the new plots display that the interesting areas were distributed very closed to the control samples. Analysing figure 30 it appears clear that toxic treatments resulted to be well distributed in the four clusters: among the 29 toxic treatments just 1 resulted to be contained in cluster 1 (and this cluster disappears) while 7 were contained in cluster 2, 10 in cluster 3 and 11 in cluster 4. Looking at figure 31 the 81 toxic treatments resulted to be contained mostly in three clusters: 22 in cluster 1, with this cluster again almost completely deleted from the scatter plot (just 1 treatment stay on the plot), 27 were included in cluster 2, 7 in cluster 3 and 25 in cluster 4.



Cluster1 – Other Not ToxicPhenotypes

/

Cluster2 – Ctrl-Like Not Toxic Phenotypes

Ctrl	EAGOT_0,01mg/ml	
CtrlDMSO	EAGOT_0,02mg/ml	
EAGRC_0,01mg/ml	EAGOT_0,05mg/ml	
EAGRC_0,02mg/ml	EAGOT_0,1mg/ml	
EAGPS_0,05mg/ml	ERKL_0,01mg/ml	
EAGPS_0,1mg/ml	ERKS_0,01mg/ml	
EAGPS_0,1mg/ml_Starvation	ERKS_0,02mg/ml	
EFIAAlop_0,01mg/ml	ERKS_0,05mg/ml	
EFIAAlop_0,02mg/ml	ERKS_0,1mg/ml	
EFIAAlop_0,05mg/ml	ESHA_0,01mg/ml	
EFIAAlop_0,1mg/ml	ESHA_0,02mg/ml	
ERHA_0,01mg/ml	ESHA_0,05mg/ml	
ERHA_0,02mg/ml	ESHA_0,1mg/ml	
ERHA_0,05mg/ml	U-2C_0,01mg/ml	
ERHA_0,1mg/ml	U-2C_0,02mg/ml	
EAGAF_0,01mg/ml	U-2C_0,05mg/ml	
EAGAF_0,02mg/ml	U-2C_0,1mg/ml	
EAGAF_0,05mg/ml	C-3B_0,01mg/ml	
EAGAF_0,1mg/ml	C-3B_0,02mg/ml	
EAGAA_0,01mg/ml	C-3B_0,05mg/ml	
EAGAA_0,02mg/ml	C-3B_0,1mg/ml	
EAGAA_0,05mg/ml	C-3B_0,01mg/ml_Starvation	
EAGAA_0,1mg/ml	C-3B_0,02mg/ml_Starvation	
EAGFO_0,01mg/ml	C-3B_0,1mg/ml_Starvation	
EAGFO_0,02mg/ml	C-4B_0,01mg/ml	
EAGFO_0,05mg/ml	C-4B_0,02mg/ml	
EAGFO_0,1mg/ml	C-4B_0,05mg/ml	
EAGPD_0,01mg/ml	C-4B_0,02mg/ml_Starvation	
EAGPD_0,02mg/ml	C-4B_0,05mg/ml_Starvation	
EAGPD_0,05mg/ml	T-1B_0,01mg/ml	
EAGPD_0,1mg/ml	T-1B_0,02mg/ml	
EAGTM_0,01mg/ml	T-1B_0,05mg/ml	
EAGTM_0,02mg/ml	T-1B_0,1mg/ml	
EAGTM_0,05mg/ml	T-1C_0,01mg/ml	
EAGTM_0,1mg/ml	T-1C_0,05mg/ml	
EAGC_0,01mg/ml	T-1C_0,1mg/ml	
EAGC_0,02mg/ml	Verbascum_blattaria_0,01mg/ml	
EAGC_0,05mg/ml	Verbascum_blattaria_0,02mg/ml	
EAGC_0,1mg/ml	Verbascum_blattaria_0,05mg/ml	
EFHA_0,01mg/ml	Verbascum_blattaria_0,1mg/ml	
EFHA_0,02mg/ml	Stachys_hissarica_0,01mg/ml	
EFHA_0,05mg/ml	Stachys_hissarica_0,02mg/ml	
EFHA_0,1mg/ml	Stachys_hissarica_0,05mg/ml	
EAGSS_0,01mg/ml	Stachys_hissarica_0,1mg/ml	
EAGSS_0,02mg/ml	Verbascum_songoricum_0,01mg/ml	
EAGSS_0,05mg/ml	Verbascum_songoricum_0,02mg/ml	
EAGSS_0,1mg/ml	Verbascum_songoricum_0,05mg/ml	
EFIPS_0,01mg/ml	Verbascum_songoricum_0,1mg/ml	
EFIPS_0,02mg/ml	Stachys_betonicifiora_0,01mg/ml	
EFIPS_0,05mg/ml	Stachys_betonicifiora_0,02mg/ml	
EFIPS_0,1mg/ml	Stachys_betonicifiora_0,05mg/ml	
EFIPS_0,1mg/ml_Starvation	Stachys_betonicifiora_0,1mg/ml	
EAGCB_0,02mg/ml	Stachys_betonicifiora_0,02mg/ml_Starvation	
EAGCB_0,05mg/ml	Phlomis_salicifolia_0,1mg/ml_Starvation	
EAGCB_0,1mg/ml	Phlomis_tadschikistanica_0,1mg/ml	
EAGCB_0,05mg/ml_Starvation	Cousina_umbrosa_0,01mg/ml	
EAGCT_0,01mg/ml	Cousina_umbrosa_0,05mg/ml	
EAGCT_0,02mg/ml	Cousina_umbrosa_0,01mg/ml_Starvation	
EAGCT_0,05mg/ml	Cousina_umbrosa_0,02mg/ml_Starvation	
EAGCT_0,1mg/ml	Cousina_umbrosa_0,05mg/ml_Starvation	

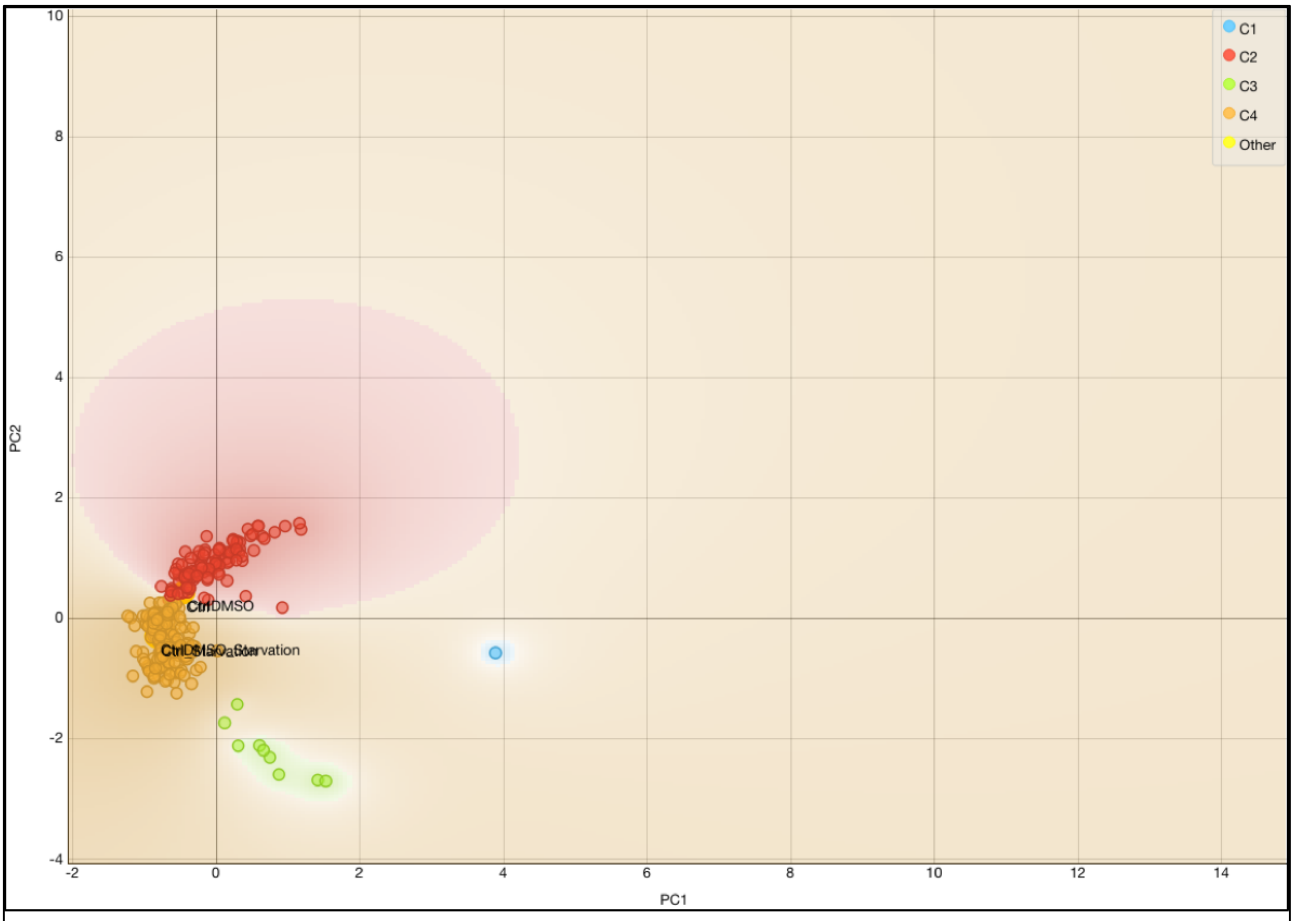
Cluster3 – Ctrl Starvation-Like Not Toxic Phenotypes

Ctrl_Starvation	EAGAS_0,05mg/ml	EAGC_0,1mg/ml_Starvation	T-1C_0,02mg/ml
CtrlDMSO_Starvation	EAGAS_0,1mg/ml	EFHA_0,01mg/ml_Starvation	T-1C_0,01mg/ml_Starvation
EAGRC_0,05mg/ml	EAGAS_0,05mg/ml_Starvation	EFHA_0,02mg/ml_Starvation	T-1C_0,02mg/ml_Starvation
EAGRC_0,1mg/ml	EAGAS_0,1mg/ml_Starvation	EFHA_0,05mg/ml_Starvation	T-1C_0,05mg/ml_Starvation
EAGRC_0,02mg/ml_Starvation	EAGAT_0,02mg/ml_Starvation	EAGSS_0,01mg/ml_Starvation	T-1C_0,1mg/ml_Starvation
EAGRC_0,05mg/ml_Starvation	EAGAT_0,05mg/ml_Starvation	EAGSS_0,02mg/ml_Starvation	Verbascum_blattaria_0,01mg/ml_Starvation
EAGRC_0,1mg/ml_Starvation	EAGAT_0,1mg/ml_Starvation	EAGSS_0,05mg/ml_Starvation	Stachys_hissarica_0,01mg/ml_Starvation
ERAAlop_0,01mg/ml	EAGAU_0,02mg/ml	EAGSS_0,1mg/ml_Starvation	Stachys_hissarica_0,02mg/ml_Starvation
ERAAlop_0,02mg/ml	EAGAU_0,01mg/ml_Starvation	EFIPS_0,01mg/ml_Starvation	Stachys_betonicifiora_0,01mg/ml_Starvation
ERAAlop_0,05mg/ml	EAGAU_0,02mg/ml_Starvation	EFIPS_0,05mg/ml_Starvation	Stachys_betonicifiora_0,1mg/ml_Starvation
ERAAlop_0,1mg/ml	EAGAU_0,05mg/ml_Starvation	EAGCB_0,01mg/ml_Starvation	Phlomis_sewertzovii_0,01mg/ml
EAGPS_0,01mg/ml	EAGAU_0,1mg/ml_Starvation	EAGCB_0,1mg/ml_Starvation	Phlomis_sewertzovii_0,02mg/ml
EAGPS_0,02mg/ml	ERPS_0,01mg/ml	EAGCT_0,01mg/ml_Starvation	Phlomis_sewertzovii_0,05mg/ml
EAGPS_0,01mg/ml_Starvation	ERPS_0,02mg/ml	EAGCT_0,02mg/ml_Starvation	Phlomis_salicifolia_0,01mg/ml
EAGPS_0,02mg/ml_Starvation	ERPS_0,05mg/ml	EAGCT_0,05mg/ml_Starvation	Phlomis_salicifolia_0,02mg/ml
EAGPS_0,05mg/ml_Starvation	EFIAAlop_0,01mg/ml_Starvation	EAGCT_0,1mg/ml_Starvation	Phlomis_salicifolia_0,05mg/ml
EAGAAsia_0,01mg/ml	EFIAAlop_0,02mg/ml_Starvation	EAGOT_0,01mg/ml_Starvation	Phlomis_salicifolia_0,1mg/ml
EAGAAsia_0,02mg/ml	EFIAAlop_0,05mg/ml_Starvation	EAGOT_0,02mg/ml_Starvation	Phlomis_salicifolia_0,02mg/ml_Starvation
EAGAAsia_0,05mg/ml	EFIAAlop_0,1mg/ml_Starvation	EAGOT_0,05mg/ml_Starvation	Silene_oreina_0,01mg/ml
EAGAAsia_0,1mg/ml	ERHA_0,01mg/ml_Starvation	EAGOT_0,1mg/ml_Starvation	Silene_oreina_0,02mg/ml
EAGAAsia_0,01mg/ml_Starvation	ERHA_0,05mg/ml_Starvation	ERKL_0,02mg/ml	Silene_oreina_0,05mg/ml
EAGAAlop_0,01mg/ml	ERHA_0,1mg/ml_Starvation	ERKL_0,05mg/ml	Silene_oreina_0,1mg/ml
EAGAAlop_0,02mg/ml	EAGAF_0,01mg/ml_Starvation	ERKL_0,1mg/ml	Phlomis_tadschikistanica_0,01mg/ml
EAGAAlop_0,05mg/ml	EAGAF_0,02mg/ml_Starvation	ERKL_0,01mg/ml_Starvation	Phlomis_tadschikistanica_0,02mg/ml
EAGAAlop_0,1mg/ml	EAGAF_0,05mg/ml_Starvation	ERKL_0,02mg/ml_Starvation	Phlomis_tadschikistanica_0,05mg/ml
EAGAAlop_0,02mg/ml_Starvation	EAGAF_0,1mg/ml_Starvation	ERKS_0,01mg/ml_Starvation	Cousina_umbrosa_0,02mg/ml
EAGAAlop_0,05mg/ml_Starvation	EAGAA_0,01mg/ml_Starvation	ERKS_0,02mg/ml_Starvation	Cousina_umbrosa_0,1mg/ml
EAGAAlop_0,1mg/ml_Starvation	EAGAA_0,02mg/ml_Starvation	ERKS_0,05mg/ml_Starvation	Nepeta_olgae_0,01mg/ml
EAGMA_0,01mg/ml	EAGAA_0,1mg/ml_Starvation	ERKS_0,1mg/ml_Starvation	Nepeta_olgae_0,02mg/ml
EAGMA_0,02mg/ml	EAGFO_0,01mg/ml_Starvation	ESHA_0,01mg/ml_Starvation	Nepeta_olgae_0,05mg/ml
EAGMA_0,05mg/ml	EAGFO_0,02mg/ml_Starvation	ESHA_0,02mg/ml_Starvation	Nepeta_olgae_0,1mg/ml
EAGMA_0,1mg/ml	EAGFO_0,05mg/ml_Starvation	ESHA_0,05mg/ml_Starvation	Scutellaria_scharistanica_0,01mg/ml
EAGMD_0,01mg/ml	EAGFO_0,1mg/ml_Starvation	ESHA_0,1mg/ml_Starvation	Scutellaria_scharistanica_0,02mg/ml
EAGMD_0,02mg/ml	EAGPD_0,01mg/ml_Starvation	U-2C_0,01mg/ml_Starvation	Scutellaria_scharistanica_0,1mg/ml
EAGMD_0,05mg/ml	EAGPD_0,02mg/ml_Starvation	U-2C_0,02mg/ml_Starvation	Schrophullaria_sp_0,01mg/ml
EAGMD_0,1mg/ml	EAGPD_0,05mg/ml_Starvation	U-2C_0,05mg/ml_Starvation	Schrophullaria_sp_0,02mg/ml
EAGKL_0,01mg/ml	EAGPD_0,1mg/ml_Starvation	U-2C_0,1mg/ml_Starvation	Schrophullaria_sp_0,05mg/ml
EAGKL_0,02mg/ml	EAGTM_0,01mg/ml_Starvation	C-3B_0,05mg/ml_Starvation	Schrophullaria_sp_0,1mg/ml
EAGKL_0,05mg/ml	EAGTM_0,02mg/ml_Starvation	C-4B_0,01mg/ml_Starvation	Leonurus_panzeroides_0,01mg/ml
EAGKL_0,1mg/ml	EAGTM_0,05mg/ml_Starvation	T-1B_0,01mg/ml_Starvation	Leonurus_panzeroides_0,02mg/ml
EAGAS_0,01mg/ml	EAGTM_0,1mg/ml_Starvation	T-1B_0,02mg/ml_Starvation	Leonurus_panzeroides_0,1mg/ml
EAGAS_0,02mg/ml	EAGC_0,01mg/ml_Starvation	T-1B_0,05mg/ml_Starvation	Leonurus_panzeroides_0,1mg/ml_Starvation
	EAGC_0,02mg/ml_Starvation		
	EAGC_0,05mg/ml_Starvation		

Cluster4 – Other Not Toxic Phenotypes

EAGRC_0,01mg/ml_Starvation	EAGKL_0,05mg/ml_Starvation	Verbascum_songoricum_0,01mg/ml_Starvation	Phlomis_tadschikistanica_0,1mg/ml_Starvation
ERAAlop_0,01mg/ml_Starvation	EAGKL_0,1mg/ml_Starvation	Verbascum_songoricum_0,02mg/ml_Starvation	Cousina_umbrosa_0,1mg/ml_Starvation
ERAAlop_0,02mg/ml_Starvation	EAGAS_0,01mg/ml_Starvation	Verbascum_songoricum_0,05mg/ml_Starvation	Nepeta_olgae_0,01mg/ml_Starvation
ERAAlop_0,05mg/ml_Starvation	EAGAS_0,02mg/ml_Starvation	Phlomis_sewertzovii_0,01mg/ml_Starvation	Nepeta_olgae_0,02mg/ml_Starvation
ERAAlop_0,1mg/ml_Starvation	EAGAT_0,01mg/ml	Phlomis_sewertzovii_0,02mg/ml_Starvation	Nepeta_olgae_0,05mg/ml_Starvation
EAGAAsia_0,02mg/ml_Starvation	EAGAT_0,02mg/ml	Phlomis_sewertzovii_0,05mg/ml_Starvation	Nepeta_olgae_0,1mg/ml_Starvation
EAGAAlop_0,01mg/ml_Starvation	EAGAT_0,05mg/ml	Phlomis_sewertzovii_0,1mg/ml_Starvation	Scutellaria_scharistanica_0,01mg/ml_Starvation
EAGMA_0,02mg/ml_Starvation	EAGAT_0,1mg/ml	Phlomis_salicifolia_0,01mg/ml_Starvation	Scutellaria_scharistanica_0,02mg/ml_Starvation
EAGMA_0,05mg/ml_Starvation	EAGAU_0,01mg/ml	Phlomis_salicifolia_0,05mg/ml_Starvation	Scutellaria_scharistanica_0,05mg/ml_Starvation
EAGMA_0,1mg/ml_Starvation	EAGAU_0,05mg/ml	Silene_oreina_0,01mg/ml_Starvation	Scutellaria_scharistanica_0,1mg/ml_Starvation
EAGMD_0,01mg/ml_Starvation	EAGAU_0,1mg/ml	Silene_oreina_0,02mg/ml_Starvation	Schrophullaria_sp_0,01mg/ml_Starvation
EAGMD_0,02mg/ml_Starvation	ERPS_0,02mg/ml_Starvation	Silene_oreina_0,05mg/ml_Starvation	Schrophullaria_sp_0,02mg/ml_Starvation
EAGMD_0,05mg/ml_Starvation	ERKL_0,05mg/ml_Starvation	Silene_oreina_0,1mg/ml_Starvation	Leonurus_panzeroides_0,01mg/ml_Starvation
EAGMD_0,1mg/ml_Starvation	Verbascum_blattaria_0,02mg/ml_Starvation	Phlomis_tadschikistanica_0,01mg/ml_Starvation	Leonurus_panzeroides_0,02mg/ml_Starvation
EAGKL_0,01mg/ml_Starvation	Verbascum_blattaria_0,05mg/ml_Starvation	Phlomis_tadschikistanica_0,02mg/ml_Starvation	Leonurus_panzeroides_0,05mg/ml_Starvation
EAGKL_0,02mg/ml_Starvation	Stachys_hissarica_0,05mg/ml_Starvation	Phlomis_tadschikistanica_0,05mg/ml_Starvation	

Figure 30. Scatter-plot reporting the samples regarding 2 hour treatments considered as not toxic; tables in the lower side reports treatments contained in each obtained cluster.



Cluster2 – Ctrl-Like Not Toxic Treatments

Ctrl	C-3B_0,01mg/ml
CtrlDMSO	C-3B_0,02mg/ml
EFIAAlop_0,01mg/ml	C-3B_0,05mg/ml
EFIAAlop_0,02mg/ml	C-3B_0,1mg/ml
EFIAAlop_0,05mg/ml	C-3B_0,02mg/ml_Starvation
EFIAAlop_0,1mg/ml	C-3B_0,05mg/ml_Starvation
ERHA_0,01mg/ml	C-4B_0,01mg/ml
ERHA_0,02mg/ml	C-4B_0,02mg/ml
ERHA_0,1mg/ml	T-1B_0,01mg/ml
EAGAF_0,01mg/ml	T-1C_0,01mg/ml
EAGAF_0,02mg/ml	T-1C_0,02mg/ml
EAGAF_0,05mg/ml	T-1C_0,05mg/ml
EAGAF_0,1mg/ml	Verbascum_blattaria_0,01mg/ml
EAGAA_0,01mg/ml	Verbascum_blattaria_0,02mg/ml
EAGAA_0,05mg/ml	Verbascum_blattaria_0,05mg/ml
EAGAA_0,1mg/ml	Verbascum_blattaria_0,1mg/ml
EAGFO_0,01mg/ml	Stachys_hissarica_0,01mg/ml
EAGFO_0,02mg/ml	Stachys_hissarica_0,02mg/ml
EAGPD_0,02mg/ml	Stachys_hissarica_0,05mg/ml
EAGTM_0,01mg/ml	Verbascum_songoricum_0,01mg/ml
EAGTM_0,02mg/ml	Verbascum_songoricum_0,02mg/ml
EAGTM_0,05mg/ml	Verbascum_songoricum_0,05mg/ml
EAGTM_0,1mg/ml	Stachys_betonicifiora_0,01mg/ml
EFHA_0,01mg/ml	Stachys_betonicifiora_0,02mg/ml
EFHA_0,02mg/ml	Stachys_betonicifiora_0,05mg/ml
EFHA_0,05mg/ml	Stachys_betonicifiora_0,1mg/ml
EFHA_0,1mg/ml	Stachys_betonicifiora_0,1mg/ml_Starvation
EAGSS_0,01mg/ml	Phlomis_sewertzovii_0,01mg/ml
EAGSS_0,05mg/ml	Phlomis_sewertzovii_0,02mg/ml
EFIPS_0,01mg/ml	Phlomis_sewertzovii_0,05mg/ml
EFIPS_0,05mg/ml	Phlomis_sewertzovii_0,1mg/ml
EAGCB_0,01mg/ml	Phlomis_salicifolia_0,01mg/ml
EAGCB_0,1mg/ml	Phlomis_salicifolia_0,02mg/ml
EAGCT_0,01mg/ml	Phlomis_salicifolia_0,05mg/ml
EAGCT_0,02mg/ml	Phlomis_salicifolia_0,1mg/ml
EAGCT_0,05mg/ml	Phlomis_tadschikistanica_0,01mg/ml
EAGCT_0,1mg/ml	Phlomis_tadschikistanica_0,02mg/ml
EAGCT_0,02mg/ml_Starvation	Phlomis_tadschikistanica_0,05mg/ml
EAGCT_0,05mg/ml_Starvation	Phlomis_tadschikistanica_0,1mg/ml
EAGOT_0,01mg/ml	Cousina_umbrosa_0,01mg/ml
EAGOT_0,02mg/ml	Cousina_umbrosa_0,02mg/ml
EAGOT_0,05mg/ml	Cousina_umbrosa_0,1mg/ml
EAGOT_0,1mg/ml	Nepeta_olgae_0,01mg/ml
ERKS_0,01mg/ml	Nepeta_olgae_0,02mg/ml
ERKS_0,02mg/ml	Nepeta_olgae_0,05mg/ml
ERKS_0,05mg/ml	Nepeta_olgae_0,1mg/ml
ESHA_0,01mg/ml	Scutellaria_scharistanica_0,01mg/ml
ESHA_0,02mg/ml	Scutellaria_scharistanica_0,05mg/ml
ESHA_0,05mg/ml	Schrophullaria_sp_0,01mg/ml
ESHA_0,1mg/ml	Schrophullaria_sp_0,02mg/ml
U-2C_0,01mg/ml	Schrophullaria_sp_0,05mg/ml
U-2C_0,02mg/ml	Schrophullaria_sp_0,1mg/ml
U-2C_0,05mg/ml	Leonurus_panzeroides_0,01mg/ml
U-2C_0,1mg/ml	Leonurus_panzeroides_0,05mg/ml

Cluster1 – Other Not Toxic Treatments

ERPS_0,01mg/ml_Starvation

Cluster3 – Other Not Toxic Treatments

EAGPS_0,1mg/ml	EAGAAlop_0,1mg/ml_Starvation
EAGPS_0,01mg/ml_Starvation	EAGKL_0,05mg/ml_Starvation
EAGPS_0,02mg/ml_Starvation	EAGPD_0,01mg/ml_Starvation
EAGPS_0,05mg/ml_Starvation	ERKS_0,02mg/ml_Starvation
EAGPS_0,1mg/ml_Starvation	

Cluster4 – Ctrl Starvation-Like Not Toxic Treatments

Ctrl_Starvation	EAGKL_0,01mg/ml	EAGPD_0,01mg/ml	Verbascum_blattaria_0,05mg/ml_Starvation
CtrlDMSO_Starvation	EAGKL_0,02mg/ml	EAGPD_0,02mg/ml_Starvation	Stachys_hissarica_0,01mg/ml_Starvation
EAGRC_0,01mg/ml	EAGKL_0,05mg/ml	EAGTM_0,02mg/ml_Starvation	Stachys_hissarica_0,02mg/ml_Starvation
EAGRC_0,02mg/ml	EAGKL_0,1mg/ml	EAGTM_0,05mg/ml_Starvation	Stachys_hissarica_0,05mg/ml_Starvation
EAGRC_0,05mg/ml	EAGKL_0,01mg/ml_Starvation	EFHA_0,01mg/ml_Starvation	Stachys_hissarica_0,1mg/ml_Starvation
EAGRC_0,1mg/ml	EAGKL_0,02mg/ml_Starvation	EFHA_0,02mg/ml_Starvation	Verbascum_songoricum_0,01mg/ml_Starvation
EAGRC_0,01mg/ml_Starvation	EAGAS_0,01mg/ml	EFHA_0,05mg/ml_Starvation	Verbascum_songoricum_0,02mg/ml_Starvation
EAGRC_0,02mg/ml_Starvation	EAGAS_0,02mg/ml	EAGSS_0,01mg/ml_Starvation	Verbascum_songoricum_0,05mg/ml_Starvation
EAGRC_0,05mg/ml_Starvation	EAGAS_0,05mg/ml	EAGSS_0,05mg/ml_Starvation	Stachys_betonicifiora_0,01mg/ml_Starvation
ERAAlop_0,01mg/ml	EAGAS_0,1mg/ml	EAGSS_0,1mg/ml_Starvation	Stachys_betonicifiora_0,02mg/ml_Starvation
ERAAlop_0,02mg/ml	EAGAS_0,01mg/ml_Starvation	EFIPS_0,01mg/ml_Starvation	Stachys_betonicifiora_0,05mg/ml_Starvation
ERAAlop_0,05mg/ml	EAGAS_0,02mg/ml_Starvation	EFIPS_0,02mg/ml_Starvation	Phlomis_sewertzovii_0,01mg/ml_Starvation
ERAAlop_0,01mg/ml_Starvation	EAGAS_0,05mg/ml_Starvation	EAGCB_0,05mg/ml	Phlomis_sewertzovii_0,02mg/ml_Starvation
ERAAlop_0,02mg/ml_Starvation	EAGAS_0,1mg/ml_Starvation	EAGCB_0,02mg/ml_Starvation	Phlomis_sewertzovii_0,05mg/ml_Starvation
ERAAlop_0,05mg/ml_Starvation	EAGAT_0,01mg/ml	EAGCB_0,1mg/ml_Starvation	Phlomis_sewertzovii_0,1mg/ml_Starvation
ERAAlop_0,1mg/ml_Starvation	EAGAT_0,02mg/ml	EAGCT_0,01mg/ml_Starvation	Phlomis_salicifolia_0,01mg/ml_Starvation
EAGPS_0,01mg/ml	EAGAT_0,05mg/ml	EAGOT_0,01mg/ml_Starvation	Phlomis_salicifolia_0,02mg/ml_Starvation
EAGPS_0,02mg/ml	EAGAT_0,1mg/ml	EAGOT_0,02mg/ml_Starvation	Phlomis_salicifolia_0,05mg/ml_Starvation
EAGPS_0,05mg/ml	EAGAT_0,01mg/ml_Starvation	EAGOT_0,05mg/ml_Starvation	Phlomis_salicifolia_0,1mg/ml_Starvation
EAGAAsia_0,01mg/ml	EAGAT_0,02mg/ml_Starvation	EAGOT_0,1mg/ml_Starvation	Silene_oreina_0,01mg/ml
EAGAAsia_0,02mg/ml	EAGAT_0,05mg/ml_Starvation	ERKL_0,01mg/ml	Silene_oreina_0,02mg/ml
EAGAAsia_0,05mg/ml	EAGAT_0,1mg/ml_Starvation	ERKL_0,02mg/ml	Silene_oreina_0,05mg/ml
EAGAAsia_0,1mg/ml	EAGAU_0,01mg/ml	ERKL_0,05mg/ml	Silene_oreina_0,1mg/ml
EAGAAsia_0,01mg/ml_Starvation	EAGAU_0,02mg/ml	ERKL_0,01mg/ml_Starvation	Silene_oreina_0,01mg/ml_Starvation
EAGAAlop_0,01mg/ml	EAGAU_0,05mg/ml	ERKL_0,02mg/ml_Starvation	Silene_oreina_0,02mg/ml_Starvation
EAGAAlop_0,02mg/ml	EAGAU_0,1mg/ml	ERKS_0,1mg/ml	Silene_oreina_0,05mg/ml_Starvation
EAGAAlop_0,05mg/ml	EAGAU_0,01mg/ml_Starvation	ERKS_0,01mg/ml_Starvation	Phlomis_tadschikistanica_0,01mg/ml_Starvation
EAGAAlop_0,1mg/ml	EAGAU_0,02mg/ml_Starvation	ERKS_0,05mg/ml_Starvation	Phlomis_tadschikistanica_0,02mg/ml_Starvation
EAGAAlop_0,01mg/ml_Starvation	EAGAU_0,05mg/ml_Starvation	ERKS_0,1mg/ml_Starvation	Phlomis_tadschikistanica_0,05mg/ml_Starvation
EAGAAlop_0,02mg/ml_Starvation	EAGAU_0,1mg/ml_Starvation	ESHA_0,01mg/ml_Starvation	Phlomis_tadschikistanica_0,1mg/ml_Starvation
EAGAAlop_0,05mg/ml_Starvation	ERPS_0,01mg/ml	ESHA_0,02mg/ml_Starvation	Cousina_umbrosa_0,02mg/ml_Starvation
EAGMA_0,01mg/ml	ERPS_0,02mg/ml	ESHA_0,05mg/ml_Starvation	Cousina_umbrosa_0,05mg/ml_Starvation
EAGMA_0,02mg/ml	ERPS_0,05mg/ml	ESHA_0,1mg/ml_Starvation	Nepeta_olgae_0,01mg/ml_Starvation
EAGMA_0,05mg/ml	EFIAAlop_0,01mg/ml_Starvation	U-2C_0,01mg/ml_Starvation	Nepeta_olgae_0,02mg/ml_Starvation
EAGMA_0,1mg/ml	EFIAAlop_0,02mg/ml_Starvation	U-2C_0,02mg/ml_Starvation	Nepeta_olgae_0,05mg/ml_Starvation
EAGMA_0,01mg/ml_Starvation	EFIAAlop_0,05mg/ml_Starvation	U-2C_0,05mg/ml_Starvation	Nepeta_olgae_0,1mg/ml_Starvation
EAGMA_0,02mg/ml_Starvation	EFIAAlop_0,1mg/ml_Starvation	U-2C_0,1mg/ml_Starvation	Scutellaria_scharistanica_0,01mg/ml_Starvation
EAGMA_0,05mg/ml_Starvation	ERHA_0,01mg/ml_Starvation	C-3B_0,01mg/ml_Starvation	Scutellaria_scharistanica_0,02mg/ml_Starvation
EAGMA_0,1mg/ml_Starvation	ERHA_0,02mg/ml_Starvation	C-4B_0,01mg/ml_Starvation	Scutellaria_scharistanica_0,05mg/ml_Starvation
EAGMD_0,01mg/ml	EAGAF_0,01mg/ml_Starvation	C-4B_0,02mg/ml_Starvation	Scutellaria_scharistanica_0,1mg/ml_Starvation
EAGMD_0,02mg/ml	EAGAF_0,02mg/ml_Starvation	T-1C_0,01mg/ml_Starvation	Schrophullaria_sp_0,01mg/ml_Starvation
EAGMD_0,05mg/ml	EAGAA_0,01mg/ml_Starvation	T-1C_0,02mg/ml_Starvation	Schrophullaria_sp_0,02mg/ml_Starvation
EAGMD_0,1mg/ml	EAGAA_0,02mg/ml_Starvation	T-1C_0,05mg/ml_Starvation	Leonurus_panzeroides_0,01mg/ml_Starvation
EAGMD_0,01mg/ml_Starvation	EAGAA_0,05mg/ml_Starvation	Verbascum_blattaria_0,01mg/ml_Starvation	Leonurus_panzeroides_0,02mg/ml_Starvation
EAGMD_0,02mg/ml_Starvation	EAGFO_0,01mg/ml_Starvation	Verbascum_blattaria_0,02mg/ml_Starvation	Leonurus_panzeroides_0,05mg/ml_Starvation
EAGMD_0,05mg/ml_Starvation			

Figure 31. Scatter-plot reporting the samples regarding 20 hour treatments considered as not toxic; tables in the lower side reports treatments contained in each obtained cluster.

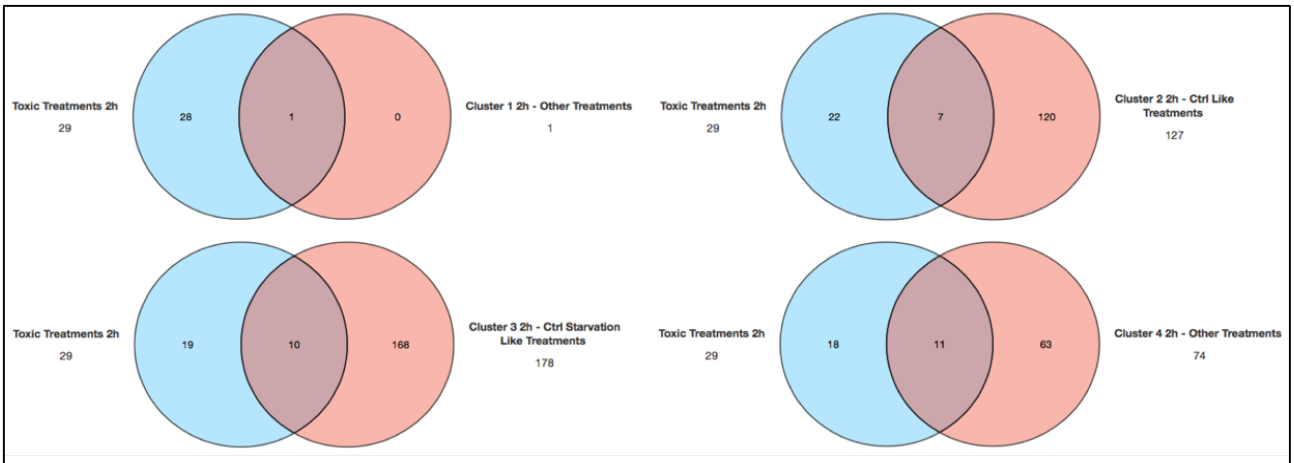
4.3.3 Evaluation of toxicity' affection and selection of the most interesting treatments

After clusters definition, further analyses have been carried out in order to select samples producing effects considered as interesting. The selection has been performed following three main rules: 1) samples in each cluster were divided in two sections named toxic and not toxic treatments (depending on the mortality percentage calculated with respect to the control samples selected by setting the 50% as thresholding percentage); 2) samples in clusters containing controls were selected as interesting when their effects has been produced in culturing conditions different from those of the specific control samples (treatments in normal conditions were considered as interesting when they were clustered with starvation controls while treatments in starvation conditions when they were grouped along with normal control samples); 3) samples in clusters not containing any control samples were selected as interesting.

Figure 32 and 33 report clusters resulting after the application of these rules for samples treated with extracts for 2 and 20 hours, respectively. In the upper side of these figures, four Venn diagrams composed by two circles have been reported: such diagrams combine the number of samples which produce a mortality rate greater than 50%, conventionally named "toxic extracts" (light blue circles), with the number of samples contained in each one of the four clusters (faint red circles), displaying the number of the toxic extracts per cluster in the overlapped circles area of each Venn diagram. The information about what is defined inside are specified next to every circle: the number of treatments considered as toxic, contained for each couple in the light blue circle, is specified at the left side with the writing "Toxic Treatments" for 2 and 20 hour analyses; the number of treatments contained in each cluster, reported in the faint red circle, is specified at the right side by mean a numerical code which identify the cluster and the further specification of the type of treatments contained inside, such as "Ctrl-Like Phenotypes", "Ctrl Starvation-Like Phenotypes" and "Other Phenotypes". Tables reported in the lower side of these figures contain the name of the samples belonging to each cluster ordered as presented in table 2. In each table the samples are divided in two groups, depending on the mortality percentage associated to each one: the extracts causing a percentage of living cells greater than 50% were reported on a white background in the upper side of each table, while samples considered toxic (percentage of detected living cells lower than 50%) are reported in the lower side of each table on a grey background. Moreover, a colour system has been used to distinguish samples considered interesting from the not interesting. Samples which clustered without any control samples in clusters named "Other Phenotypes" have been stained in blue; samples concerning treatments administrated in starvation conditions and clustered along with normal control samples treated and untreated with DMSO were grouped in clusters defined as "Control-Like Phenotypes" and colored in green; samples regarding treatments performed in normal conditions and clustered with starvation controls and starvation controls DMSO samples, grouped in clusters under the name "Control Starvation-Like Phenotypes", have been colored in red. The lists of the samples divided per cluster, along with the mortality rate produced and the associated factor scores, ordered as in table 2 and defined by a colours code, have been reported in table A5 and A6 in the section "supplementary data": the boundaries of each cluster have been delimited by thicker lines and the background stained with the same colour used to identify clusters after the hierarchical cluster analysis (figures 26, 27, 28, 29), while the samples considered as toxic have been written in white bold on a red background.

Results about the effects produced by extracts administrated for 2 hours and showed in figure 32, reveal that among the 380 administrated treatments, 29 samples have produced a toxicity rate greater than 50% and that 169 treatments show effects potentially interesting. More specifically, results of the analysis per clusters have revealed as following:

- Cluster 1 ("Other Phenotypes") contains just 1 treatment producing an effect different from both control type samples and resulted to be toxic;



Cluster2 – Ctrl-Like Phenotypes

Ctrl	100	EAGOT_0,01mg/ml	98
CtrlDMSO	107	EAGOT_0,02mg/ml	98
EAGRC_0,01mg/ml	102	EAGOT_0,05mg/ml	88
EAGRC_0,02mg/ml	90	EAGOT_0,1mg/ml	89
EAGPS_0,05mg/ml	90	ERKL_0,01mg/ml	98
EAGPS_0,1mg/ml	84	ERKS_0,01mg/ml	96
EAGPS_0,1mg/ml_Starvation	66	ERKS_0,02mg/ml	87
EFIAAlop_0,01mg/ml	102	ERKS_0,05mg/ml	77
EFIAAlop_0,02mg/ml	86	ERKS_0,1mg/ml	81
EFIAAlop_0,05mg/ml	85	ESHA_0,01mg/ml	98
EFIAAlop_0,1mg/ml	79	ESHA_0,02mg/ml	83
ERHA_0,01mg/ml	113	ESHA_0,05mg/ml	92
ERHA_0,02mg/ml	115	ESHA_0,1mg/ml	78
ERHA_0,05mg/ml	80	U-2C_0,01mg/ml	78
ERHA_0,1mg/ml	83	U-2C_0,02mg/ml	87
EAGAF_0,01mg/ml	61	U-2C_0,05mg/ml	90
EAGAF_0,02mg/ml	114	U-2C_0,1mg/ml	85
EAGAF_0,05mg/ml	87	C-3B_0,01mg/ml	63
EAGAF_0,1mg/ml	67	C-3B_0,02mg/ml	65
EAGAA_0,01mg/ml	105	C-3B_0,05mg/ml	78
EAGAA_0,02mg/ml	96	C-3B_0,1mg/ml	72
EAGAA_0,05mg/ml	76	C-3B_0,01mg/ml_Starvation	57
EAGAA_0,1mg/ml	85	C-3B_0,02mg/ml_Starvation	82
EAGFO_0,01mg/ml	102	C-3B_0,1mg/ml_Starvation	71
EAGFO_0,02mg/ml	71	C-4B_0,01mg/ml	59
EAGFO_0,05mg/ml	67	C-4B_0,02mg/ml	63
EAGFO_0,1mg/ml	88	C-4B_0,05mg/ml	73
EAGPD_0,01mg/ml	108	C-4B_0,02mg/ml_Starvation	68
EAGPD_0,02mg/ml	108	C-4B_0,05mg/ml_Starvation	71
EAGPD_0,05mg/ml	71	T-1B_0,01mg/ml	67
EAGPD_0,1mg/ml	97	T-1B_0,02mg/ml	67
EAGTM_0,01mg/ml	71	T-1B_0,05mg/ml	79
EAGTM_0,02mg/ml	105	T-1B_0,1mg/ml	51
EAGTM_0,05mg/ml	94	T-1C_0,01mg/ml	69
EAGTM_0,1mg/ml	62	T-1C_0,05mg/ml	88
EAGC_0,01mg/ml	94	T-1C_0,1mg/ml	64
EAGC_0,02mg/ml	96	Verbascum_blattaria_0,01mg/ml	81
EAGC_0,05mg/ml	108	Verbascum_blattaria_0,02mg/ml	97
EAGC_0,1mg/ml	91	Verbascum_blattaria_0,05mg/ml	96
EFHA_0,01mg/ml	95	Verbascum_blattaria_0,1mg/ml	70
EFHA_0,02mg/ml	83	Stachys_hissarica_0,01mg/ml	91
EFHA_0,05mg/ml	83	Stachys_hissarica_0,02mg/ml	85
EFHA_0,1mg/ml	95	Stachys_hissarica_0,05mg/ml	51
EAGSS_0,01mg/ml	113	Stachys_hissarica_0,1mg/ml	91
EAGSS_0,02mg/ml	87	Verbascum_songoricum_0,01mg/ml	84
EAGSS_0,05mg/ml	73	Verbascum_songoricum_0,02mg/ml	105
EAGSS_0,1mg/ml	109	Verbascum_songoricum_0,05mg/ml	60
EFIPS_0,01mg/ml	70	Verbascum_songoricum_0,1mg/ml	85
EFIPS_0,02mg/ml	80	Stachys_betonicifiora_0,01mg/ml	82
EFIPS_0,05mg/ml	75	Stachys_betonicifiora_0,02mg/ml	88
EFIPS_0,1mg/ml	50	Stachys_betonicifiora_0,05mg/ml	78
EFIPS_0,1mg/ml_Starvation	59	Stachys_betonicifiora_0,1mg/ml	79
EAGCB_0,02mg/ml	59	Stachys_betonicifiora_0,02mg/ml_Starvation	104
EAGCB_0,05mg/ml	69	Phlomis_salicifolia_0,1mg/ml_Starvation	55
EAGCB_0,1mg/ml	59	Phlomis_tadschikistanica_0,1mg/ml	108
EAGCB_0,05mg/ml_Starvation	68	Cousina_umbrosa_0,01mg/ml	61
EAGCT_0,01mg/ml	104	Cousina_umbrosa_0,05mg/ml	56
EAGCT_0,02mg/ml	82	Cousina_umbrosa_0,01mg/ml_Starvation	91
EAGCT_0,05mg/ml	88	Cousina_umbrosa_0,02mg/ml_Starvation	111
EAGCT_0,1mg/ml	72	Cousina_umbrosa_0,05mg/ml_Starvation	77
ERPS_0,1mg/ml_Starvation	5	C-4B_0,1mg/ml	45
EAGFO_0,05mg/ml_Starvation	24	C-4B_0,1mg/ml_Starvation	37
EAGFO_0,1mg/ml_Starvation	3	Phlomis_sewertzovii_0,1mg/ml	43
EAGCB_0,01mg/ml	49		

Cluster1 – Other Phenotypes

Schrophullaria_sp_0,1mg/ml_Starvation	2

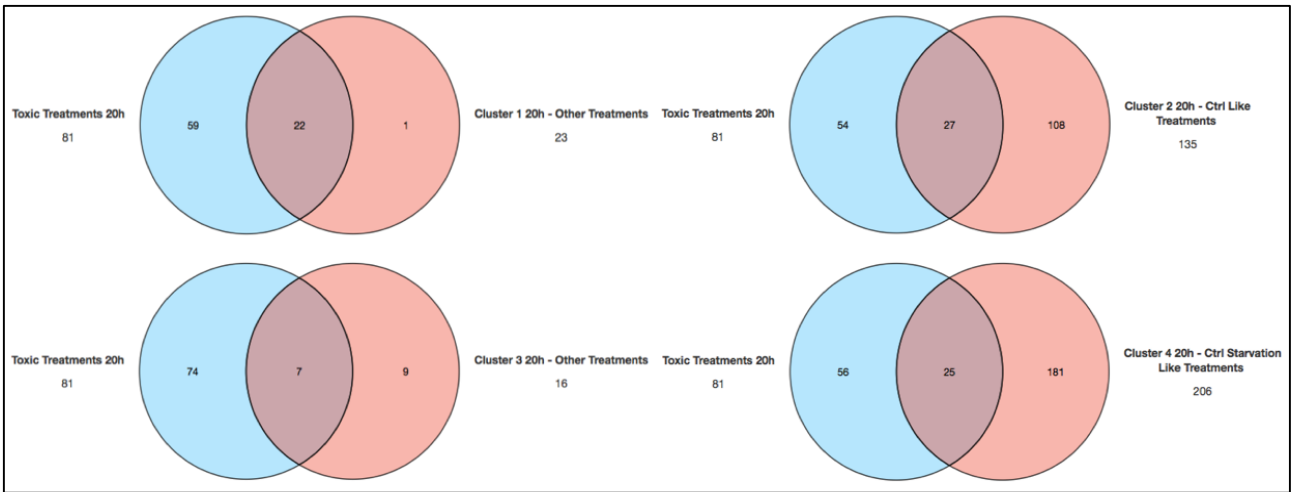
Cluster3 – Ctrl Starvation-Like Phenotypes

Ctrl_Starvation	100	ERPS_0,05mg/ml	60	ERKS_0,1mg/ml_Starvation	79
CtrlDMSO_Starvation	87	EFIAAlop_0,01mg/ml_Starvation	95	ESHA_0,01mg/ml_Starvation	91
EAGRC_0,05mg/ml	69	EFIAAlop_0,02mg/ml_Starvation	97	ESHA_0,02mg/ml_Starvation	84
EAGRC_0,1mg/ml	88	EFIAAlop_0,05mg/ml_Starvation	87	ESHA_0,05mg/ml_Starvation	85
EAGRC_0,02mg/ml_Starvation	106	EFIAAlop_0,1mg/ml_Starvation	123	ESHA_0,1mg/ml_Starvation	93
EAGRC_0,05mg/ml_Starvation	94	ERHA_0,01mg/ml_Starvation	144	U-2C_0,01mg/ml_Starvation	108
EAGRC_0,1mg/ml_Starvation	94	ERHA_0,05mg/ml_Starvation	138	U-2C_0,02mg/ml_Starvation	94
ERAAlop_0,01mg/ml	88	ERHA_0,1mg/ml_Starvation	72	U-2C_0,05mg/ml_Starvation	97
ERAAlop_0,02mg/ml	90	EAGAF_0,01mg/ml_Starvation	95	U-2C_0,1mg/ml_Starvation	94
ERAAlop_0,05mg/ml	75	EAGAF_0,02mg/ml_Starvation	65	C-3B_0,05mg/ml_Starvation	76
ERAAlop_0,1mg/ml	65	EAGAF_0,05mg/ml_Starvation	108	C-4B_0,01mg/ml_Starvation	71
EAGPS_0,01mg/ml	98	EAGAF_0,1mg/ml_Starvation	60	T-1B_0,01mg/ml_Starvation	70
EAGPS_0,02mg/ml	95	EAGAA_0,01mg/ml_Starvation	77	T-1B_0,02mg/ml_Starvation	65
EAGPS_0,01mg/ml_Starvation	100	EAGAA_0,02mg/ml_Starvation	101	T-1B_0,05mg/ml_Starvation	59
EAGPS_0,02mg/ml_Starvation	100	EAGAA_0,1mg/ml_Starvation	83	T-1C_0,02mg/ml	63
EAGPS_0,05mg/ml_Starvation	55	EAGFO_0,01mg/ml_Starvation	54	T-1C_0,01mg/ml_Starvation	85
EAGAAsia_0,01mg/ml	83	EAGFO_0,02mg/ml_Starvation	86	T-1C_0,02mg/ml_Starvation	82
EAGAAsia_0,02mg/ml	78	EAGPD_0,01mg/ml_Starvation	135	T-1C_0,05mg/ml_Starvation	86
EAGAAsia_0,05mg/ml	72	EAGPD_0,02mg/ml_Starvation	84	T-1C_0,1mg/ml_Starvation	91
EAGAAsia_0,1mg/ml	72	EAGPD_0,05mg/ml_Starvation	111	Verbascum_blattaria_0,01mg/ml_Starvation	109
EAGAAsia_0,01mg/ml_Starvation	61	EAGPD_0,1mg/ml_Starvation	100	Stachys_hissarica_0,01mg/ml_Starvation	106
EAGAAlop_0,01mg/ml	101	EAGTM_0,01mg/ml_Starvation	130	Stachys_hissarica_0,02mg/ml_Starvation	124
EAGAAlop_0,02mg/ml	77	EAGTM_0,02mg/ml_Starvation	62	Stachys_betonicifiora_0,01mg/ml_Starvation	142
EAGAAlop_0,05mg/ml	80	EAGTM_0,05mg/ml_Starvation	137	Stachys_betonicifiora_0,1mg/ml_Starvation	107
EAGAAlop_0,1mg/ml	79	EAGTM_0,1mg/ml_Starvation	110	Phlomis_sewertzovii_0,01mg/ml	71
EAGAAlop_0,02mg/ml_Starvation	85	EAGC_0,01mg/ml_Starvation	95	Phlomis_sewertzovii_0,02mg/ml	63
EAGAAlop_0,05mg/ml_Starvation	66	EAGC_0,02mg/ml_Starvation	52	Phlomis_sewertzovii_0,05mg/ml	76
EAGAAlop_0,1mg/ml_Starvation	83	EAGC_0,05mg/ml_Starvation	69	Phlomis_salicifolia_0,01mg/ml	78
EAGMA_0,01mg/ml	109	EAGC_0,1mg/ml_Starvation	79	Phlomis_salicifolia_0,02mg/ml	70
EAGMA_0,02mg/ml	83	EFHA_0,01mg/ml_Starvation	100	Phlomis_salicifolia_0,05mg/ml	83
EAGMA_0,05mg/ml	74	EFHA_0,02mg/ml_Starvation	68	Phlomis_salicifolia_0,1mg/ml	55
EAGMA_0,1mg/ml	73	EFHA_0,05mg/ml_Starvation	100	Phlomis_salicifolia_0,02mg/ml_Starvation	139
EAGMD_0,01mg/ml	96	EAGSS_0,01mg/ml_Starvation	76	Silene_oreina_0,01mg/ml	54
EAGMD_0,02mg/ml	84	EAGSS_0,02mg/ml_Starvation	98	Silene_oreina_0,02mg/ml	74
EAGMD_0,05mg/ml	81	EAGSS_0,05mg/ml_Starvation	99	Silene_oreina_0,05mg/ml	56
EAGMD_0,1mg/ml	83	EAGSS_0,1mg/ml_Starvation	107	Silene_oreina_0,1mg/ml	88
EAGKL_0,01mg/ml	76	EFIPS_0,01mg/ml_Starvation	119	Phlomis_tadschikistanica_0,01mg/ml	90
EAGKL_0,02mg/ml	79	EFIPS_0,05mg/ml_Starvation	88	Phlomis_tadschikistanica_0,02mg/ml	97
EAGKL_0,05mg/ml	92	EAGCB_0,01mg/ml_Starvation	84	Phlomis_tadschikistanica_0,05mg/ml	63
EAGKL_0,1mg/ml	63	EAGCB_0,1mg/ml_Starvation	56	Cousina_umbrosa_0,02mg/ml	53
EAGAS_0,01mg/ml	99	EAGCT_0,01mg/ml_Starvation	91	Cousina_umbrosa_0,1mg/ml	62
EAGAS_0,02mg/ml	79	EAGCT_0,02mg/ml_Starvation	92	Nepeta_olgae_0,01mg/ml	51
EAGAS_0,05mg/ml	76	EAGCT_0,05mg/ml_Starvation	72	Nepeta_olgae_0,02mg/ml	65
EAGAS_0,1mg/ml	83	EAGCT_0,1mg/ml_Starvation	64	Nepeta_olgae_0,05mg/ml	115
EAGAS_0,05mg/ml_Starvation	79	EAGOT_0,01mg/ml_Starvation	102	Nepeta_olgae_0,1mg/ml	74
EAGAS_0,1mg/ml_Starvation	73	EAGOT_0,02mg/ml_Starvation	103	Scutellaria_scharistanica_0,01mg/ml	57
EAGAT_0,02mg/ml_Starvation	73	EAGOT_0,05mg/ml_Starvation	97	Scutellaria_scharistanica_0,02mg/ml	53
EAGAT_0,05mg/ml_Starvation	62	EAGOT_0,1mg/ml_Starvation	100	Scutellaria_scharistanica_0,1mg/ml	50
EAGAT_0,1mg/ml_Starvation	69	ERKL_0,02mg/ml	88	Schrophullaria_sp_0,01mg/ml	63
EAGAU_0,02mg/ml	81	ERKL_0,05mg/ml	87	Schrophullaria_sp_0,02mg/ml	57
EAGAU_0,01mg/ml_Starvation	93	ERKL_0,1mg/ml	82	Schrophullaria_sp_0,05mg/ml	73
EAGAU_0,02mg/ml_Starvation	73	ERKL_0,01mg/ml_Starvation	100	Schrophullaria_sp_0,1mg/ml	92
EAGAU_0,05mg/ml_Starvation	78	ERKL_0,02mg/ml_Starvation	98	Leonurus_panzeroides_0,01mg/ml	59
EAGAU_0,1mg/ml_Starvation	82	ERKS_0,01mg/ml_Starvation	78	Leonurus_panzeroides_0,02mg/ml	87
ERPS_0,01mg/ml	72	ERKS_0,02mg/ml_Starvation	84	Leonurus_panzeroides_0,1mg/ml	85
ERPS_0,02mg/ml	69	ERKS_0,05mg/ml_Starvation	75	Leonurus_panzeroides_0,1mg/ml_Starvation	93
EAGAAsia_0,1mg/ml_Starvation	42	EFHA_0,1mg/ml_Starvation	35	T-1B_0,1mg/ml_Starvation	26
ERPS_0,1mg/ml	48	EFIPS_0,02mg/ml_Starvation	40	Scutellaria_scharistanica_0,05mg/ml	48
ERHA_0,02mg/ml_Starvation	47	EAGCB_0,02mg/ml_Starvation	41	Leonurus_panzeroides_0,05mg/ml	49
EAGAA_0,05mg/ml_Starvation	36				

Cluster4 – Other Phenotypes

EAGRC_0,01mg/ml_Starvation	99	EAGKL_0,05mg/ml_Starvation	83	Verbascum_songoricum_0,01mg/ml_Starvation	107	Phlomis_tadschikistanica_0,1mg/ml_Starvation	85
ERAAlop_0,01mg/ml_Starvation	60	EAGKL_0,1mg/ml_Starvation	74	Verbascum_songoricum_0,02mg/ml_Starvation	103	Cousina_umbrosa_0,1mg/ml_Starvation	120
ERAAlop_0,02mg/ml_Starvation	67	EAGAS_0,01mg/ml_Starvation	113	Verbascum_songoricum_0,05mg/ml_Starvation	59	Nepeta_olgae_0,01mg/ml_Starvation	141
ERAAlop_0,05mg/ml_Starvation	67	EAGAS_0,02mg/ml_Starvation	77	Phlomis_sewertzovii_0,01mg/ml_Starvation	132	Nepeta_olgae_0,02mg/ml_Starvation	130
ERAAlop_0,1mg/ml_Starvation	51	EAGAT_0,01mg/ml	91	Phlomis_sewertzovii_0,02mg/ml_Starvation	120	Nepeta_olgae_0,05mg/ml_Starvation	94
EAGAAsia_0,02mg/ml_Starvation	64	EAGAT_0,02mg/ml	74	Phlomis_sewertzovii_0,05mg/ml_Starvation	91	Nepeta_olgae_0,1mg/ml_Starvation	95
EAGAAlop_0,01mg/ml_Starvation	83	EAGAT_0,05mg/ml	60	Phlomis_sewertzovii_0,1mg/ml_Starvation	75	Scutellaria_scharistanica_0,01mg/ml_Starvation	91
EAGMA_0,02mg/ml_Starvation	68	EAGAT_0,1mg/ml	78	Phlomis_salicifolia_0,01mg/ml_Starvation	114	Scutellaria_scharistanica_0,02mg/ml_Starvation	102
EAGMA_0,05mg/ml_Starvation	57	EAGAU_0,01mg/ml	85	Phlomis_salicifolia_0,05mg/ml_Starvation	59	Scutellaria_scharistanica_0,05mg/ml_Starvation	105
EAGMA_0,1mg/ml_Starvation	58	EAGAU_0,05mg/ml	77	Silene_oreina_0,01mg/ml_Starvation	123	Scutellaria_scharistanica_0,1mg/ml_Starvation	64
EAGMD_0,01mg/ml_Starvation	94	EAGAU_0,1mg/ml	70	Silene_oreina_0,02mg/ml_Starvation	113	Schrophullaria_sp_0,01mg/ml_Starvation	97
EAGMD_0,02mg/ml_Starvation	109	ERPS_0,02mg/ml_Starvation	53	Silene_oreina_0,05mg/ml_Starvation	95	Schrophullaria_sp_0,02mg/ml_Starvation	115
EAGMD_0,05mg/ml_Starvation	77	ERKL_0,05mg/ml_Starvation	96	Silene_oreina_0,1mg/ml_Starvation	84	Leonurus_panzeroides_0,01mg/ml_Starvation	128
EAGMD_0,1mg/ml_Starvation	74	Verbascum_blattaria_0,02mg/ml_Starvation	102	Phlomis_tadschikistanica_0,01mg/ml_Starvation	128	Leonurus_panzeroides_0,02mg/ml_Starvation	112
EAGKL_0,01mg/ml_Starvation	51	Verbascum_blattaria_0,05mg/ml_Starvation	65	Phlomis_tadschikistanica_0,02mg/ml_Starvation	103	Leonurus_panzeroides_0,05mg/ml_Starvation	152
EAGKL_0,02mg/ml_Starvation	79	Stachys_hissarica_0,05mg/ml_Starvation	82	Phlomis_tadschikistanica_0,05mg/ml_Starvation	120		
EAGAAsia_0,05mg/ml_Starvation	45	ERPS_0,01mg/ml_Starvation	43	Verbascum_blattaria_0,1mg/ml_Starvation	43	Stachys_betonicifiora_0,05mg/ml_Starvation	46
EAGMA_0,01mg/ml_Starvation	36	ERPS_0,05mg/ml_Starvation	30	Stachys_hissarica_0,1mg/ml_Starvation	24	Schrophullaria_sp_0,05mg/ml_Starvation	24
EAGAT_0,01mg/ml_Starvation	32	ERKL_0,1mg/ml_Starvation	47	Verbascum_songoricum_0,1mg/ml_Starvation	42		

Figure 32. The legend is reported in the following pages.



Cluster1 – Other Treatments

ERPS_0,01mg/ml_Starvation	58
ERPS_0,1mg/ml	5
ERPS_0,02mg/ml_Starvation	18
ERPS_0,05mg/ml_Starvation	16
ERPS_0,1mg/ml_Starvation	13
EAGFO_0,1mg/ml	3
EAGFO_0,05mg/ml_Starvation	4
EAGFO_0,1mg/ml_Starvation	5
EAGPD_0,1mg/ml_Starvation	6
EFHA_0,1mg/ml_Starvation	7
EFIPS_0,1mg/ml_Starvation	6
ERKL_0,1mg/ml	2
ERKL_0,05mg/ml_Starvation	3
ERKL_0,1mg/ml_Starvation	2
C-4B_0,1mg/ml_Starvation	1
T-1B_0,05mg/ml	2
T-1B_0,1mg/ml	3
T-1B_0,02mg/ml_Starvation	6
T-1B_0,05mg/ml_Starvation	3
T-1B_0,1mg/ml_Starvation	2
Verbascum_blattaria_0,1mg/ml_Starvation	23
Schrophullaria_sp_0,05mg/ml_Starvation	3
Schrophullaria_sp_0,1mg/ml_Starvation	3

Cluster2 – Ctrl-Like Treatments

Ctrl	100	EAGCT_0,1mg/ml	83	Stachys_hissarica_0,05mg/ml	73
CtrlDMSO	93	EAGCT_0,02mg/ml_Starvation	82	Verbascum_songoricum_0,01mg/ml	91
EFIAAlop_0,01mg/ml	91	EAGCT_0,05mg/ml_Starvation	55	Verbascum_songoricum_0,02mg/ml	88
EFIAAlop_0,02mg/ml	76	EAGOT_0,01mg/ml	92	Verbascum_songoricum_0,05mg/ml	84
EFIAAlop_0,05mg/ml	93	EAGOT_0,02mg/ml	99	Stachys_betonicifiora_0,01mg/ml	93
EFIAAlop_0,1mg/ml	60	EAGOT_0,05mg/ml	101	Stachys_betonicifiora_0,02mg/ml	104
ERHA_0,01mg/ml	75	EAGOT_0,1mg/ml	89	Stachys_betonicifiora_0,05mg/ml	90
ERHA_0,02mg/ml	53	ERKS_0,01mg/ml	90	Stachys_betonicifiora_0,1mg/ml	111
ERHA_0,1mg/ml	53	ERKS_0,02mg/ml	91	Stachys_betonicifiora_0,1mg/ml_Starvation	56
EAGAF_0,01mg/ml	122	ERKS_0,05mg/ml	86	Phlomis_sewertzovii_0,01mg/ml	113
EAGAF_0,02mg/ml	92	ESHA_0,01mg/ml	96	Phlomis_sewertzovii_0,02mg/ml	102
EAGAF_0,05mg/ml	82	ESHA_0,02mg/ml	88	Phlomis_sewertzovii_0,05mg/ml	95
EAGAF_0,1mg/ml	113	ESHA_0,05mg/ml	94	Phlomis_sewertzovii_0,1mg/ml	87
EAGAA_0,01mg/ml	66	ESHA_0,1mg/ml	89	Phlomis_salicifolia_0,01mg/ml	94
EAGAA_0,05mg/ml	57	U-2C_0,01mg/ml	97	Phlomis_salicifolia_0,02mg/ml	99
EAGAA_0,1mg/ml	76	U-2C_0,02mg/ml	82	Phlomis_salicifolia_0,05mg/ml	96
EAGFO_0,01mg/ml	88	U-2C_0,05mg/ml	77	Phlomis_salicifolia_0,1mg/ml	67
EAGFO_0,02mg/ml	50	U-2C_0,1mg/ml	87	Phlomis_tadschikistanica_0,01mg/ml	90
EAGPD_0,02mg/ml	70	C-3B_0,01mg/ml	82	Phlomis_tadschikistanica_0,02mg/ml	76
EAGTM_0,01mg/ml	84	C-3B_0,02mg/ml	79	Phlomis_tadschikistanica_0,05mg/ml	70
EAGTM_0,02mg/ml	67	C-3B_0,05mg/ml	66	Phlomis_tadschikistanica_0,1mg/ml	57
EAGTM_0,05mg/ml	60	C-3B_0,1mg/ml	66	Cousina_umbrosa_0,01mg/ml	62
EAGTM_0,1mg/ml	58	C-3B_0,02mg/ml_Starvation	70	Cousina_umbrosa_0,02mg/ml	50
EFHA_0,01mg/ml	75	C-3B_0,05mg/ml_Starvation	57	Cousina_umbrosa_0,1mg/ml	51
EFHA_0,02mg/ml	61	C-4B_0,01mg/ml	88	Nepeta_olgae_0,01mg/ml	84
EFHA_0,05mg/ml	95	C-4B_0,02mg/ml	80	Nepeta_olgae_0,02mg/ml	78
EFHA_0,1mg/ml	65	T-1B_0,01mg/ml	58	Nepeta_olgae_0,05mg/ml	64
EAGSS_0,01mg/ml	68	T-1C_0,01mg/ml	77	Nepeta_olgae_0,1mg/ml	82
EAGSS_0,05mg/ml	59	T-1C_0,02mg/ml	82	Scutellaria_scharistanica_0,01mg/ml	59
EFIPS_0,01mg/ml	87	T-1C_0,05mg/ml	70	Scutellaria_scharistanica_0,05mg/ml	59
EFIPS_0,05mg/ml	70	Verbascum_blattaria_0,01mg/ml	94	Schrophullaria_sp_0,01mg/ml	54
EAGCB_0,01mg/ml	80	Verbascum_blattaria_0,02mg/ml	94	Schrophullaria_sp_0,02mg/ml	64
EAGCB_0,1mg/ml	65	Verbascum_blattaria_0,05mg/ml	81	Schrophullaria_sp_0,05mg/ml	69
EAGCT_0,01mg/ml	90	Verbascum_blattaria_0,1mg/ml	56	Schrophullaria_sp_0,1mg/ml	61
EAGCT_0,02mg/ml	95	Stachys_hissarica_0,01mg/ml	112	Leonurus_panzeroides_0,01mg/ml	52
EAGCT_0,05mg/ml	84	Stachys_hissarica_0,02mg/ml	82	Leonurus_panzeroides_0,05mg/ml	72
ERHA_0,05mg/ml	48	EAGC_0,05mg/ml	33	C-4B_0,05mg/ml_Starvation	15
EAGAA_0,02mg/ml	48	EAGSS_0,02mg/ml	39	T-1B_0,02mg/ml	26
EAGAA_0,1mg/ml_Starvation	29	EFIPS_0,02mg/ml	47	Stachys_hissarica_0,1mg/ml	45
EAGFO_0,05mg/ml	40	EFIPS_0,1mg/ml	27	Verbascum_songoricum_0,1mg/ml	39
EAGFO_0,02mg/ml_Starvation	14	EAGCB_0,02mg/ml	45	Cousina_umbrosa_0,05mg/ml	36
EAGPD_0,05mg/ml	40	EAGCT_0,1mg/ml_Starvation	49	Scutellaria_scharistanica_0,02mg/ml	41
EAGPD_0,1mg/ml	22	C-3B_0,1mg/ml_Starvation	34	Scutellaria_scharistanica_0,1mg/ml	29
EAGC_0,01mg/ml	43	C-4B_0,05mg/ml	49	Leonurus_panzeroides_0,02mg/ml	49
EAGC_0,02mg/ml	26	C-4B_0,1mg/ml	14	Leonurus_panzeroides_0,1mg/ml	46

Cluster3 – Other Treatments

EAGPS_0,1mg/ml	88
EAGPS_0,01mg/ml_Starvation	180
EAGPS_0,02mg/ml_Starvation	122
EAGPS_0,05mg/ml_Starvation	127
EAGPS_0,1mg/ml_Starvation	72
EAGAAlop_0,1mg/ml_Starvation	51
EAGKL_0,05mg/ml_Starvation	74
EAGPD_0,01mg/ml_Starvation	68
ERKS_0,02mg/ml_Starvation	103
EAGRC_0,1mg/ml_Starvation	28
EAGAAsia_0,02mg/ml_Starvation	49
EAGAAsia_0,05mg/ml_Starvation	23
EAGAAsia_0,1mg/ml_Starvation	40
EAGKL_0,1mg/ml_Starvation	31
Verbascum_songoricum_0,1mg/ml_Starvation	15
Silene_oreina_0,1mg/ml_Starvation	49

Cluster4 – Ctrl Starvation-Like Treatments

Ctrl_Starvation	100	EAGMA_0,05mg/ml_Starvation	74	EAGAU_0,02mg/ml_Starvation	72	EAGOT_0,05mg/ml_Starvation	93	Stachys_betonicifiora_0,02mg/ml_Starvation	133
CtrlDMSO_Starvation	97	EAGMA_0,1mg/ml_Starvation	84	EAGAU_0,05mg/ml_Starvation	104	EAGOT_0,1mg/ml_Starvation	103	Stachys_betonicifiora_0,05mg/ml_Starvation	165
EAGRC_0,01mg/ml	104	EAGMD_0,01mg/ml	84	EAGAU_0,1mg/ml_Starvation	72	ERKL_0,01mg/ml	95	Phlomis_sewertzovii_0,01mg/ml_Starvation	92
EAGRC_0,02mg/ml	98	EAGMD_0,02mg/ml	81	ERPS_0,01mg/ml	91	ERKL_0,02mg/ml	94	Phlomis_sewertzovii_0,02mg/ml_Starvation	126
EAGRC_0,05mg/ml	106	EAGMD_0,05mg/ml	77	ERPS_0,02mg/ml	70	ERKL_0,05mg/ml	53	Phlomis_sewertzovii_0,05mg/ml_Starvation	117
EAGRC_0,1mg/ml	96	EAGMD_0,1mg/ml	67	ERPS_0,05mg/ml	78	ERKL_0,01mg/ml_Starvation	84	Phlomis_sewertzovii_0,1mg/ml_Starvation	50
EAGRC_0,01mg/ml_Starvation	134	EAGMD_0,01mg/ml_Starvation	95	EFIAAlop_0,01mg/ml_Starvation	86	ERKL_0,02mg/ml_Starvation	79	Phlomis_salicifolia_0,01mg/ml_Starvation	111
EAGRC_0,02mg/ml_Starvation	123	EAGMD_0,02mg/ml_Starvation	107	EFIAAlop_0,02mg/ml_Starvation	75	ERKS_0,1mg/ml	84	Phlomis_salicifolia_0,02mg/ml_Starvation	115
EAGRC_0,05mg/ml_Starvation	119	EAGMD_0,05mg/ml_Starvation	75	EFIAAlop_0,05mg/ml_Starvation	69	ERKS_0,01mg/ml_Starvation	108	Phlomis_salicifolia_0,05mg/ml_Starvation	122
ERAAlop_0,01mg/ml	85	EAGKL_0,01mg/ml	86	EFIAAlop_0,1mg/ml_Starvation	117	ERKS_0,05mg/ml_Starvation	95	Phlomis_salicifolia_0,1mg/ml_Starvation	95
ERAAlop_0,02mg/ml	73	EAGKL_0,02mg/ml	80	ERHA_0,01mg/ml_Starvation	57	ERKS_0,1mg/ml_Starvation	85	Silene_oreina_0,01mg/ml	72
ERAAlop_0,05mg/ml	62	EAGKL_0,05mg/ml	74	ERHA_0,02mg/ml_Starvation	59	ESHA_0,01mg/ml_Starvation	99	Silene_oreina_0,02mg/ml	79
ERAAlop_0,01mg/ml_Starvation	83	EAGKL_0,1mg/ml	78	EAGAF_0,01mg/ml_Starvation	83	ESHA_0,02mg/ml_Starvation	95	Silene_oreina_0,05mg/ml	76
ERAAlop_0,02mg/ml_Starvation	100	EAGKL_0,01mg/ml_Starvation	60	EAGAF_0,02mg/ml_Starvation	111	ESHA_0,05mg/ml_Starvation	87	Silene_oreina_0,1mg/ml	61
ERAAlop_0,05mg/ml_Starvation	62	EAGKL_0,02mg/ml_Starvation	103	EAGAA_0,01mg/ml_Starvation	80	ESHA_0,1mg/ml_Starvation	80	Silene_oreina_0,01mg/ml_Starvation	101
ERAAlop_0,1mg/ml_Starvation	68	EAGAS_0,01mg/ml	92	EAGAA_0,02mg/ml_Starvation	58	U-2C_0,01mg/ml_Starvation	90	Silene_oreina_0,02mg/ml_Starvation	118
EAGPS_0,01mg/ml	104	EAGAS_0,02mg/ml	85	EAGAA_0,05mg/ml_Starvation	59	U-2C_0,02mg/ml_Starvation	80	Silene_oreina_0,05mg/ml_Starvation	78
EAGPS_0,02mg/ml	91	EAGAS_0,05mg/ml	88	EAGFO_0,01mg/ml_Starvation	58	U-2C_0,05mg/ml_Starvation	90	Phlomis_tadschikistanica_0,01mg/ml_Starvation	100
EAGPS_0,05mg/ml	84	EAGAS_0,1mg/ml	72	EAGPD_0,01mg/ml	70	U-2C_0,1mg/ml_Starvation	70	Phlomis_tadschikistanica_0,02mg/ml_Starvation	116
EAGAAsia_0,01mg/ml	75	EAGAS_0,01mg/ml_Starvation	110	EAGPD_0,02mg/ml_Starvation	62	C-3B_0,01mg/ml_Starvation	82	Phlomis_tadschikistanica_0,05mg/ml_Starvation	109
EAGAAsia_0,02mg/ml	93	EAGAS_0,02mg/ml_Starvation	121	EAGTM_0,02mg/ml_Starvation	105	C-4B_0,01mg/ml_Starvation	71	Phlomis_tadschikistanica_0,1mg/ml_Starvation	76
EAGAAsia_0,05mg/ml	69	EAGAS_0,05mg/ml_Starvation	113	EAGTM_0,05mg/ml_Starvation	70	C-4B_0,02mg/ml_Starvation	64	Cousina_umbrosa_0,02mg/ml_Starvation	62
EAGAAsia_0,1mg/ml	58	EAGAS_0,1mg/ml_Starvation	212	EFHA_0,01mg/ml_Starvation	90	T-1C_0,01mg/ml_Starvation	98	Cousina_umbrosa_0,05mg/ml_Starvation	58
EAGAAsia_0,01mg/ml_Starvation	59	EAGAT_0,01mg/ml	81	EFHA_0,02mg/ml_Starvation	106	T-1C_0,02mg/ml_Starvation	81	Nepeta_olgae_0,01mg/ml_Starvation	103
EAGAAlop_0,01mg/ml	105	EAGAT_0,02mg/ml	87	EFHA_0,05mg/ml_Starvation	103	T-1C_0,05mg/ml_Starvation	84	Nepeta_olgae_0,02mg/ml_Starvation	110
EAGAAlop_0,02mg/ml	88	EAGAT_0,05mg/ml	80	EAGSS_0,01mg/ml_Starvation	82	Verbascum_blattaria_0,01mg/ml_Starvation	112	Nepeta_olgae_0,05mg/ml_Starvation	97
EAGAAlop_0,05mg/ml	98	EAGAT_0,1mg/ml	91	EAGSS_0,05mg/ml_Starvation	66	Verbascum_blattaria_0,02mg/ml_Starvation	103	Nepeta_olgae_0,1mg/ml_Starvation	81
EAGAAlop_0,1mg/ml	109	EAGAT_0,01mg/ml_Starvation	81	EAGSS_0,1mg/ml_Starvation	61	Verbascum_blattaria_0,05mg/ml_Starvation	82	Scutellaria_scharistanica_0,01mg/ml_Starvation	107
EAGAAlop_0,01mg/ml_Starvation	133	EAGAT_0,02mg/ml_Starvation	107	EFIPS_0,01mg/ml_Starvation	79	Stachys_hissarica_0,01mg/ml_Starvation	106	Scutellaria_scharistanica_0,02mg/ml_Starvation	82
EAGAAlop_0,02mg/ml_Starvation	222	EAGAT_0,05mg/ml_Starvation	81	EFIPS_0,02mg/ml_Starvation	93	Stachys_hissarica_0,02mg/ml_Starvation	84	Scutellaria_scharistanica_0,05mg/ml_Starvation	63
EAGAAlop_0,05mg/ml_Starvation	88	EAGAT_0,1mg/ml_Starvation	113	EAGCB_0,05mg/ml	50	Stachys_hissarica_0,05mg/ml_Starvation	91	Scutellaria_scharistanica_0,1mg/ml_Starvation	98
EAGMA_0,01mg/ml	62	EAGAU_0,01mg/ml	88	EAGCB_0,02mg/ml_Starvation	57	Stachys_hissarica_0,1mg/ml_Starvation	60	Schrophullaria_sp_0,01mg/ml_Starvation	103
EAGMA_0,02mg/ml	68	EAGAU_0,02mg/ml	83	EAGCB_0,1mg/ml_Starvation	52	Verbascum_songoricum_0,01mg/ml_Starvation	154	Schrophullaria_sp_0,02mg/ml_Starvation	70
EAGMA_0,05mg/ml	84	EAGAU_0,05mg/ml	118	EAGCT_0,01mg/ml_Starvation	74	Verbascum_songoricum_0,02mg/ml_Starvation	103	Leonurus_panzeroides_0,01mg/ml_Starvation	90
EAGMA_0,1mg/ml	81	EAGAU_0,1mg/ml	68	EAGOT_0,01mg/ml_Starvation	106	Verbascum_songoricum_0,05mg/ml_Starvation	58	Leonurus_panzeroides_0,02mg/ml_Starvation	85
EAGMA_0,01mg/ml_Starvation	88	EAGAU_0,01mg/ml_Starvation	88	EAGOT_0,02mg/ml_Starvation	111	Stachys_betonicifiora_0,01mg/ml_Starvation	110	Leonurus_panzeroides_0,05mg/ml_Starvation	61
EAGMA_0,02mg/ml_Starvation	85								
ERAAlop_0,1mg/ml	47	EAGAF_0,1mg/ml_Starvation	29	EAGC_0,01mg/ml_Starvation	48	EAGSS_0,02mg/ml_Starvation	47	T-1C_0,1mg/ml	10
EAGMD_0,1mg/ml_Starvation	44	EAGPD_0,05mg/ml_Starvation	39	EAGC_0,02mg/ml_Starvation	39	EFIPS_0,05mg/ml_Starvation	30	T-1C_0,1mg/ml_Starvation	27
ERHA_0,05mg/ml_Starvation	44	EAGTM_0,01mg/ml_Starvation	47	EAGC_0,05mg/ml_Starvation	23	EAGCB_0,01mg/ml_Starvation	41	Cousina_umbrosa_0,01mg/ml_Starvation	48
ERHA_0,1mg/ml_Starvation	39	EAGTM_0,1mg/ml_Starvation	44	EAGC_0,1mg/ml_Starvation	13	EAGCB_0,05mg/ml_Starvation	48	Cousina_umbrosa_0,1mg/ml_Starvation	32
EAGAF_0,05mg/ml_Starvation	42	EAGC_0,1mg/ml	24	EAGSS_0,1mg/ml	39	T-1B_0,01mg/ml_Starvation	39	Leonurus_panzeroides_0,1mg/ml_Starvation	34

Figure 33. The legend is reported in the following pages.

Figure 32. The four Venn diagrams in the upper side of the figure show the correspondences among samples producing a mortality percentage greater then 50%, conventionally named "toxic extracts", and samples contained in each cluster after 2 hours of incubation: the number of toxic samples cultured in normal conditions are reported in faint red circles, the number of toxic samples cultured in starvation conditions are reported in green circles while the number of samples contained in each extracts are reported in light blue circles. The overlapped areas in each Venn diagrams report the number of samples belonging to all the two or three involved circles. Tables in the lower side of the figure report the lists of the samples contained in each clusters and the respective percentage of living cells remaining after treatments, with toxic extracts reported at the bottom of each table on a grey background and the other samples on a white background. Moreover, samples contained in cluster without any control sample (clusters 1 and 3) were considered interesting and colored in blue, samples cultured in starvation conditions and clustering with normal controls (cluster 2) were considered interesting and colored in green and samples cultured in normal conditions and clustering with starvation controls (cluster 2) were considered interesting and colored in red.

Figure 33. The four Venn diagrams in the upper side of the figure show the correspondences among samples producing a mortality percentage greater then 50%, conventionally named "toxic extracts", and samples contained in each cluster after 20 hours of incubation: the number of toxic samples cultured in normal conditions are reported in faint red circles, the number of toxic samples cultured in starvation conditions are reported in green circles while the number of samples contained in each extracts are reported in light blue circles. The overlapped areas in each Venn diagrams report the number of samples belonging to all the two or three involved circles. Tables in the lower side of the figure report the lists of the samples contained in each clusters and the respective percentage of living cells remaining after treatments, with toxic extracts reported at the bottom of each table on a grey background and the other samples on a white background. Moreover, samples contained in cluster without any control sample (clusters 1 and 2) were considered interesting and colored in blue, samples cultured in starvation conditions and clustering with normal controls (cluster 4) were considered interesting and colored in green and samples cultured in normal conditions and clustering with starvation controls (cluster 3) were considered interesting and colored in red.

- Cluster 2 ("Control-Like Phenotypes") contains 125 treatments producing effects similar to those produced by Control and Control DMSO samples, 7 of them causing a toxicity greater than 50%. Among the 127 total treatments, 17 have resulted to be potentially interesting because administrated in starvation conditions, 13 of which associated to a toxicity rate lower than 50% and 4 considered as toxic treatments;
- Cluster 3 ("Control Starvation-Like Phenotypes") contains 176 treatments. The results were similar to Control Starvation and Control DMSO Starvation and 10 of them were considered as toxic. Treatments administrated in normal conditions, considered as potentially interesting, were 70 and just 3 of them felt into the category of the toxic treatments;
- Cluster 4 ("Other Phenotypes") contains 74 treatments producing effects different from both control type samples, 11 of which, basing on the rules explained above, fall into the category "toxic treatments".

Figure 33, in the following page, reports results about the effects produced by the 380 treatments administrated for 20 hours, revealing that 81 of them have produced toxicity rate greater than 50% and 114 produced potentially interesting effects. Below the results of the analysis specific for each cluster have been reported:

- Cluster 1 ("Other Phenotypes") contains just 23 treatment producing an effect different from both control type samples and 22 of them resulted to produce a toxicity rate greater then 50%;
- Cluster 2 ("Control-Like Phenotypes"), contains 133 treatments the effects of which resulted similar to Control and Control DMSO samples, among them 27 were considered as toxic. In this case, treatments which were considered potentially interesting because administrated in starvation conditions were 10, and 5 of them were comprised among the toxic treatments;
- Cluster 3 ("Other Phenotypes"), contains 16 treatments producing effects different from both control type samples, 7 of which were resulted to be toxic;
- Cluster 4 ("Control Starvation-Like Phenotypes"), in the end, contains 204 treatments producing effects similar to Control Starvation and Control DMSO Starvation, 25 of which were considered as toxic. Among the total amount of these treatments, 59 were considered as potentially interesting because they were administrated and tested in normal conditions; 4 of these were resulted to match the condition to be considered toxic.

4.3.4 Further analysis of the effects produced by the extracts treatments administrated for 2 and/or 20 hours

The final outgoing data were then further analyzed to compare the results about the effects of the same treatments after 2 and 20 hours since their administration, in order to evaluate the toxicity effect and their influences on autophagy pathway in shorter and longer time durations. The results have been shown below using a series of Venn diagrams.

4.3.4.1 Comparison of the effects produced by treatments on cell viability after 2 and 20 hours since administration

The analysis of the toxicity associated to each treatment have been completed by comparing toxic and not toxic effects produced after 2 and 20 hours since administration. Treatments have been then grouped in 4 new sets identified through four different Venn diagrams reported below (figures 34, 35, 36 and 37): the first diagram (figure 34) reports the analysis of the treatments producing effects considered as toxic after both 2 and 20 hours since their administration; the second diagram (figure 35) reports the analysis of the treatments producing effects considered as not toxic after administration for both 20 and 20 hours; the third diagram (figure 36) reports the analysis of all the treatments producing effects considered as not toxic after 2 hours and resulting able to produce toxic effects after 20 hours since their administration; the fourth diagram (figure 37) shows all the treatments able to produce toxic effects after 2 hours and not toxic effects after 20 hours since their administration.
All the figures have been built following the same scheme: the light blue circle at the left side of each diagram reports the results about treatments after 2 hours since their administration, specified by the writing at the left side of the circle, while the faint red circle at the right side reports the results concerning the 20 hour treatments specified by the writing at the right side of the circle. The overlapped area reports the number of treatments matching the conditions associated to both the circles, thus grouping all the extracts potentially interesting for their cytotoxic activity.

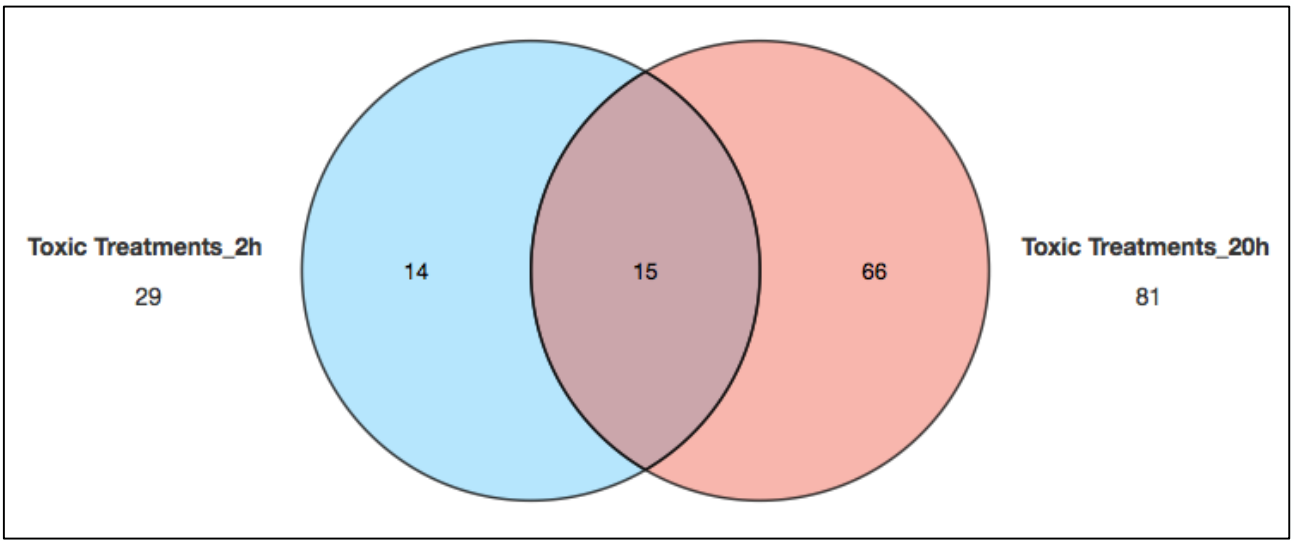


Figure 34. Analysis of the treatments producing effects considered as toxic after both 2 and 20 hours since their administration.

Toxic Treatments after both 2 and 20 hours					
1	EAGAAsia_0,05mg/ml_Starvation	6	EAGFO_0,05mg/ml_Starvation	11	T-1B_0,1mg/ml_Starvation
2	EAGAAsia_0,1mg/ml_Starvation	7	EAGFO_0,1mg/ml_Starvation	12	*Verbascum_blattaria*_0,1mg/ml_Starvation
3	ERPS_0,1mg/ml	8	ERKL_0,1mg/ml_Starvation	13	*Verbascum_songoricum*_0,1mg/ml_Starvation
4	ERPS_0,05mg/ml_Starvation	9	C-4B_0,1mg/ml	14	*Schrophullaria_sp*_0,05mg/ml_Starvation
5	ERPS_0,1mg/ml_Starvation	10	C-4B_0,1mg/ml_Starvation	15	*Schrophullaria_sp*_0,1mg/ml_Starvation

Table 3. List of the treatments producing a mortality rate higher than 50% after both 2 and 20 hours since treatments administration.

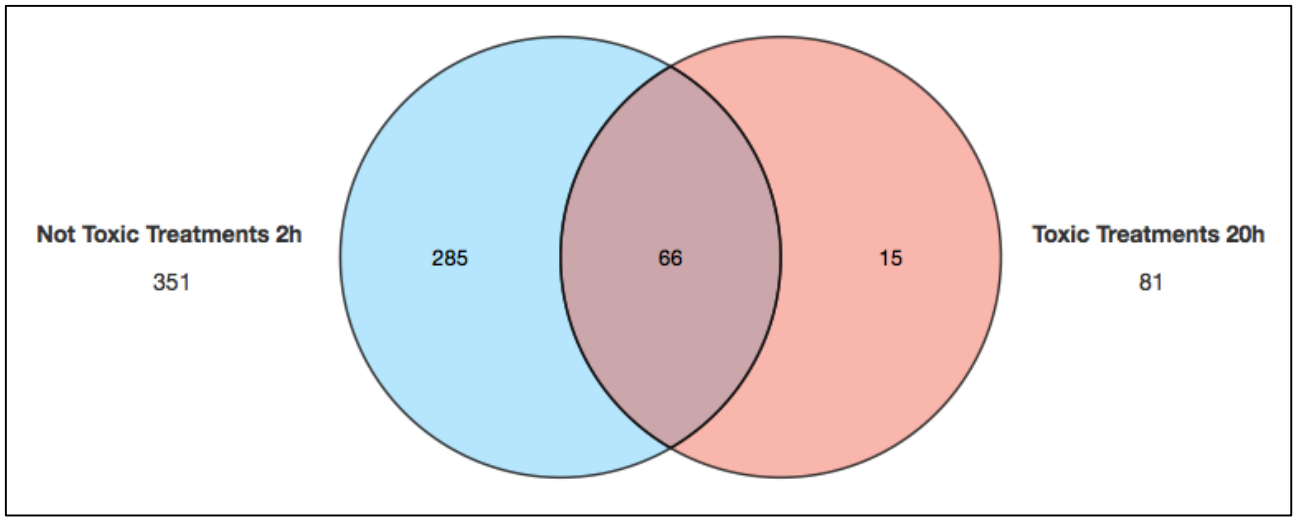


Figure 35. Analysis of the treatments producing effects considered as not toxic after 2 hours but considered as toxic after 20 hours since their administration.

Treatments Not Toxic after 2 and Toxic after 20 hours									
1	EAGRC_0,1mg/ml_Starv.	15	EAGFO_0,1mg/ml	28	EAGC_0,02mg/ml_Starv.	41	EAGCB_0,05mg/ml_Starv.	54	T-1C_0,1mg/ml
2	ERAAlop_0,1mg/ml	16	EAGFO_0,02mg/ml_Starv.	29	EAGC_0,05mg/ml_Starv.	42	EAGCT_0,1mg/ml_Starv.	55	T-1C_0,1mg/ml_Starv.
3	EAGAAsia_0,02mg/ml_Starv.	17	EAGPD_0,05mg/ml	30	EAGC_0,1mg/ml_Starv.	43	ERKL_0,1mg/ml	56	*Stachys_hissarica*_0,1mg/ml
4	EAGMD_0,1mg/ml_Starv.	18	EAGPD_0,1mg/ml	31	EFHA_0,1mg/ml_Starv.	44	ERKL_0,05mg/ml_Starv.	57	*Verbascum_songoricum*_0,1mg/ml
5	EAGKL_0,1mg/ml_Starv.	19	EAGPD_0,05mg/ml_Starv.	32	EAGSS_0,02mg/ml	45	C-3B_0,1mg/ml_Starv.	58	*Silene_oreina*_0,1mg/ml_Starv.
6	ERPS_0,02mg/ml_Starv.	20	EAGPD_0,1mg/ml_Starv.	33	EAGSS_0,1mg/ml	46	C-4B_0,05mg/ml	59	*Cousina_umbrosa*_0,05mg/ml
7	ERHA_0,05mg/ml	21	EAGTM_0,01mg/ml_Starv.	34	EAGSS_0,02mg/ml_Starv.	47	C-4B_0,05mg/ml_Starv.	60	*Cousina_umbrosa*_0,01mg/ml_Starv.
8	ERHA_0,05mg/ml_Starv.	22	EAGTM_0,1mg/ml_Starv.	35	EFIPS_0,02mg/ml	48	T-1B_0,02mg/ml	61	*Cousina_umbrosa*_0,1mg/ml_Starv.
9	ERHA_0,1mg/ml_Starv.	23	EAGC_0,01mg/ml	36	EFIPS_0,1mg/ml	49	T-1B_0,05mg/ml	62	*Scutellaria_scharistanica*_0,02mg/ml
10	EAGAF_0,05mg/ml_Starv.	24	EAGC_0,02mg/ml	37	EFIPS_0,05mg/ml_Starv.	50	T-1B_0,1mg/ml	63	*Scutellaria_scharistanica*_0,1mg/ml
11	EAGAF_0,1mg/ml_Starv.	25	EAGC_0,05mg/ml	38	EFIPS_0,1mg/ml_Starv.	51	T-1B_0,01mg/ml_Starv.	64	*Schrophullaria_sp*_0,02mg/ml
12	EAGAA_0,02mg/ml	26	EAGC_0,1mg/ml	39	EAGCB_0,02mg/ml	52	T-1B_0,02mg/ml_Starv.	65	*Schrophullaria_sp*_0,1mg/ml
13	EAGAA_0,1mg/ml_Starv.	27	EAGC_0,01mg/ml_Starv.	40	EAGCB_0,01mg/ml_Starv.	53	T-1B_0,05mg/ml_Starv.	66	*Leonurus_panzeroides*_0,1mg/ml_Starv.
14	EAGFO_0,05mg/ml								

Table 4. List of the treatments producing a mortality rate lower than 50% after 2 hours but higher than the threshold value after 20 hours since treatments administration.

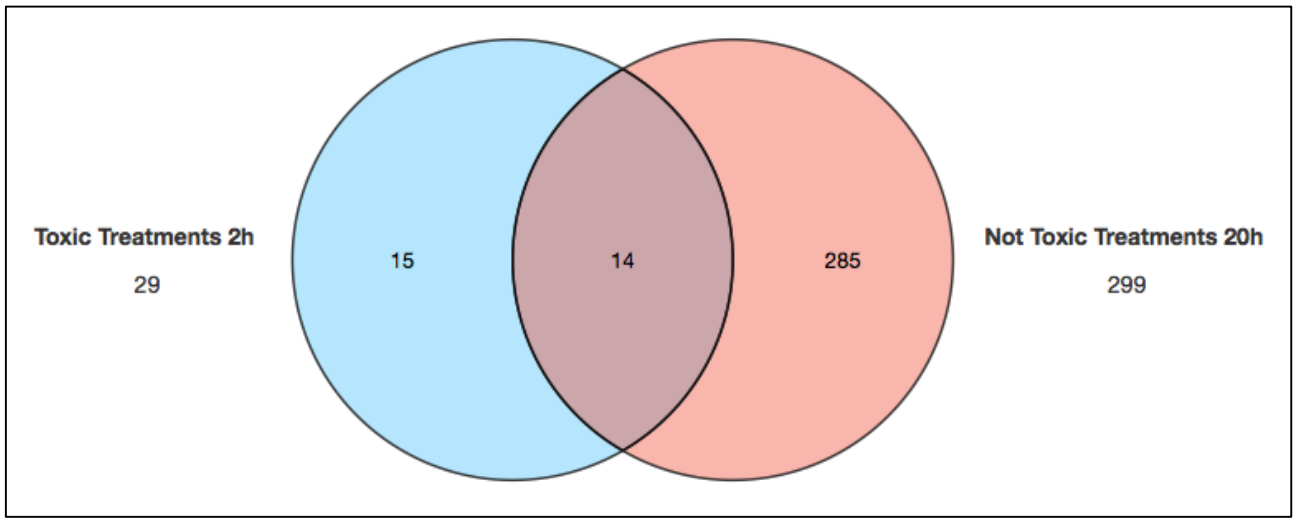


Figure 36. Analysis of the treatments producing effects considered as toxic after 2 hours but considered as not toxic after 20 hours since their administration.

Treatments Toxic after 2 and Not Toxic after 20 hours					
1	EAGMA_0,01mg/ml_Starvation	6	EFHA_0,01mg/ml_Starvation	11	*Stachys_betoniciflora*_0,05mg/ml_Starvation
2	EAGAT_0,01mg/ml_Starvation	7	EFIPS_0,02mg/ml_Starvation	12	*Phlomis_sewertzovii*_0,1mg/ml
3	ERPS_0,01mg/ml_Starvation	8	EAGCB_0,01mg/ml	13	*Scutellaria_scharistanica*_0,05mg/ml
4	ERHA_0,02mg/ml_Starvation	9	EAGCB_0,02mg/ml_Starvation	14	*Leonurus_panzeroides*_0,05mg/ml
5	EAGAA_0,05mg/ml_Starvation	10	*Stachys_hissarica*_0,1mg/ml_Starvation		

Table 5. List of the treatments producing a mortality rate lower than 50% after 2 hours but lower than the threshold value after 20 hours since their administration.

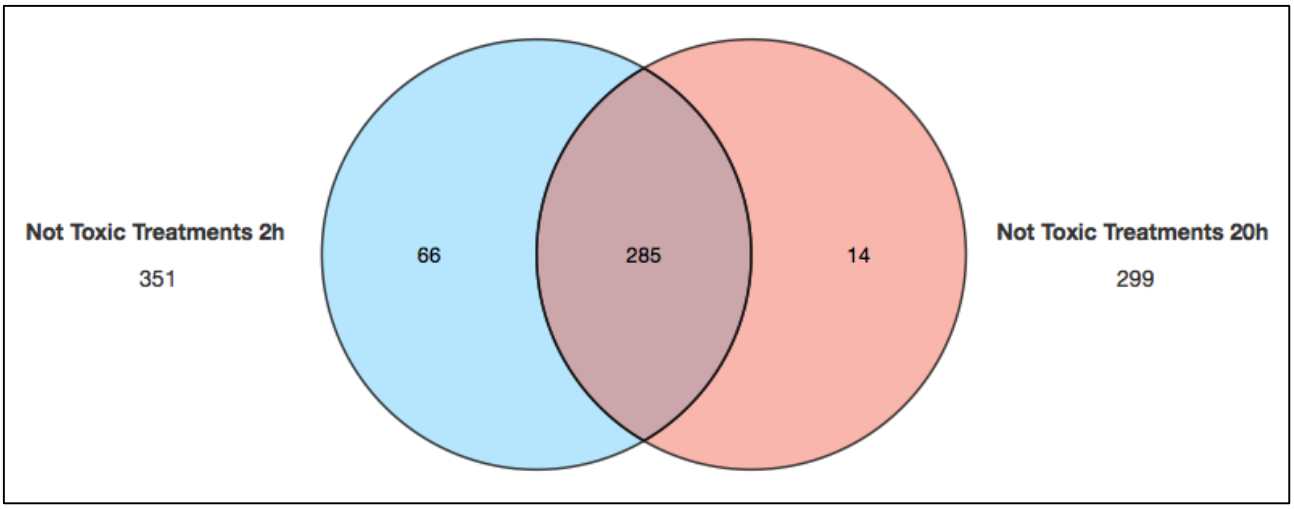


Figure 37. Analysis of the treatments producing effects considered as not toxic after both 2 and 20 hours since their administration.

Not Toxic Treatments after both 2 and 20 hours									
1	Ctrl	**58**	EAGKL_0,01mg/ml_Starv.	**115**	EAGPD_0,01mg/ml_Starv.	**172**	ESHA_0,01mg/ml_Starv.	**229**	*Phlomis_sewertzovii*_0,02mg/ml
2	CtrlDMSO	**59**	EAGKL_0,02mg/ml_Starv.	**116**	EAGPD_0,02mg/ml_Starv.	**173**	ESHA_0,02mg/ml_Starv.	**230**	*Phlomis_sewertzovii*_0,05mg/ml
3	Ctrl_Starvation	**60**	EAGKL_0,05mg/ml_Starv.	**117**	EAGTM_0,01mg/ml	**174**	ESHA_0,05mg/ml_Starv.	**231**	*Phlomis_sewertzovii*_0,01mg/ml_Starv.
4	CtrlDMSO_Starvation	**61**	EAGAS_0,01mg/ml	**118**	EAGTM_0,02mg/ml	**175**	ESHA_0,1mg/ml_Starv.	**232**	*Phlomis_sewertzovii*_0,02mg/ml_Starv.
5	EAGRC_0,01mg/ml	**62**	EAGAS_0,02mg/ml	**119**	EAGTM_0,05mg/ml	**176**	U-2C_0,01mg/ml	**233**	*Phlomis_sewertzovii*_0,05mg/ml_Starv.
6	EAGRC_0,02mg/ml	**63**	EAGAS_0,05mg/ml	**120**	EAGTM_0,1mg/ml	**177**	U-2C_0,02mg/ml	**234**	*Phlomis_sewertzovii*_0,1mg/ml_Starv.
7	EAGRC_0,05mg/ml	**64**	EAGAS_0,1mg/ml	**121**	EAGTM_0,02mg/ml_Starv.	**178**	U-2C_0,05mg/ml	**235**	*Phlomis_salicifolia*_0,01mg/ml
8	EAGRC_0,1mg/ml	**65**	EAGAS_0,01mg/ml_Starv.	**122**	EAGTM_0,05mg/ml_Starv.	**179**	U-2C_0,1mg/ml	**236**	*Phlomis_salicifolia*_0,02mg/ml
9	EAGRC_0,01mg/ml_Starv.	**66**	EAGAS_0,02mg/ml_Starv.	**123**	EFHA_**0,01**mg/ml	**180**	U-2C_0,01mg/ml_Starv.	**237**	*Phlomis_salicifolia*_0,05mg/ml
10	EAGRC_0,02mg/ml_Starv.	**67**	EAGAS_0,05mg/ml_Starv.	**124**	EFHA_0,02mg/ml	**181**	U-2C_0,02mg/ml_Starv.	**238**	*Phlomis_salicifolia*_0,1mg/ml
11	EAGRC_0,05mg/ml_Starv.	**68**	EAGAS_0,1mg/ml_Starv.	**125**	EFHA_0,05mg/ml	**182**	U-2C_0,05mg/ml_Starv.	**239**	*Phlomis_salicifolia*_0,01mg/ml_Starv.
12	ERAAlop_0,01mg/ml	**69**	EAGAT_0,01mg/ml	**126**	EFHA_0,1mg/ml	**183**	U-2C_0,1mg/ml_Starv.	**240**	*Phlomis_salicifolia*_0,02mg/ml_Starv.
13	ERAAlop_0,02mg/ml	**70**	EAGAT_0,02mg/ml	**127**	EFHA_0,02mg/ml_Starv.	**184**	C-3B_0,01mg/ml	**241**	*Phlomis_salicifolia*_0,05mg/ml_Starv.
14	ERAAlop_0,05mg/ml	**71**	EAGAT_0,05mg/ml	**128**	EFHA_0,05mg/ml_Starv.	**185**	C-3B_0,02mg/ml	**242**	*Phlomis_salicifolia*_0,1mg/ml_Starv.
15	ERAAlop_0,01mg/ml_Starv.	**72**	EAGAT_0,1mg/ml	**129**	EAGSS_0,01mg/ml	**186**	C-3B_0,05mg/ml	**243**	*Silene_oreina*_0,01mg/ml
16	ERAAlop_0,02mg/ml_Starv.	**73**	EAGAT_0,02mg/ml_Starv.	**130**	EAGSS_0,05mg/ml	**187**	C-3B_0,1mg/ml	**244**	*Silene_oreina*_0,02mg/ml
17	ERAAlop_0,05mg/ml_Starv.	**74**	EAGAT_0,05mg/ml_Starv.	**131**	EAGSS_0,01mg/ml_Starv.	**188**	C-3B_0,01mg/ml_Starv.	**245**	*Silene_oreina*_0,05mg/ml
18	ERAAlop_0,1mg/ml_Starv.	**75**	EAGAT_0,1mg/ml_Starv.	**132**	EAGSS_0,05mg/ml_Starv.	**189**	C-3B_0,02mg/ml_Starv.	**246**	*Silene_oreina*_0,1mg/ml
19	EAGPS_0,01mg/ml	**76**	EAGAU_0,01mg/ml	**133**	EAGSS_0,1mg/ml_Starv.	**190**	C-3B_0,05mg/ml_Starv.	**247**	*Silene_oreina*_0,01mg/ml_Starv.
20	EAGPS_0,02mg/ml	**77**	EAGAU_0,02mg/ml	**134**	EFIPS_0,01mg/ml	**191**	C-4B_0,01mg/ml	**248**	*Silene_oreina*_0,02mg/ml_Starv.
21	EAGPS_0,05mg/ml	**78**	EAGAU_0,05mg/ml	**135**	EFIPS_0,05mg/ml	**192**	C-4B_0,02mg/ml	**249**	*Silene_oreina*_0,05mg/ml_Starv.
22	EAGPS_0,1mg/ml	**79**	EAGAU_0,1mg/ml	**136**	EFIPS_0,01mg/ml_Starv.	**193**	C-4B_0,01mg/ml_Starv:	**250**	*Phlomis_tadschikistanica*_0,01mg/ml
23	EAGPS_0,01mg/ml_Starv.	**80**	EAGAU_0,01mg/ml_Starv.	**137**	EAGCB_0,05mg/ml	**194**	C-4B_0,02mg/ml_Starv.	**251**	*Phlomis_tadschikistanica*_0,02mg/ml
24	EAGPS_0,02mg/ml_Starv.	**81**	EAGAU_0,02mg/ml_Starv.	**138**	EAGCB_0,1mg/ml	**195**	T-1B_0,01mg/ml	**252**	*Phlomis_tadschikistanica*_0,05mg/ml
25	EAGPS_0,05mg/ml_Starv.	**82**	EAGAU_0,05mg/ml_Starv.	**139**	EAGCB_0,1mg/ml_Starv.	**196**	T-1C_0,01mg/ml	**253**	*Phlomis_tadschikistanica*_0,1mg/ml
26	EAGPS_0,1mg/ml_Starv.	**83**	EAGAU_0,1mg/ml_Starv.	**140**	EAGCT_0,01mg/ml	**197**	T-1C_0,02mg/ml	**254**	*Phlomis_tadschikistanica*_0,01mg/ml_Starv
27	EAGAAsia_0,01mg/ml	**84**	ERPS_0,01mg/ml	**141**	EAGCT_0,02mg/ml	**198**	T-1C_0,05mg/ml	**255**	*Phlomis_tadschikistanica*_0,02mg/ml_Starv
28	EAGAAsia_0,02mg/ml	**85**	ERPS_0,02mg/ml	**142**	EAGCT_0,05mg/ml	**199**	T-1C_0,01mg/ml_Starv.	**256**	*Phlomis_tadschikistanica*_0,05mg/ml_Starv
29	EAGAAsia_0,05mg/ml	**86**	ERPS_0,05mg/ml	**143**	EAGCT_0,1mg/ml	**200**	T-1C_0,02mg/ml_Starv.	**257**	*Phlomis_tadschikistanica*_0,1mg/ml_Starv.
30	EAGAAsia_0,1mg/ml	**87**	EFIAAlop_0,01mg/ml	**144**	EAGCT_0,01mg/ml_Starv.	**201**	T-1C_0,05mg/ml_Starv.	**258**	*Cousina_umbrosa*_0,01mg/ml
31	EAGAAsia_0,01mg/ml_Starv.	**88**	EFIAAlop_0,02mg/ml	**145**	EAGCT_0,02mg/ml_Starv.	**202**	*Verbascum_blattaria*_0,01mg/ml	**259**	*Cousina_umbrosa*_0,02mg/ml
32	EAGAAlop_0,01mg/ml	**89**	EFIAAlop_0,05mg/ml	**146**	EAGCT_0,05mg/ml_Starv.	**203**	*Verbascum_blattaria*_0,02mg/ml	**260**	*Cousina_umbrosa*_0,1mg/ml
33	EAGAAlop_0,02mg/ml	**90**	EFIAAlop_0,1mg/ml	**147**	EAGOT_0,01mg/ml	**204**	*Verbascum_blattaria*_0,05mg/ml	**261**	*Cousina_umbrosa*_0,02mg/ml_Starv.
34	EAGAAlop_0,05mg/ml	**91**	EFIAAlop_0,01mg/ml_Starv.	**148**	EAGOT_0,02mg/ml	**205**	*Verbascum_blattaria*_0,1mg/ml	**262**	*Cousina_umbrosa*_0,05mg/ml_Starv.
35	EAGAAlop_0,1mg/ml	**92**	EFIAAlop_0,02mg/ml_Starv.	**149**	EAGOT_0,05mg/ml	**206**	*Verbascum_blattaria*_0,01mg/ml_Starv.	**263**	*Nepeta_olgae*_0,01mg/ml
36	EAGAAlop_0,01mg/ml_Starv.	**93**	EFIAAlop_0,05mg/ml_Starv.	**150**	EAGOT_0,1mg/ml	**207**	*Verbascum_blattaria*_0,02mg/ml_Starv.	**264**	*Nepeta_olgae*_0,02mg/ml
37	EAGAAlop_0,02mg/ml_Starv.	**94**	EFIAAlop_0,1mg/ml_Starv.	**151**	EAGOT_0,01mg/ml_Starv.	**208**	*Verbascum_blattaria*_0,05mg/ml_Starv.	**265**	*Nepeta_olgae*_0,05mg/ml
38	EAGAAlop_0,05mg/ml_Starv.	**95**	ERHA_0,01mg/ml	**152**	EAGOT_0,02mg/ml_Starv.	**209**	*Stachys_hissarica*_0,01mg/ml	**266**	*Nepeta_olgae*_0,1mg/ml
39	EAGAAlop_0,1mg/ml_Starv.	**96**	ERHA_0,02mg/ml	**153**	EAGOT_0,05mg/ml_Starv.	**210**	*Stachys_hissarica*_0,02mg/ml	**267**	*Nepeta_olgae*_0,01mg/ml_Starv.
40	EAGMA_0,01mg/ml	**97**	ERHA_0,1mg/ml	**154**	EAGOT_0,1mg/ml_Starv:	**211**	*Stachys_hissarica*_0,05mg/ml	**268**	*Nepeta_olgae*_0,02mg/ml_Starv.
41	EAGMA_0,02mg/ml	**98**	ERHA_0,01mg/ml_Starv.	**155**	ERKL_0,01mg/ml	**212**	*Stachys_hissarica*_0,01mg/ml_Starv.	**269**	*Nepeta_olgae*_0,05mg/ml_Starv.
42	EAGMA_0,05mg/ml	**99**	EAGAF_0,01mg/ml	**156**	ERKL_0,02mg/ml	**213**	*Stachys_hissarica*_0,02mg/ml_Starv.	**270**	*Nepeta_olgae*_0,1mg/ml_Starv.
43	EAGMA_0,1mg/ml	**100**	EAGAF_0,02mg/ml	**157**	ERKL_0,05mg/ml	**214**	*Stachys_hissarica*_0,05mg/ml_Starv.	**271**	*Scutellaria_scharistanica*_0,01mg/ml
44	EAGMA_0,02mg/ml_Starv.	**101**	EAGAF_0,05mg/ml	**158**	ERKL_0,01mg/ml_Starv.	**215**	*Verbascum_songoricum*_0,01mg/ml	**272**	*Scutellaria_scharistanica*_0,01mg/ml_Starv
45	EAGMA_0,05mg/ml_Starv.	**102**	EAGAF_0,1mg/ml	**159**	ERKL_0,02mg/ml_Starv.	**216**	*Verbascum_songoricum*_0,02mg/ml	**273**	*Scutellaria_scharistanica*_0,02mg/ml_Starv
46	EAGMA_0,1mg/ml_Starv.	**103**	EAGAF_0,01mg/ml_Starv.	**160**	ERKS_0,01mg/ml	**217**	*Verbascum_songoricum*_0,05mg/ml	**274**	*Scutellaria_scharistanica*_0,05mg/ml_Starv
47	EAGMD_0,01mg/ml	**104**	EAGAF_0,02mg/ml_Starv.	**161**	ERKS_0,02mg/ml	**218**	*Verbascum_songoricum*_0,01mg/ml_Starv.	**275**	*Scutellaria_scharistanica*_0,1mg/ml_Starv.
48	EAGMD_0,02mg/ml	**105**	EAGAA_0,01mg/ml	**162**	ERKS_0,05mg/ml	**219**	*Verbascum_songoricum*_0,02mg/ml_Starv.	**276**	*Schrophullaria_sp*_0,01mg/ml
49	EAGMD_0,05mg/ml	**106**	EAGAA_0,05mg/ml	**163**	ERKS_0,1mg/ml	**220**	*Verbascum_songoricum*_0,05mg/ml_Starv.	**277**	*Schrophullaria_sp*_0,05mg/ml
50	EAGMD_0,1mg/ml	**107**	EAGAA_0,1mg/ml	**164**	ERKS_0,01mg/ml_Starv.	**221**	*Stachys_betoniciflora*_0,01mg/ml	**278**	*Schrophullaria_sp*_0,01mg/ml_Starv.
51	EAGMD_0,01mg/ml_Starv.	**108**	EAGAA_0,01mg/ml_Starv.	**165**	ERKS_0,02mg/ml_Starv.	**222**	*Stachys_betoniciflora*_0,02mg/ml	**279**	*Schrophullaria_sp*_0,02mg/ml_Starv.
52	EAGMD_0,02mg/ml_Starv.	**109**	EAGAA_0,02mg/ml_Starv.	**166**	ERKS_0,05mg/ml_Starv.	**223**	*Stachys_betoniciflora*_0,05mg/ml	**280**	*Leonurus_panzeroides*_0,01mg/ml
53	EAGMD_0,05mg/ml_Starv.	**110**	EAGFO_0,01mg/ml	**167**	ERKS_0,1mg/ml_Starv.	**224**	*Stachys_betoniciflora*_0,1mg/ml	**281**	*Leonurus_panzeroides*_0,02mg/ml
54	EAGKL_0,01mg/ml	**111**	EAGFO_0,02mg/ml	**168**	ESHA_0,01mg/ml	**225**	*Stachys_betoniciflora*_0,01mg/ml_Starv.	**282**	*Leonurus_panzeroides*_0,1mg/ml
55	EAGKL_0,02mg/ml	**112**	EAGFO_0,01mg/ml_Starv.	**169**	ESHA_0,02mg/ml	**226**	*Stachys_betoniciflora*_0,02mg/ml_Starv.	**283**	*Leonurus_panzeroides*_0,01mg/ml_Starv.
56	EAGKL_0,05mg/ml	**113**	EAGPD_0,01mg/ml	**170**	ESHA_0,05mg/ml	**227**	*Stachys_betoniciflora*_0,1mg/ml_Starv.	**284**	*Leonurus_panzeroides*_0,02mg/ml_Starv.
57	EAGKL_0,1mg/ml	**114**	EAGPD_0,02mg/ml	**171**	ESHA_0,1mg/ml	**228**	*Phlomis_sewertzovii*_0,01mg/ml	**285**	*Leonurus_panzeroides*_0,05mg/ml_Starv.

Table 6. List of the treatments producing a mortality rate lower than 50% after both 2 and 20 hours since treatments administration.

Observing the results of the analyses, figure 34 reports the number of the treatments resulted to be toxic after both 2 and 20 hours since administration and 15 treatments have matched such conditions after both treatments duration times. Figure 37 reports the analysis concerning treatments resulting to be not toxic after both 2 and 20 hours since their administration, revealing that 285 treatments have resulted to match both these investigated conditions. Figure 35 and 36 report the results concerning the cross-examination of the previous analyzed groups. In particular, in figure 35 results concerning the analysis of the treatments producing effects considered as not toxic after 2 hours but producing a toxicity rate higher than 50% after 20 hours has been reported and 66 treatments have resulted to match both these conditions. Figure 36 reports that just 14 treatments have resulted to produce a toxic effect after 2 hours but not after 20 hours since treatments administration. The lists of the extracts selected in these four analysis has been reported in table below the respective graph (tables 3, 4, 5 and 6), and the same lists associated to the respective percentage of living cells detected for each treatment have been reported in tables A7, A8, A9, A10 in the section supplementary data.

4.3.4.2 Comparison of the treatments producing interesting effects after both 2 and 20 hours since administration

Other interesting analyses have been performed considering all the treatments which showed an interesting effect after both 2 as well as 20 hours since their administration. As previously reported, after the Hierarchical Cluster Analysis the treatments were grouped in different clusters, containing at least one of the two sets of control samples ("Control" and "Control DMSO"; "Control Starvation" and "Control DMSO Starvation") or not containing anyone of these. Treatments considered as interesting were then detected basing on the culturing conditions in which they were administrated and the type of control samples possibly contained in the same cluster: for example, treatments which clustered without any control sample ("Other Phenotypes"), treatments administrated in starvation conditions that clustered along with "Control" and "Control DMSO" samples ("Control Like Phenotypes") and treatments administrated in normal conditions included in the cluster also containing "Control Starvation" and "Control DMSO Starvation" samples ("Control Starvation Like Phenotypes") have been considered as interesting. Starting from these assumptions, treatments were analyzed in order to define their capability to produce the same interesting effect after both 2 as well as 20 hours since the administration and also to evaluate treatments not producing interesting effects after 2 and 20 hours since their administration. All the analyses have been reported using Venn diagrams similarly organized as in paragraph 4.3.4.1: for each diagram, the number of the treatments considered as interesting after 2 hours has been reported in the light blue circle at the left side, with the type of interesting category written at the left, while in the faint red circle at the right side is reported the number of treatments considered interesting after 20 hours and belonging to the category written on the right side; the overlapped areas contain all the treatments matching both conditions considered in the diagram.

In details, figure 38 reports that just 3 treatments administrated in starvation conditions produced an effect considered as interesting because similar to those produced by "Control" and "Control DMSO" samples after both shorter as well as longer duration of the treatments. In a similar fashion, figure 39 reports the same kind of results, but concerning treatments administrated in normal conditions which produce effects comparable to "Control Starvation" and "Control DMSO Starvation" samples, revealing that 43 extracts were selected for matching both such conditions. Just 1 treatment has been selected for producing an effect different from that produced by both types of control samples ("Control" and "Control DMSO"; "Control Starvation" and "Control DMSO Starvation") after 2 and also after 20 hours since its administration, as reported in figure 40. Data reported in figure 41 complete the picture by showing that 139 treatments

have produced effects considered as not interesting, because included into the cluster containing also the control samples cultured in the same conditions (normal or starvation), after administration for both 2 as well as 20 hours.

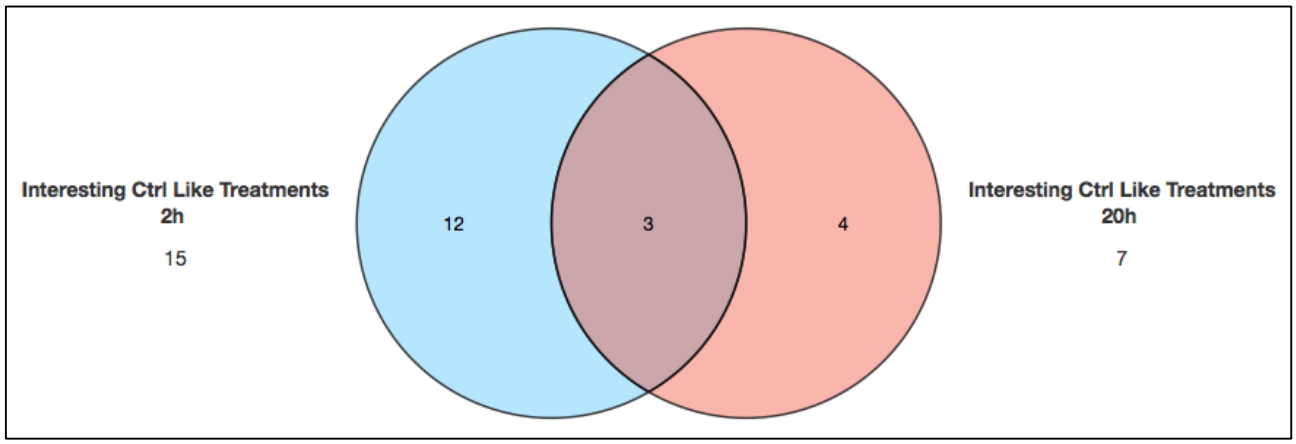


Figure 38. Analysis of the treatments administrated in starvation conditions and able to produce effects considered as interesting because similar to those produced by the control samples cultured in normal conditions after both 2 and 20 hours since their administration.

Interesting Control Like Treatments after 2 and 20 hours					
1	Ctrl	2	Ctrl DMSO	3	C-3B_0,02mg/ml_Starvation

Table 7. List of the treatments cultured in starvation conditions and producing an effect similar to those produced by the control samples cultured in normal conditions after both 2 and 20 hours since their administration.

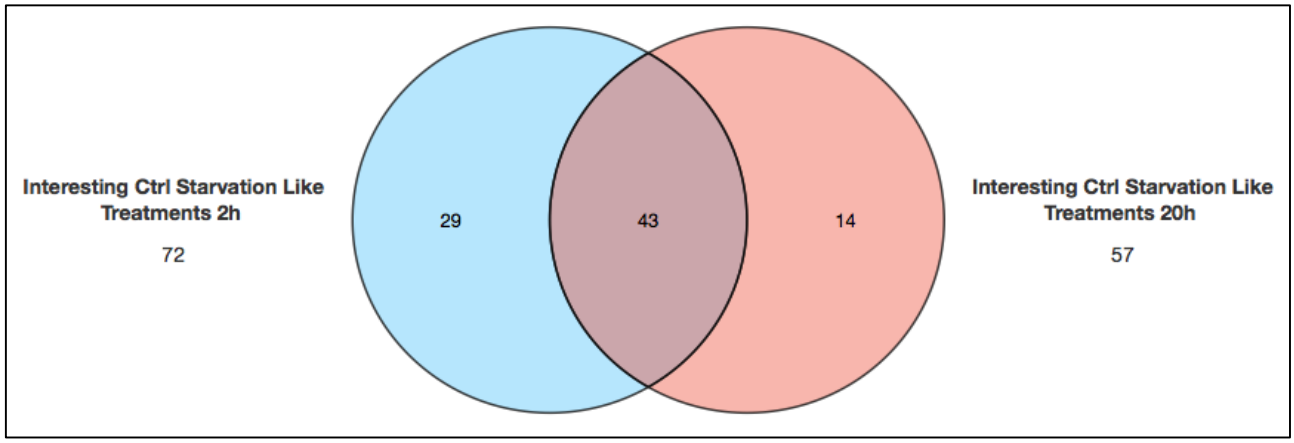


Figure 39. Analysis of the treatments administrated in normal conditions and able to produce effects considered as interesting because similar to those produced by the control samples cultured in starvation conditions after both 2 and 20 hours since their administration.

Interesting Control Starvation Like Treatments after 2 and 20 hours					
1	Ctrl_Starvation	16	EAGAAlop_0,05mg/ml	30	EAGAS_0,01mg/ml
2	CtrlDMSO_Starvation	17	EAGAAlop_0,1mg/ml	31	EAGAS_0,02mg/ml
3	EAGRC_0,05mg/ml	18	EAGMA_0,01mg/ml	32	EAGAS_0,05mg/ml
4	EAGRC_0,1mg/ml	19	EAGMA_0,02mg/ml	33	EAGAS_0,1mg/ml
5	ERAAlop_0,01mg/ml	20	EAGMA_0,05mg/ml	34	EAGAU_0,02mg/ml
6	ERAAlop_0,02mg/ml	21	EAGMA_0,1mg/ml	35	ERPS_0,01mg/ml
7	ERAAlop_0,05mg/ml	22	EAGMD_0,01mg/ml	36	ERPS_0,02mg/ml
8	EAGPS_0,01mg/ml	23	EAGMD_0,02mg/ml	37	ERPS_0,05mg/ml
9	EAGPS_0,02mg/ml	24	EAGMD_0,05mg/ml	38	ERKL_0,02mg/ml
10	EAGAAsia_0,01mg/ml	25	EAGMD_0,1mg/ml	39	ERKL_0,05mg/ml
11	EAGAAsia_0,02mg/ml	26	EAGKL_0,01mg/ml	40	*Silene_oreina*_0,01mg/ml
12	EAGAAsia_0,05mg/ml	27	EAGKL_0,02mg/ml	41	*Silene_oreina*_0,02mg/ml
13	EAGAAsia_0,1mg/ml	28	EAGKL_0,05mg/ml	42	*Silene_oreina*_0,05mg/ml
14	EAGAAlop_0,01mg/ml	29	EAGKL_0,1mg/ml	43	*Silene_oreina*_0,1mg/ml
15	EAGAAlop_0,02mg/ml				

Table 8. List of the treatments cultured in normal conditions and producing an effect similar to those produced by the control samples cultured in starvation conditions after both 2 and 20 hours since their administration.

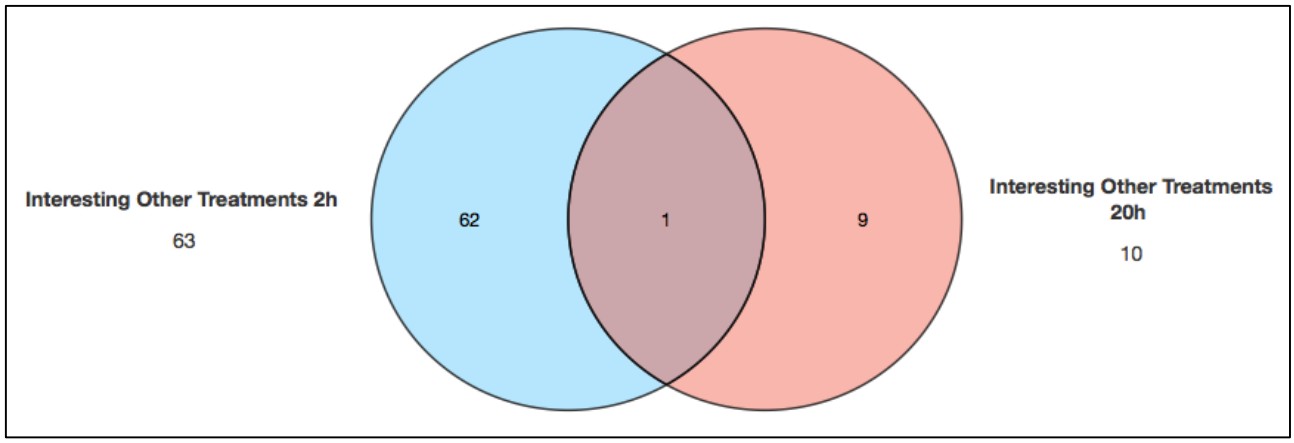


Figure 40. Analysis of the treatments producing effects considered as interesting because different to those produced by both the control sample types after both 2 and 20 hours since their administration, independently to the culturing conditions.

Interesting Other Treatments after 2 and 20 hours	
1	EAGKL_0,05mg/ml_Starvation

Table 9. List of the treatments producing effects considered as interesting because different to those produced by both the control sample types after both 2 and 20 hours since their administration, independently to the culturing conditions.

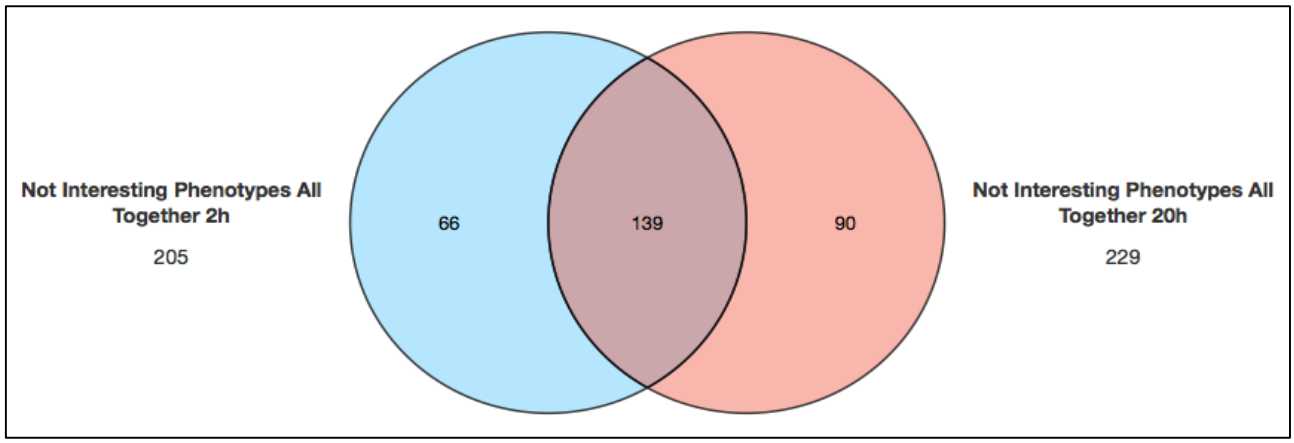


Figure 41. Analysis of the treatments producing effects considered as not interesting after both 2 and 20 hours since their administration, independently to the culturing conditions.

Not Interesting Treatments after 2 and 20 hours									
1	Ctrl	29	EAGSS_0,05mg/ml	57	C-4B_0,02mg/ml	85	EAGAS_0,1mg/ml_Starvation	113	EAGCT_0,01mg/ml_Starvation
2	CtrlDMSO	30	EFIPS_0,01mg/ml	58	T-1B_0,01mg/ml	86	EAGAT_0,02mg/ml_Starvation	114	EAGOT_0,01mg/ml_Starvation
3	EFIAAlop_0,01mg/ml	31	EFIPS_0,05mg/ml	59	T-1C_0,01mg/ml	87	EAGAT_0,05mg/ml_Starvation	115	EAGOT_0,02mg/ml_Starvation
4	EFIAAlop_0,02mg/ml	32	EAGCB_0,1mg/ml	60	T-1C_0,05mg/ml	88	EAGAT_0,1mg/ml_Starvation	116	EAGOT_0,05mg/ml_Starvation
5	EFIAAlop_0,05mg/ml	33	EAGCT_0,01mg/ml	61	*Verbascum_blattaria*_0,01mg/ml	89	EAGAU_0,01mg/ml_Starvation	117	EAGOT_0,1mg/ml_Starvation
6	EFIAAlop_0,1mg/ml	34	EAGCT_0,02mg/ml	62	*Verbascum_blattaria*_0,02mg/ml	90	EAGAU_0,02mg/ml_Starvation	118	ERKL_0,01mg/ml_Starvation
7	ERHA_0,01mg/ml	35	EAGCT_0,05mg/ml	63	*Verbascum_blattaria*_0,05mg/ml	91	EAGAU_0,05mg/ml_Starvation	119	ERKL_0,02mg/ml_Starvation
8	ERHA_0,02mg/ml	36	EAGCT_0,1mg/ml	64	*Verbascum_blattaria*_0,1mg/ml	92	EAGAU_0,1mg/ml_Starvation	120	ERKS_0,01mg/ml_Starvation
9	ERHA_0,1mg/ml	37	EAGOT_0,01mg/ml	65	*Stachys_hissarica*_0,01mg/ml	93	EFIAAlop_0,01mg/ml_Starvation	121	ERKS_0,05mg/ml_Starvation
10	EAGAF_0,01mg/ml	38	EAGOT_0,02mg/ml	66	*Stachys_hissarica*_0,02mg/ml	94	EFIAAlop_0,02mg/ml_Starvation	122	ERKS_0,1mg/ml_Starvation
11	EAGAF_0,02mg/ml	39	EAGOT_0,05mg/ml	67	*Stachys_hissarica*_0,05mg/ml	95	EFIAAlop_0,05mg/ml_Starvation	123	ESHA_0,01mg/ml_Starvation
12	EAGAF_0,05mg/ml	40	EAGOT_0,1mg/ml	68	*Verbascum_songoricum*_0,01mg/ml	96	EFIAAlop_0,1mg/ml_Starvation	124	ESHA_0,02mg/ml_Starvation
13	EAGAF_0,1mg/ml	41	ERKS_0,01mg/ml	69	*Verbascum_songoricum*_0,02mg/ml	97	ERHA_0,01mg/ml_Starvation	125	ESHA_0,05mg/ml_Starvation
14	EAGAA_0,01mg/ml	42	ERKS_0,02mg/ml	70	*Verbascum_songoricum*_0,05mg/ml	98	EAGAF_0,01mg/ml_Starvation	126	ESHA_0,1mg/ml_Starvation
15	EAGAA_0,05mg/ml	43	ERKS_0,05mg/ml	71	*Stachys_betoniciflora*_0,01mg/ml	99	EAGAF_0,02mg/ml_Starvation	127	U-2C_0,01mg/ml_Starvation
16	EAGAA_0,1mg/ml	44	ESHA_0,01mg/ml	72	*Stachys_betoniciflora*_0,02mg/ml	100	EAGAA_0,01mg/ml_Starvation	128	U-2C_0,02mg/ml_Starvation
17	EAGFO_0,01mg/ml	45	ESHA_0,02mg/ml	73	*Stachys_betoniciflora*_0,05mg/ml	101	EAGAA_0,02mg/ml_Starvation	129	U-2C_0,05mg/ml_Starvation
18	EAGFO_0,02mg/ml	46	ESHA_0,05mg/ml	74	*Stachys_betoniciflora*_0,1mg/ml	102	EAGFO_0,01mg/ml_Starvation	130	U-2C_0,1mg/ml_Starvation
19	EAGPD_0,02mg/ml	47	ESHA_0,1mg/ml	75	*Phlomis_tadschikistanica*_0,1mg/ml	103	EAGPD_0,02mg/ml_Starvation	131	C-4B_0,01mg/ml_Starvation
20	EAGTM_0,01mg/ml	48	U-2C_0,01mg/ml	76	*Cousina_umbrosa*_0,01mg/ml	104	EAGTM_0,02mg/ml_Starvation	132	T-1C_0,01mg/ml_Starvation
21	EAGTM_0,02mg/ml	49	U-2C_0,02mg/ml	77	Ctrl_Starvation	105	EAGTM_0,05mg/ml_Starvation	133	T-1C_0,02mg/ml_Starvation
22	EAGTM_0,05mg/ml	50	U-2C_0,05mg/ml	78	CtrlDMSO_Starvation	106	EFHA_0,02mg/ml_Starvation	134	T-1C_0,05mg/ml_Starvation
23	EAGTM_0,1mg/ml	51	U-2C_0,1mg/ml	79	EAGRC_0,02mg/ml_Starvation	107	EFHA_0,05mg/ml_Starvation	135	*Verbascum_blattaria*_0,01mg/ml_Starvation
24	EFHA_0,01mg/ml	52	C-3B_0,01mg/ml	80	EAGRC_0,05mg/ml_Starvation	108	EAGSS_0,01mg/ml_Starvation	136	*Stachys_hissarica*_0,01mg/ml_Starvation
25	EFHA_0,02mg/ml	53	C-3B_0,02mg/ml	81	EAGAAsia_0,01mg/ml_Starvation	109	EAGSS_0,05mg/ml_Starvation	137	*Stachys_hissarica*_0,02mg/ml_Starvation
26	EFHA_0,05mg/ml	54	C-3B_0,05mg/ml	82	EAGAAlop_0,02mg/ml_Starvation	110	EAGSS_0,1mg/ml_Starvation	138	*Stachys_betoniciflora*_0,01mg/ml_Starvation
27	EFHA_0,1mg/ml	55	C-3B_0,1mg/ml	83	EAGAAlop_0,05mg/ml_Starvation	111	EFIPS_0,01mg/ml_Starvation	139	*Phlomis_salicifolia*_0,02mg/ml_Starvation
28	EAGSS_0,01mg/ml	56	C-4B_0,01mg/ml	84	EAGAS_0,05mg/ml_Starvation	112	EAGCB_0,1mg/ml_Starvation		

Table 10. List of the treatments producing effects considered as not interesting after both 2 and 20 hours since their administration, independently from the culturing conditions.

All the treatments selected through these analyses have been reported name by name into the tables showed below; each graph contains the specific Venn diagram, respectively indicated as tables 7, 8, 9 and 10. The same tables, completed with the percentage of living cells and the factor scores associated to the PC1 and the PC2 after both 2 and 20 hours since treatments administration, have been then reported also in the section "supplementary data" as respectively tables A11, A12, A13 and A14, thus allowing to have a better look at the overall result and to help the data interpretation.

4.3.4.3 Analyses of the treatments producing effects not interesting after 2 hours but becoming interesting after 20 hours

This analysis has been performed by crossing data concerning the effects of treatments after 2 and 20 hours since their administration in order to select the extracts whose effects became interesting during the time. To provide a more complete view of the final results, all data has been analyzed in two parallel tracks and two different groups of treatments have been selected: on one side, treatments administrated in physiological conditions, whose effects produced after 2 hours were not interesting, have been selected when contained in one of the clusters without any control samples or in the cluster containing also the two control starvation samples after 20 hours, because considered as interesting; on the other side, treatments administrated in autophagy-induced conditions producing effects considered as not interesting after 2 hours, were selected when present in cluster free of control samples or in that containing the reference control samples for physiological conditions, because considered as interesting after 20 hours.
The results of the analyses have been showed in figures 42 and 43 by using Venn diagrams composed by three circles: the lowest circle, colored in green, contained data concerning the specific group of treatments not interesting after 2 hours since the administration, as written below the circle (Control-Like and Control Starvation-Like with and without DMSO 2h); the light blue and the faint red circles respectively at the high left and right corner, report data concerning the treatments that were interesting after 20 hours since the administration, as written on the left and the right sides (Control-Like, or Control Starvation-Like with and without DMSO, and Other 20h). The overlapped areas report the number of the treatments satisfying the conditions described by each of the circles involved and, in such the diagrams composed by three circles, the attention is to be focused on the areas of the green circle overlapped with the light blue circles as well as those that overlapped with the faint red circles; no data can meet the required conditions in the overlapping area between light blue and faint red circles and the one in which all the three circles were involved.
Concerning the results, in figure 42 the analysis of the treatments administrated in physiological conditions and considered as interesting only after 20 hours has been reported: among the 107 treatments not interesting after 2 hours, 7 have been selected because similar to starvation reference control samples and 1 because different from both control samples types after 20 hours since their administration. The analysis of the treatments administrated in starvation conditions which didn't produce any interesting effects after 2 hours since their administration, has been reported in figure 43: among 98 treatments not interesting after 2 hours, 4 were selected because comparable to the reference control samples for physiological conditions and other 6 because able to produce effects different from both control samples types after 20 hours since their administration.
All the selected treatments from figures 42 and 43 have been reported name by name in tables 11 and 12, also sorted per category. Tables A15 and A16 contained in the section supplementary data report a more complete version of such tables, containing the percentages of living cells and the values of the factor scores for PC1 and PC2.

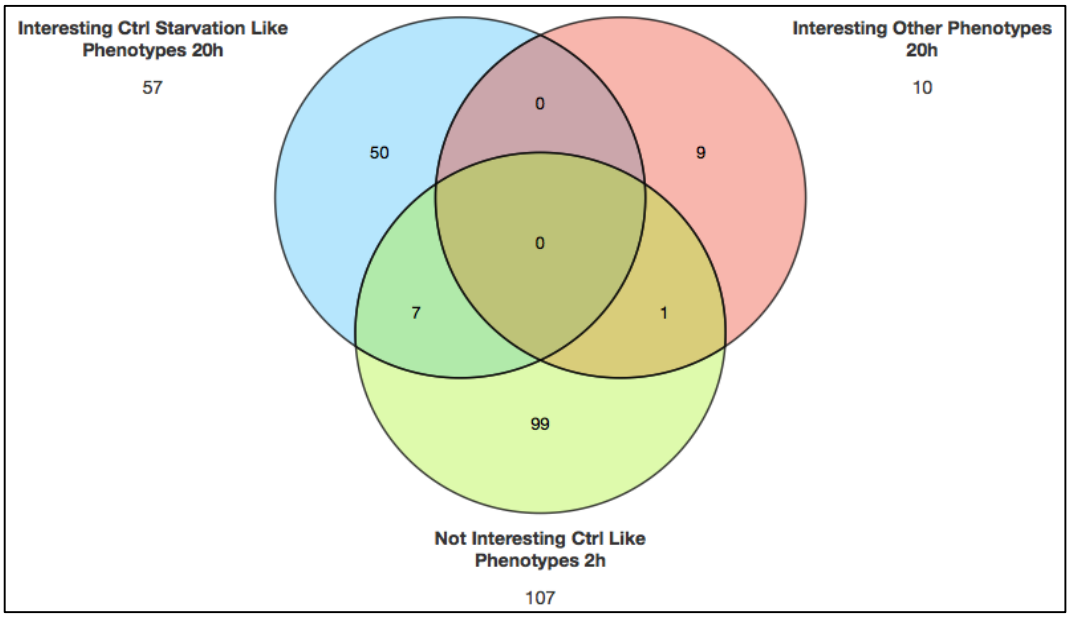


Figure 42. Analysis of the treatments administrated in normal conditions and able to produce effects considered as interesting only after 20 hours since their administration, because similar to the control starvation samples or because different from both control sample types.

Control Like Treatments considered Not Interesting after 2 hours but Interesting or different from both reference control samples after 20 hours									
Control Starvation Like Interesting Treatments 20 hours								Other Treatments 20 hours	
1	EAGRC_0,01mg/ml	3	EAGPS_0,05mg/ml	5	EAGCB_0,05mg/ml	7	ERKS_0,1mg/ml	1	EAGPS_0,1mg/ml
2	EAGRC_0,02mg/ml	4	EAGPD_0,01mg/ml	6	ERKL_0,01mg/ml				

Table 11. List of the treatments cultured in normal conditions and able to produce effects considered as interesting only after 20 hours since their administration, because similar to the control starvation samples or because different from both control sample types.

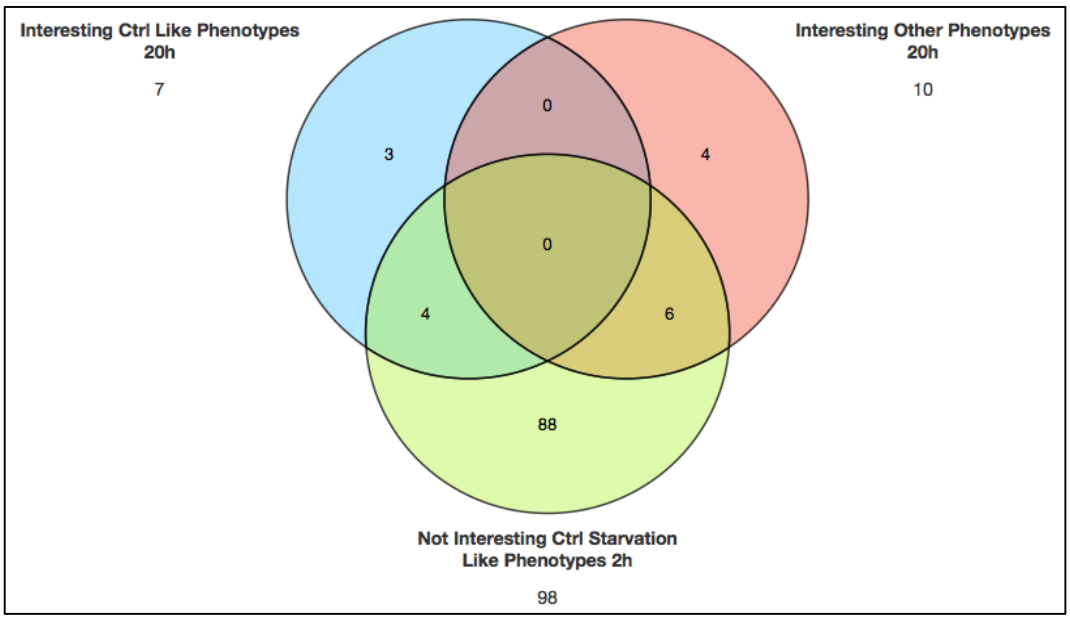


Figure 43. Analysis of the treatments administrated in starvation conditions and able to produce effects considered as interesting only after 20 hours since their administration, because similar to the normal control samples or because different from both control sample types.

Control Starvation Like Treatments considered Not Interesting after 2 hours but Interesting or different from both reference control samples after 20 hours											
Control Like Interesting Treatments after 20 hours				Other Treatments 20 hours							
1	EAGCT_0,02mg/ml_ Starv.	3	C-3B_0,05mg/ml_ Starv.	1	EAGPS_0,01mg/ml_ Starv.	3	EAGPS_0,05mg/ml_ Starv.	5	EAGPD_0,01mg/ml_ Starv.		
2	EAGCT_0,05mg/ml_ Starv.	4	*Stachys_betoniciflora*_0,1mg/ml_Starv.	2	EAGPS_0,02mg/ml_ Starv.	4	EAGAAlop_0,1mg/ml_ Starv.	6	ERKS_0,02mg/ml_ Starv.		

Table 12. List of the treatments cultured in starvation conditions and able to produce effects considered as interesting only after 20 hours since their administration, because similar to the normal control samples or because different from both control sample.

4.3.4.4 Analyses of the treatments producing effects interesting after 2 hours but changing their activity over time

Following the scheme used to carry out the analyses just reported in paragraph 4.3.4.3, also treatments which showed interesting effects after 2 hours and modified such effects over time have been detected. The results of the analyses have been reported in figures 44, 45, 46 and 47 through Venn diagrams composed by two and three circles and organized as explained in the former paragraphs. The first comparison have been reported in figure 44, containing a Venn diagram composed by three circles which report the number of the treatments administrated in autophagy-induced conditions and interesting after 2 hours since their administration because producing phenotypes comparable with those of the control samples taken as reference for physiological conditions (green circle), the number of treatments able to produce phenotypes different from both reference samples (light blue circle) and the number of those whose effects were considered toxic (faint red circle) after 20 hours since their administration. Among the 15 treatments whose effects produced after 2 hours were interesting, just 1 produced a phenotype different form the two control samples used as reference, while 5 of them showed effects considered as toxic after 20 hours since their administration. Regarding the remaining 8 treatments, 2 consisted in the control samples (with and without DMSO), 6 lost their activity and showed phenotypes comparable to those produced by starvation control samples and only 1 was able to keep the same activity after 20 hours (data not shown), thus confirming the data obtained from the former analyses and already reported in the previous paragraphs. The Venn diagram contained in figure 45 compared the numbers of the treatments administrated in physiological conditions which produced phenotypes comparable to those of the control samples taken as reference for starvation conditions after 2 hours (green circle) with those whose effects were different from both the control phenotypes (number reported into the light blue circle) and those producing toxic effects after 20 hours (number reported into the faint red circle): in this case, only 6 of the 72 treatments interesting after 2 hours acquired a cytotoxic potential over time while no one has produced effects different from both control samples after 20 hours since their administration. Among the remaining 66 treatments, 2 were the control samples references for autophagy-induced conditions (with and without DMSO), 23 lost their activity over time and 41 have showed the same interesting activity also after 20 hours since their administration (data not shown), in agreement with data previously obtained and reported. Concluding, the Venn diagram reported in figure 46 refers about the destiny of the 63 treatments showing effects considered as interesting because different from those produced by both the control samples taken as reference after 2 hours (number reported in green circle) by evaluating how many and which of them changed their activity over time. The comparison of such 63 treatments with those producing an activity interesting and comparable with one of the reference control samples after 20 hours (numbers reported in light blue and faint red circles), has produced the following results: the effects of 7 treatments interesting because different from both control samples after 2 hours resulted to be comparable with those produced by reference control samples for autophagy-induced conditions; no one was found to produce effects similar to the control sample reproducing the physiological conditions after 20 hours. The Venn diagram composed by two circles reported in figure 47 completes the analysis providing information regarding the remaining 56 “other phenotypes” and clarifies that 7 of them produced toxic effects after 20 hours. Among the remaining 49 treatments, 1 continued to produce effects different to both control samples, as reported in one of the previous analysis, while all the others lost such ability and became not interesting after 20 hours.

The list of the treatments name by name, selected and divided per categories through Venn diagrams, and reported in figure 44, 45, 46 and 47, have been reported in tables 13, 14 and 15; other versions of such tables, completed with the percentages of living cells and the values of the factor scores for PC1 and PC2, have been also reported in the section supplementary data (tables A17, A18 and A19).

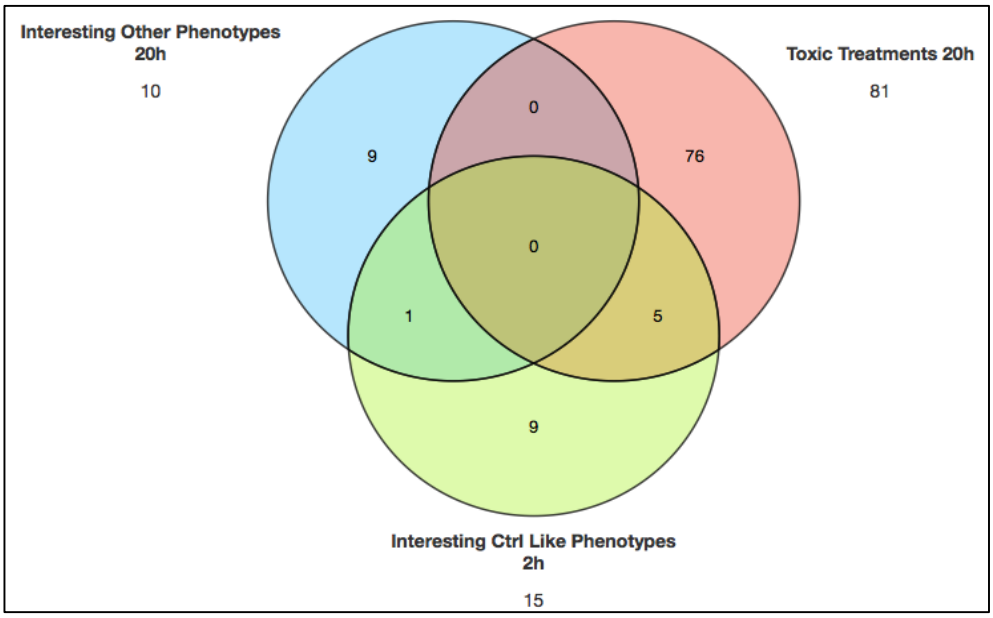


Figure 44. Analysis of the treatments administrated in starvation conditions and able to produce effects considered as interesting after 2 hours, because similar to those produced by the control samples cultured in normal conditions, but different form both control sample types taken as references or toxic after 20 hours since their administration.

Control Like Treatments considered as Interesting after 2 hours but different form both control samples or Toxic after 20 hours							
Other Treatments 20 hours		Toxic Treatments 20 hours					
1	EAGPS_0,1mg/ml_Starvation	1	EFIPS_0,1mg/ml_Starvation	3	C-3B_0,1mg/ml_Starvation	5	*Cousina_umbrosa*_0,01mg/ml_Starvation
		2	EAGCB_0,05mg/ml_Starvation	4	C-4B_0,05mg/ml_Starvation		

Table 23. List of the treatments cultured in starvation conditions and producing an effect considered as interesting after 2 hours but different form both control sample types taken as references or toxic after 20 hours since their administration.

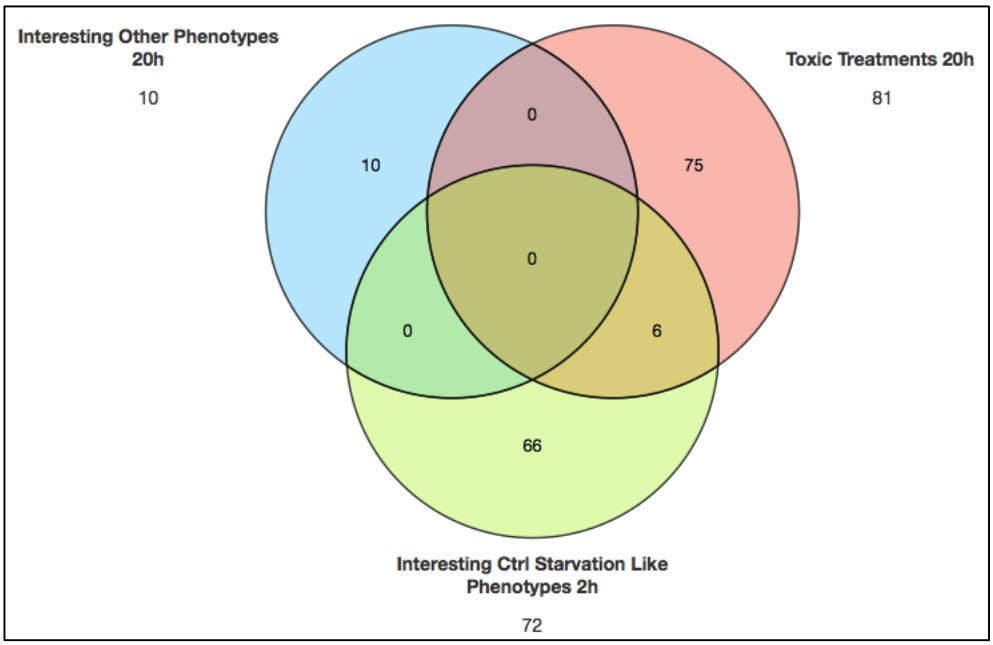


Figure 45. Analysis of the treatments administrated in normal conditions and able to produce effects considered as interesting after 2 hours, because similar to those produced by the control samples cultured in starvation conditions, but different form both control sample types taken as references or toxic after 20 hours since their administration.

Control Starvation Like Treatments considered as interesting after 2 hours but different form both control samples or toxic after 20 hours							
Other Treatments 20 hours		Toxic Treatments 20 hours					
/	/	1	ERAAlop_0,1mg/ml	3	*Scutellaria_scharistanica*_0,02mg/ml	5	*Schrophullaria_sp*_0,02mg/ml
		2	ERKL_0,1mg/ml	4	*Scutellaria_scharistanica*_0,1mg/ml	6	*Schrophullaria_sp*_0,1mg/ml

Table 14. List of the treatments cultured in normal conditions and producing an effect considered as interesting after 2 hours but different form both control sample types taken as references or toxic after 20 hours since their administration.

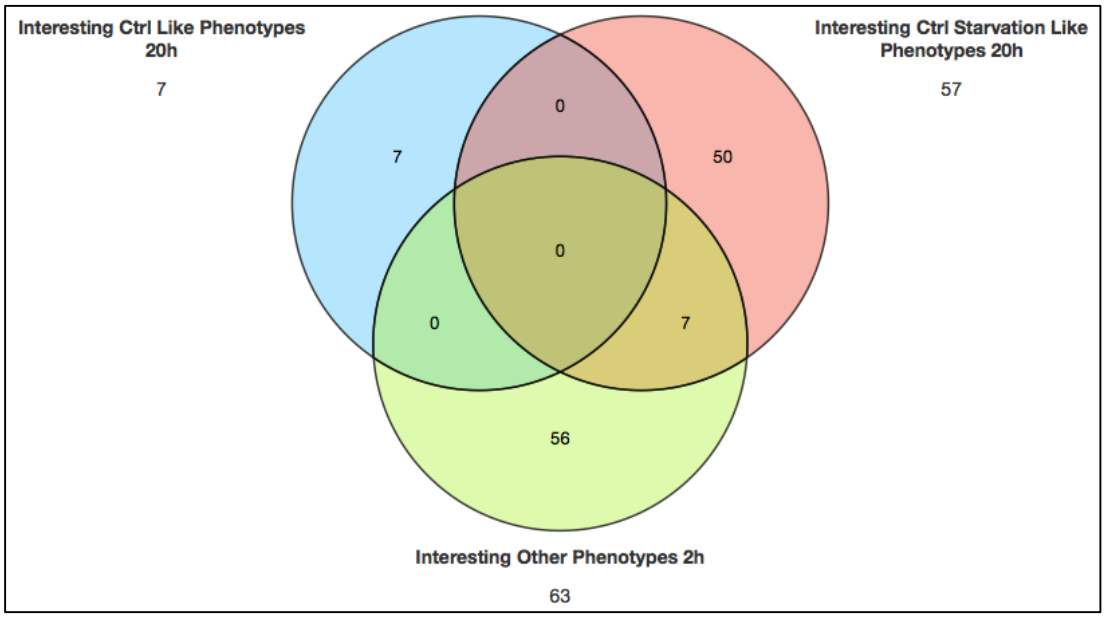


Figure 46. Analysis of the treatments considered as interesting because able to produce effects different from both control sample types after 2 hours but similar to one of the control samples after 20 hours since their administration.

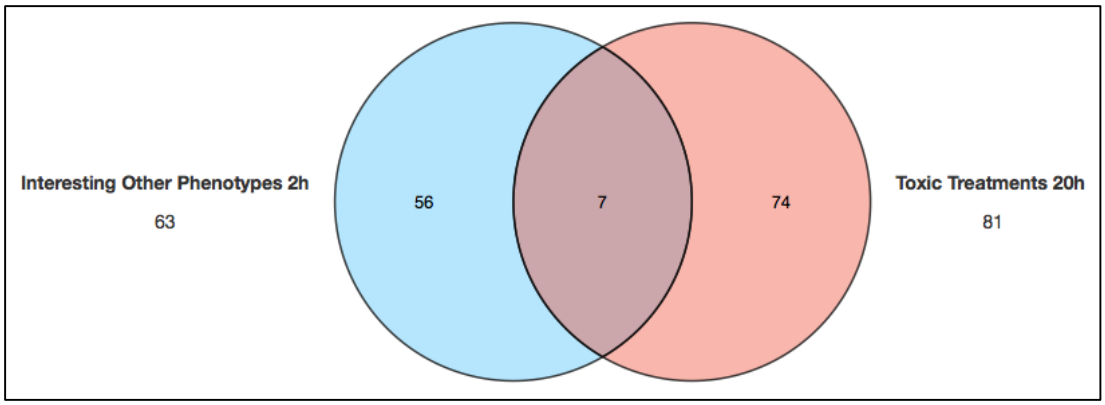


Figure 47. Analysis of the treatments considered as interesting because able to produce effects different from both control sample types after 2 hours which acquired a cytotoxic activity after 20 hours since their administration.

Treatments producing effects different from both reference control samples after 2 hours but comparable with one of the control samples or toxic after 20 hours					
Control Like Treatments 20 hours		Control Starvation Like Treatments 20 hours		Toxic Treatments 20 hours	
/	/	1	EAGAT_0,01mg/ml	1	EAGAAsia_0,02mg/ml_Starvation
		2	EAGAT_0,02mg/ml	2	EAGMD_0,1mg/ml_Starvation
		3	EAGAT_0,05mg/ml	3	EAGKL_0,1mg/ml_Starvation
		4	EAGAT_0,1mg/ml	4	ERPS_0,02mg/ml_Starvation
		5	EAGAU_0,01mg/ml	5	ERKL_0,05mg/ml_Starvation
		6	EAGAU_0,05mg/ml	6	Silene_oreina_0,1mg/ml_Starvation
		7	EAGAU_0,1mg/ml	7	Cousina_umbrosa_0,1mg/ml_Starvation

Table 15. List of the treatments producing effects different from both control sample types after 2 hours but similar to one of the control samples or toxic after 20 hours since their administration.

4.4 Comparison of effects produced by six extracts administered on both HeLa and SH-SY5Y cells

In this paragraph, the results concerning the comparative analysis of the effects of the extracts after administration on both HeLa and SH-SY5Y cell lines have been reported. This analysis, performed in parallel to the main one, has been carried out to investigate the effects produced by the extracts on autophagy in different cellular models and to evaluate if the approach developed for investigation on SH-SY5Y cells was also applicable on one other cell line. From a technical point of view, cells were firstly treated as explained in paragraph 3.4, then the datasets dimensions have been reduced by applying the PCA to obtain 2 Principal Components for each analysis and, in the end, the Euclidean distances among treatments were then measured and used to perform the Hierarchical Cluster Analysis (HCA), by which treatments were grouped in different clusters identified by cutting the dendogram at a specific height, including all the most similar within the same cluster.

Results about these investigations have been reported in the following pages: figures from 48 up to 53 display the screen diagrams concerning the PCA, dendograms referring the HCA and scatter plots showing the spatial distributions of the treatments administrated on SH-SY5Y cells for 2 and 20 hours, while figures form 54 up to 59 report the results concerning the same kind of analyses about treatment administrated for 2 and 20 hours on HeLa cells. Looking at such results reported in figure 48 the PCA performed on the data describing the treatments administrated for 2 hours on SH-SY5Y cells shows that the variance still considered after the reduction of the dataset dimensions is around 78,5%, while the variance still contained in the reduced dataset regarding treatments administrated for 20 hours on SH-SY5Y cells is almost 81,2%, as reported in figure 49. The results of the HCAs of data concerning treatments administrated for 2 and 20 hours on SH-SY5Y cells have been respectively reported in figures 50 and 51. While 4 clusters were enough to sort out all the treatments administrated for 2 hours in different clusters, depending on their Euclidean distances and maintaining the two control sets in different clusters, 5 clusters were instead needed to evaluate the distances among treatments administrated on cells for 20 hours, always keeping the normal and starvation control sets in different clusters. Further, figures 52 and 53 report the spatial distribution of the treatments depending on their effects produced after 2 and 20 hours, respectively. As evident, after 2 hours all the treatments tended closed to the control samples (highlighted in the graph) because, even if divided in 4 different clusters, the produced phenotypes were very similar. After 20 hours, when the effects became much more pronounced, one more cluster were needed to distinguish the phenotypes that, although most of them remained close to the control samples, were found to be positioned in more specific locations, with some of them significantly shifted at specific distances and directions: in particular, some samples seemed to be shifted away from the controls (highlighted in the graph) toward the high and the right side of the scatter plot.

The effects produced by treatments on HeLa cells were completely different. In fact, after the performance of the PCAs by which the variance still represented in the resized dataset resulted to be 79,1% for data regarding treatments administrated for 2 hours (figure 54) and 79,7% for those administrated for 20 hours (figure 55), the HCAs didn't allow to divide the two sets of control samples (Control and Control Starvation, with and without DMSO) in different clusters, despite dendograms resulting from both analyses (2 and 20 hours) were cut at a very low height. More specifically, dendograms reporting phenotypes produced by the treatments administrated for 2 hours has been cut at the 1,5% of its height and 26 different clusters were needed to sort the 52 samples (figure 56), with most of them placed very close to the control samples even if included in different clusters (figure 58); dendograms of the phenotypes concerning samples administrated for 20 hours has been cut at 0,1% of its height and the 52 phenotypes, mostly grouped again around those produced by the control samples (figure 59), were divided in 51 different groups with the controls included one by one in 4 different clusters.

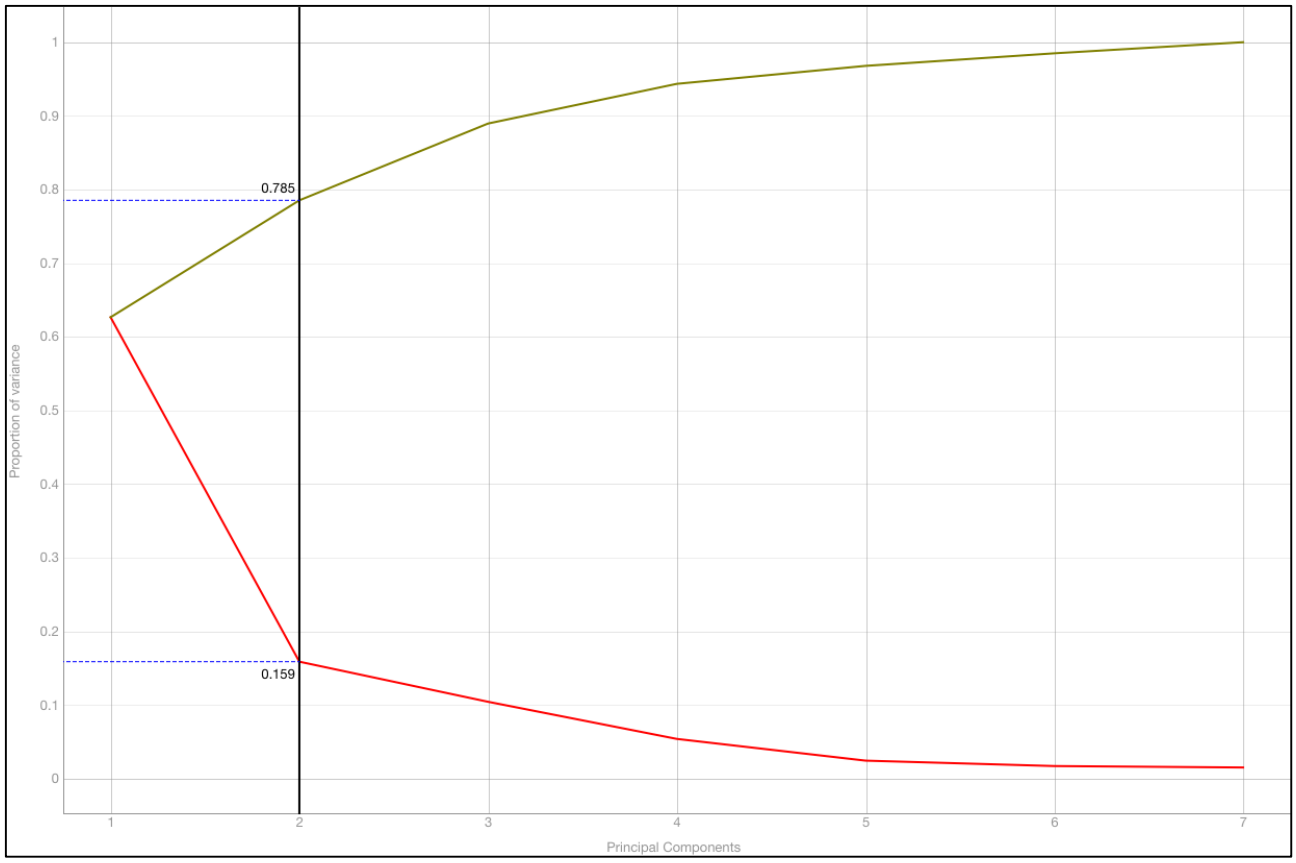


Figure 48. Screen-diagram of the Principal Component Analysis regarding dataset containing features measured from SH-SY5Y treated for 2 hours with the three extracts chosen for the analysis.

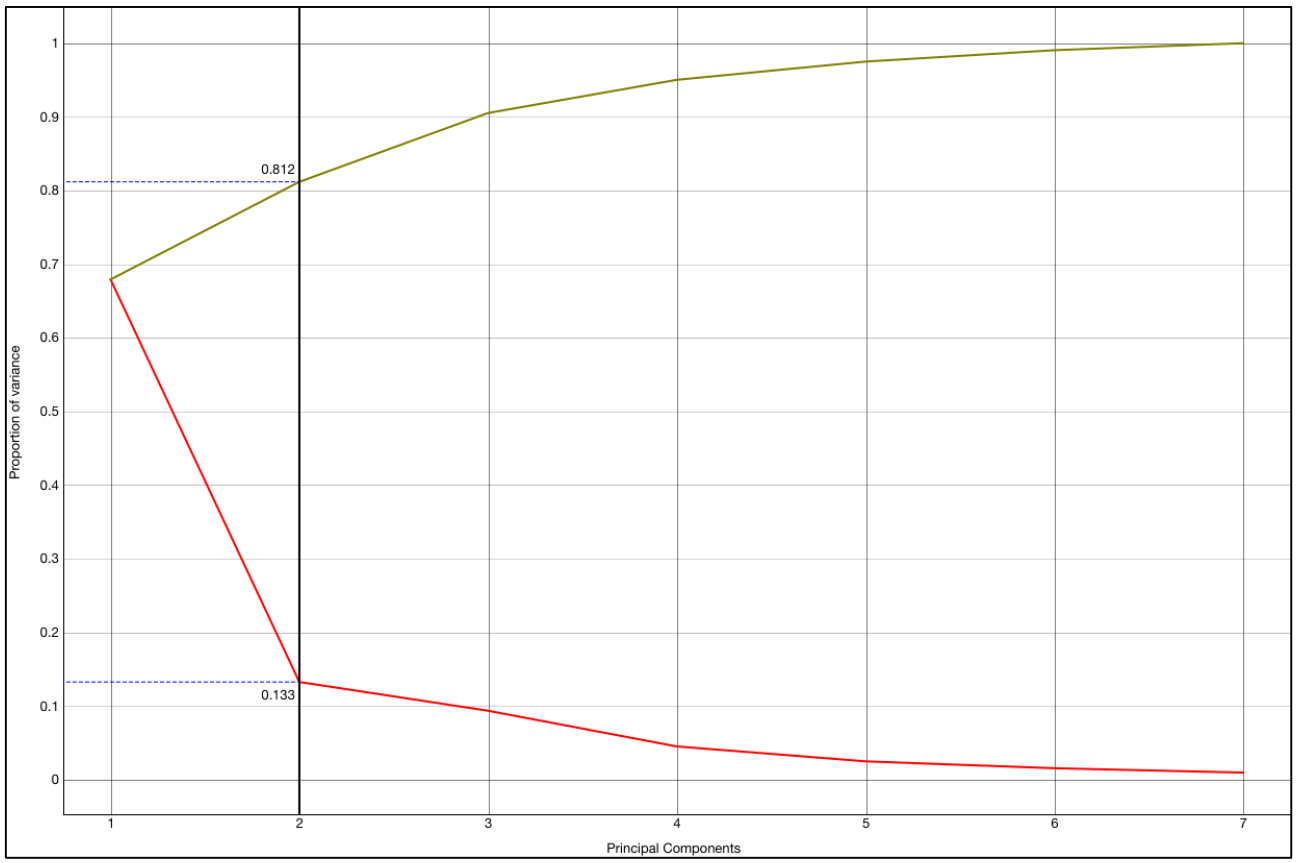


Figure 49. Screen-diagram of the Principal Component Analysis regarding dataset containing features measured from SH-SY5Y treated for 20 hours with the three extracts chosen for the analysis.

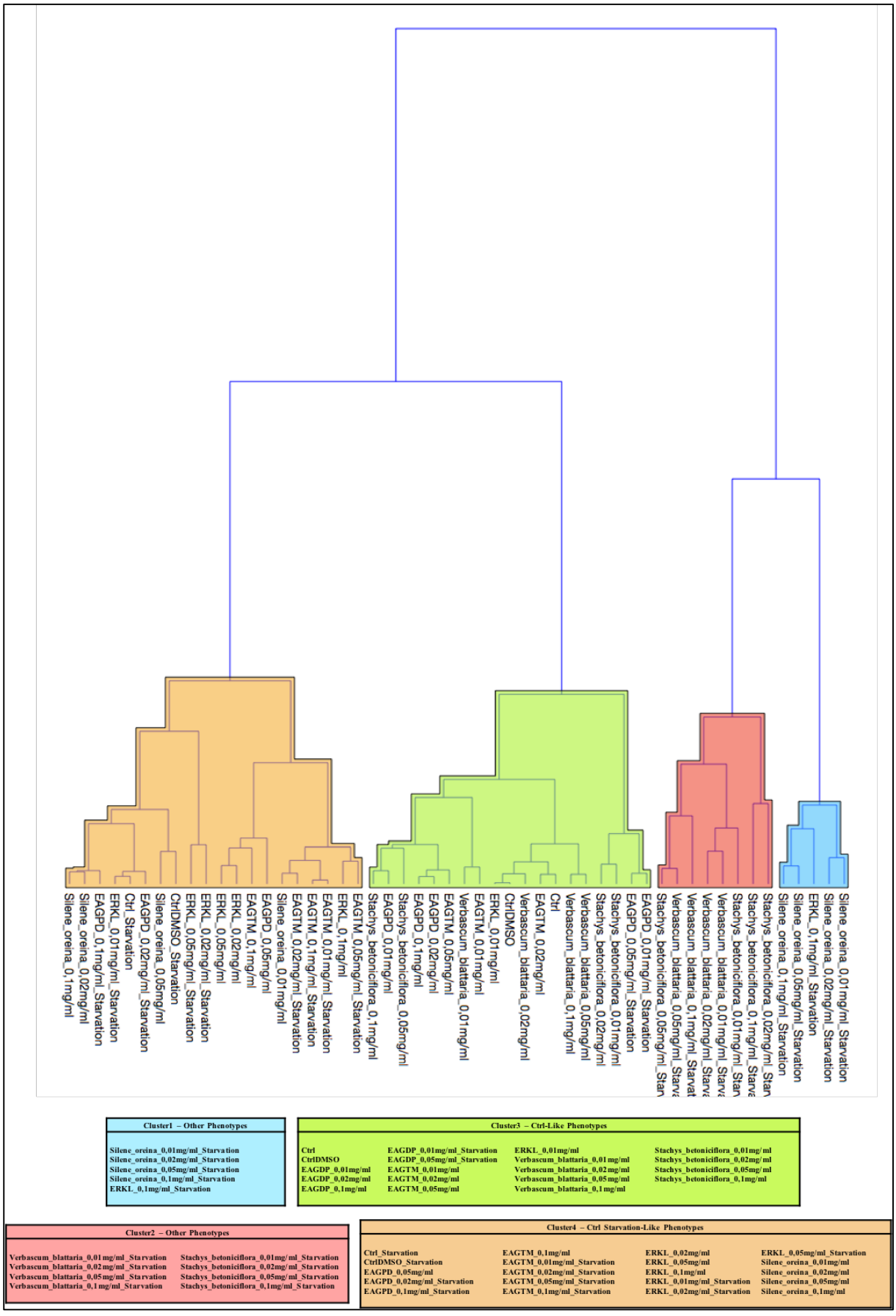


Figure 50. Hierarchical clustering analysis of the data regarding phenotypes produced on SH-SY5Y after 2 hours of treatment with extracts: the dendogram in the upper side reports the distribution of the different samples represented by every single leaf grouped basing on their Euclidean distances by using the Ward linkage strategy, while tables in the lower side report treatments contained in each obtained cluster.

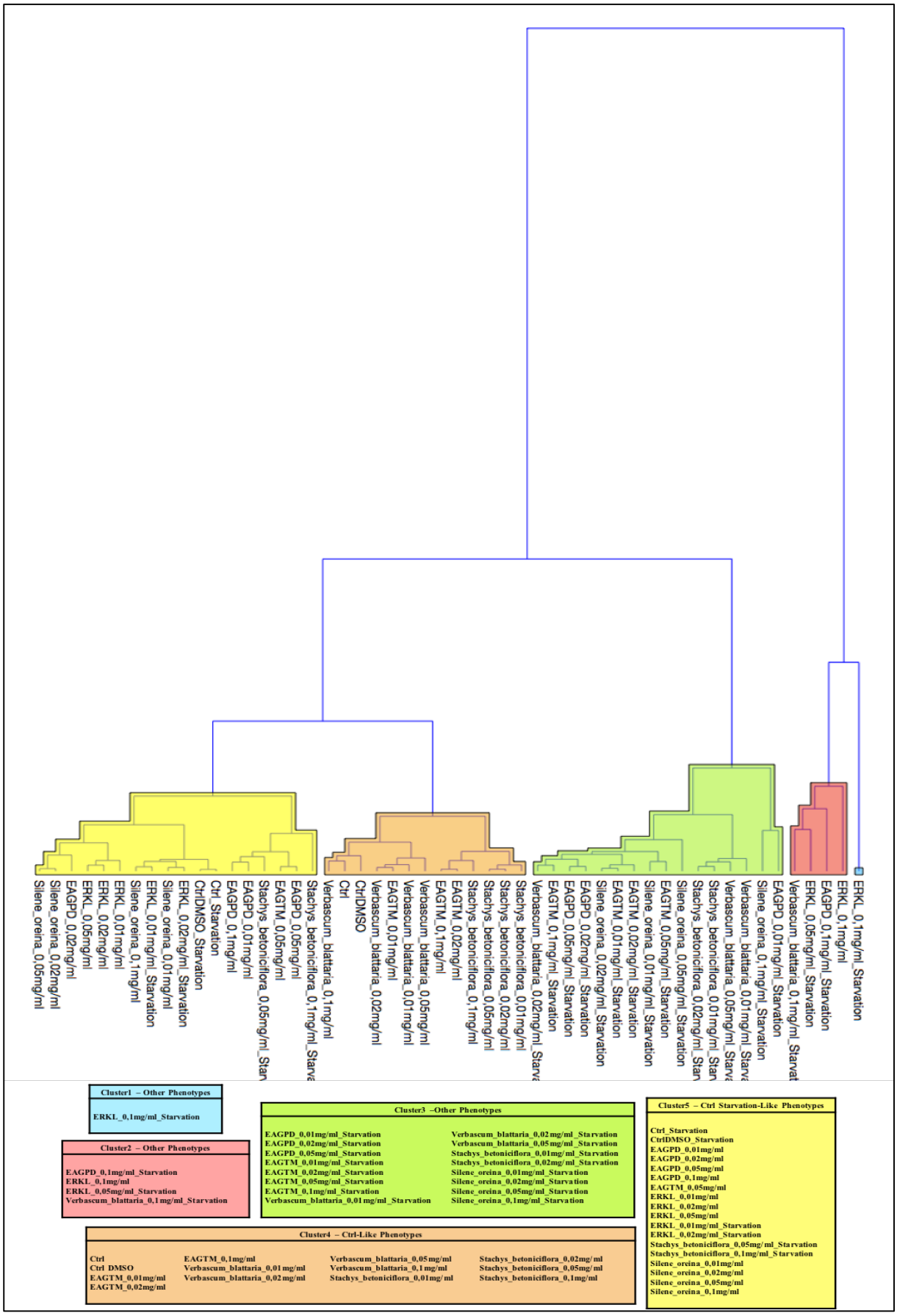


Figure 51. Hierarchical clustering analysis of the data regarding phenotypes produced on SH-SY5Y after 20 hours of treatment with extracts: the dendogram in the upper side reports the distribution of the different samples represented by every single leaf grouped basing on their Euclidean distances by using the Ward linkage strategy, while tables in the lower side report treatments contained in each obtained cluster.

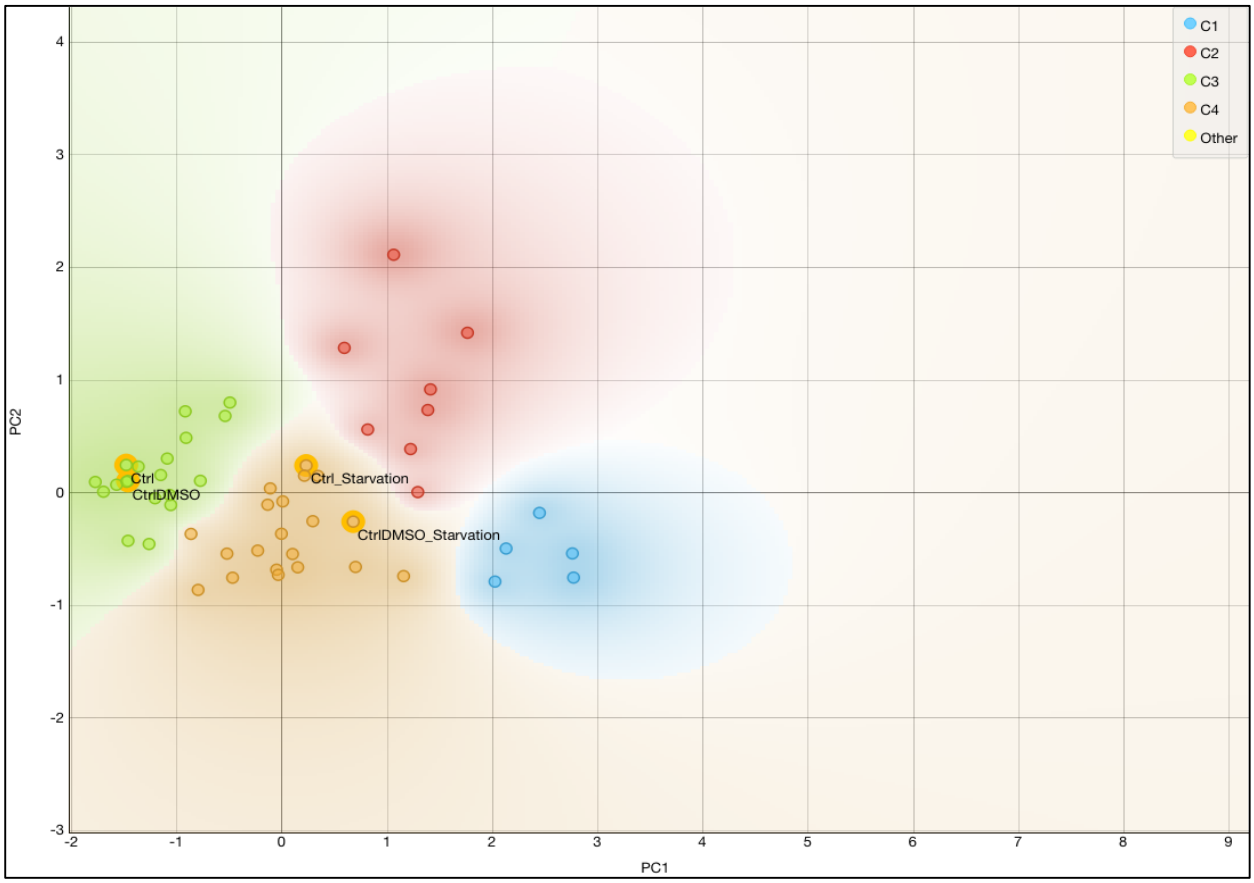


Figure 52. Spatial distribution on a 2-dimensional plane of the samples administrated for 2 hours on SH-SY5Y cells, displayed in a scatter plot in which the x- and the y-axes are defined by the PC1 and PC2, respectively.

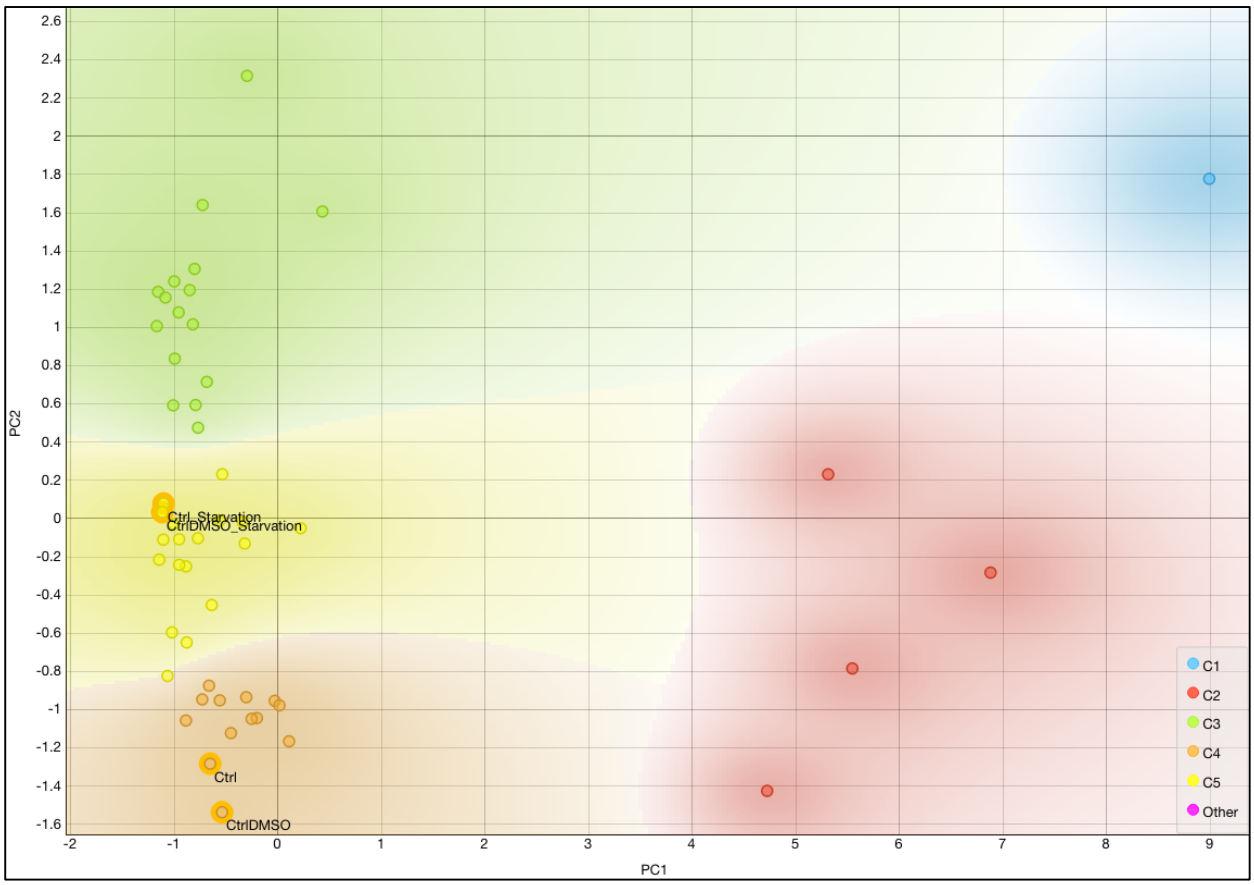


Figure 53. Spatial distribution on a 2-dimensional plane of the samples administrated for 20 hours on SH-SY5Y cells, displayed in a scatter plot in which the x- and the y-axes are defined by the PC1 and PC2, respectively.

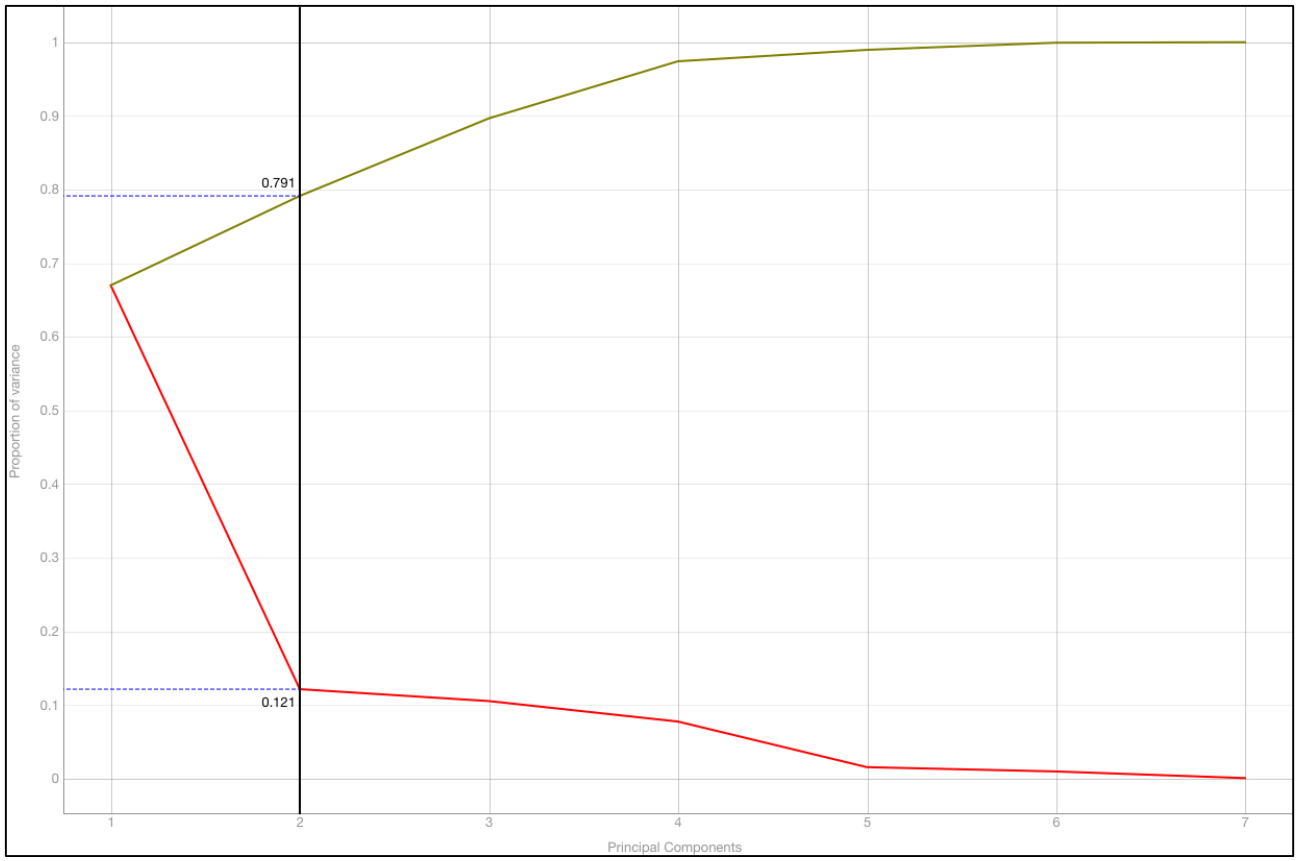


Figure 54. Screen-diagram of the Principal Component Analysis regarding dataset containing features measured from HeLa cells treated for 2 hours with the three extracts chosen for the analysis.

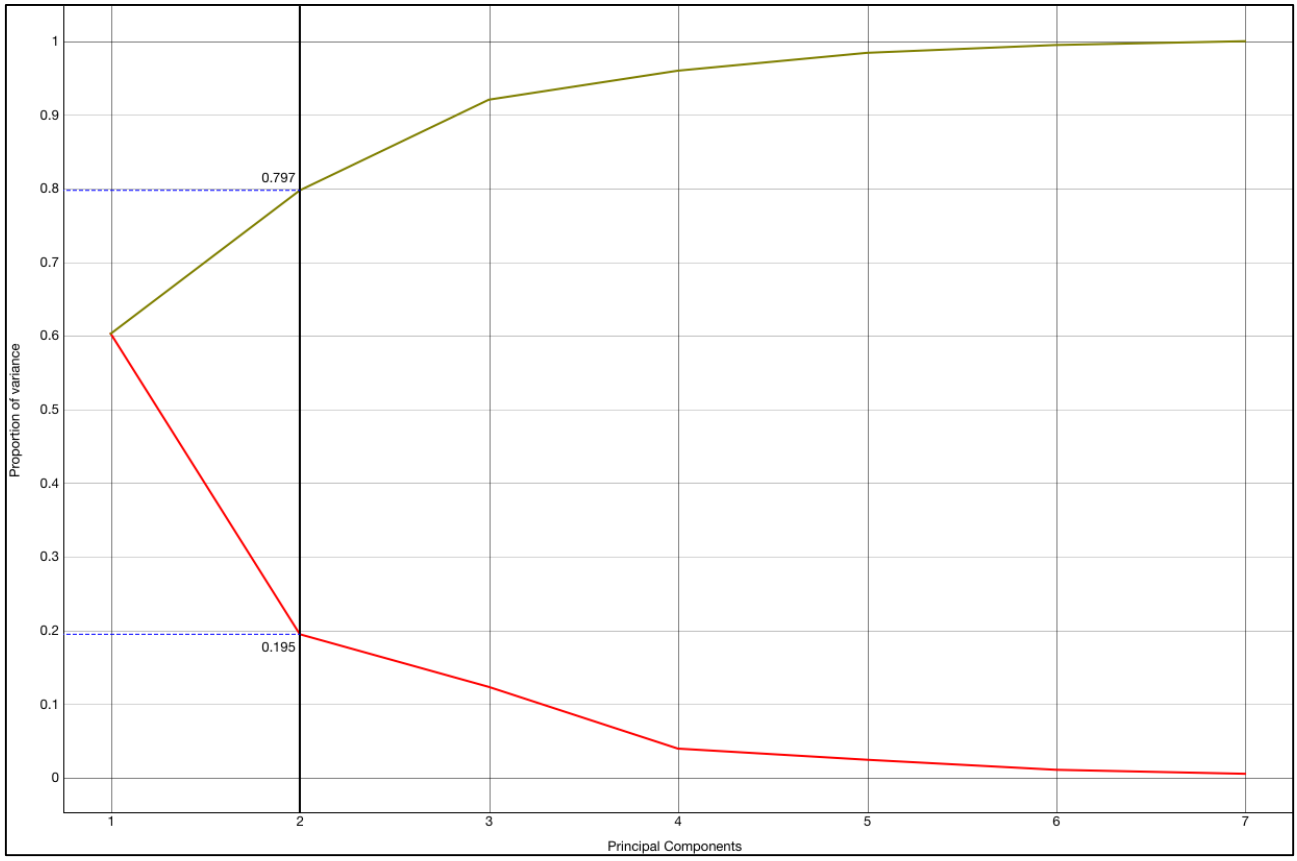


Figure 55. Screen-diagram of the Principal Component Analysis regarding dataset containing features measured from HeLa cells treated for 20 hours with the three extracts chosen for the analysis.

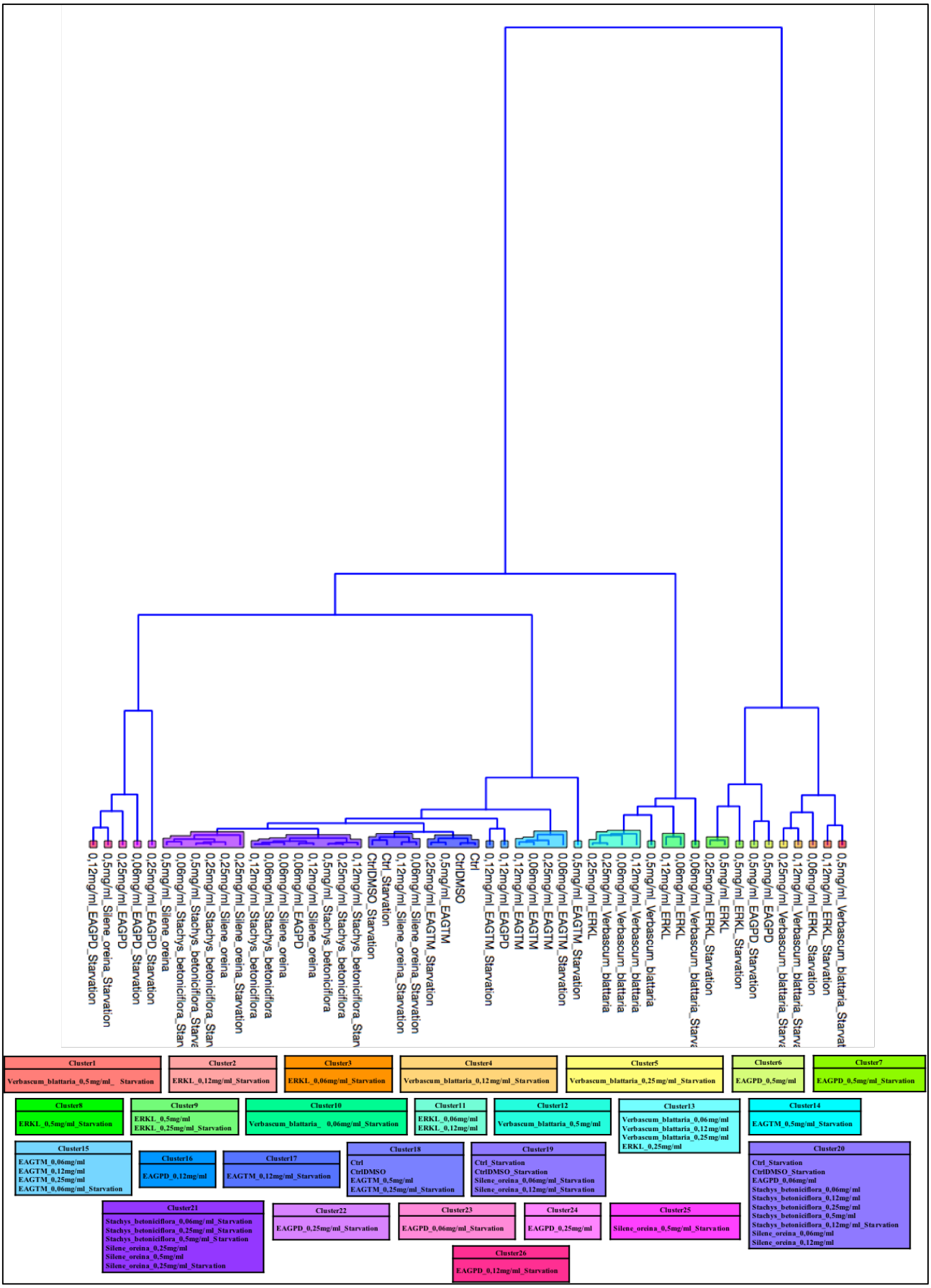


Figure 56. Hierarchical clustering analysis of the data regarding phenotypes produced on HeLa after 2 hours of treatment with extracts: the dendogram in the upper side reports the distribution of the different samples represented by every single leaf grouped basing on their Euclidean distances by using the Ward linkage strategy, while tables in the lower side report treatments contained in each obtained cluster.

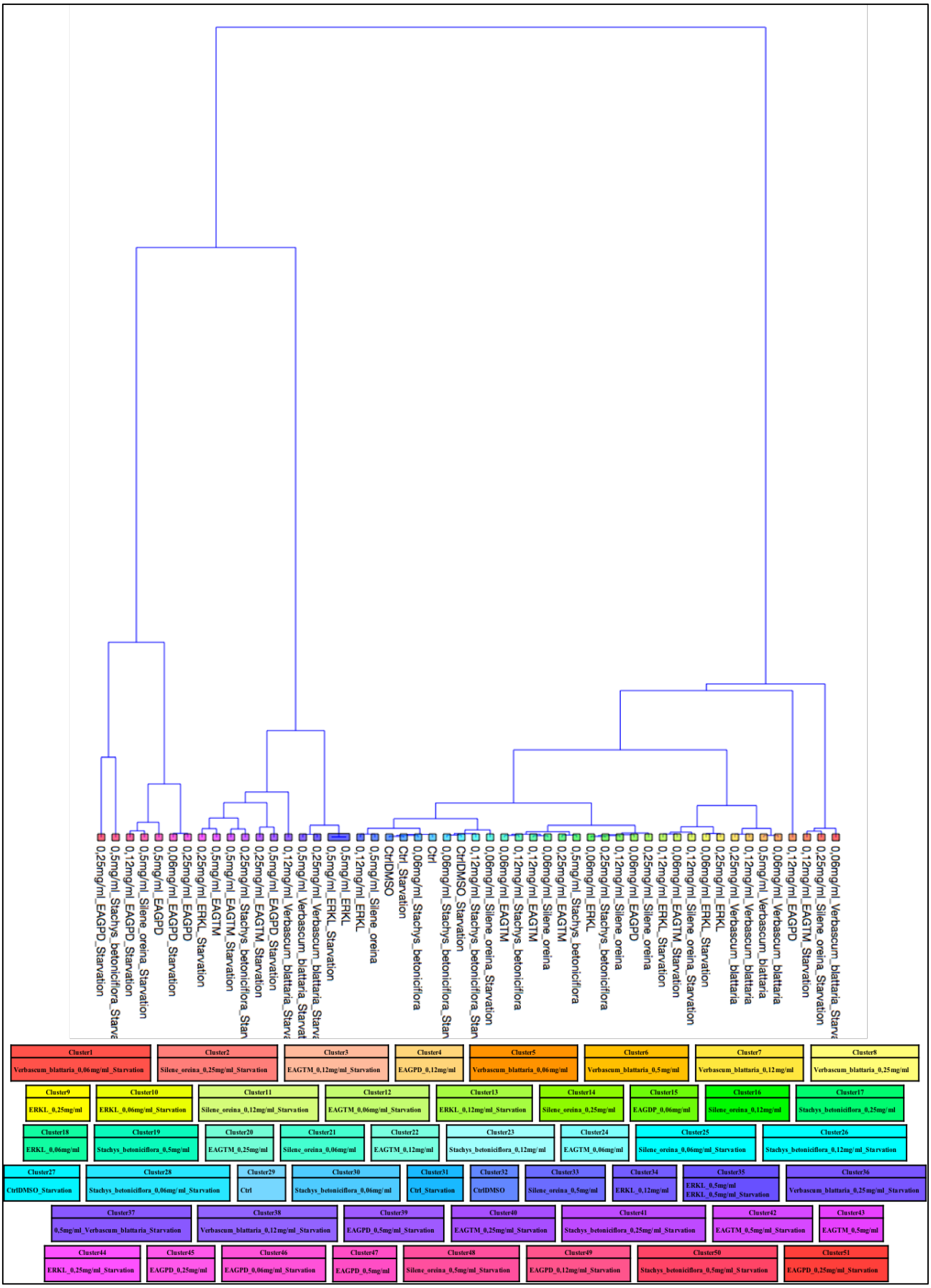


Figure 57. Hierarchical clustering analysis of the data regarding phenotypes produced on HeLa after 20 hours of treatment with extracts: the dendogram in the upper side reports the distribution of the different samples represented by every single leaf grouped basing on their Euclidean distances by using the Ward linkage strategy, while tables in the lower side report treatments contained in each obtained cluster.

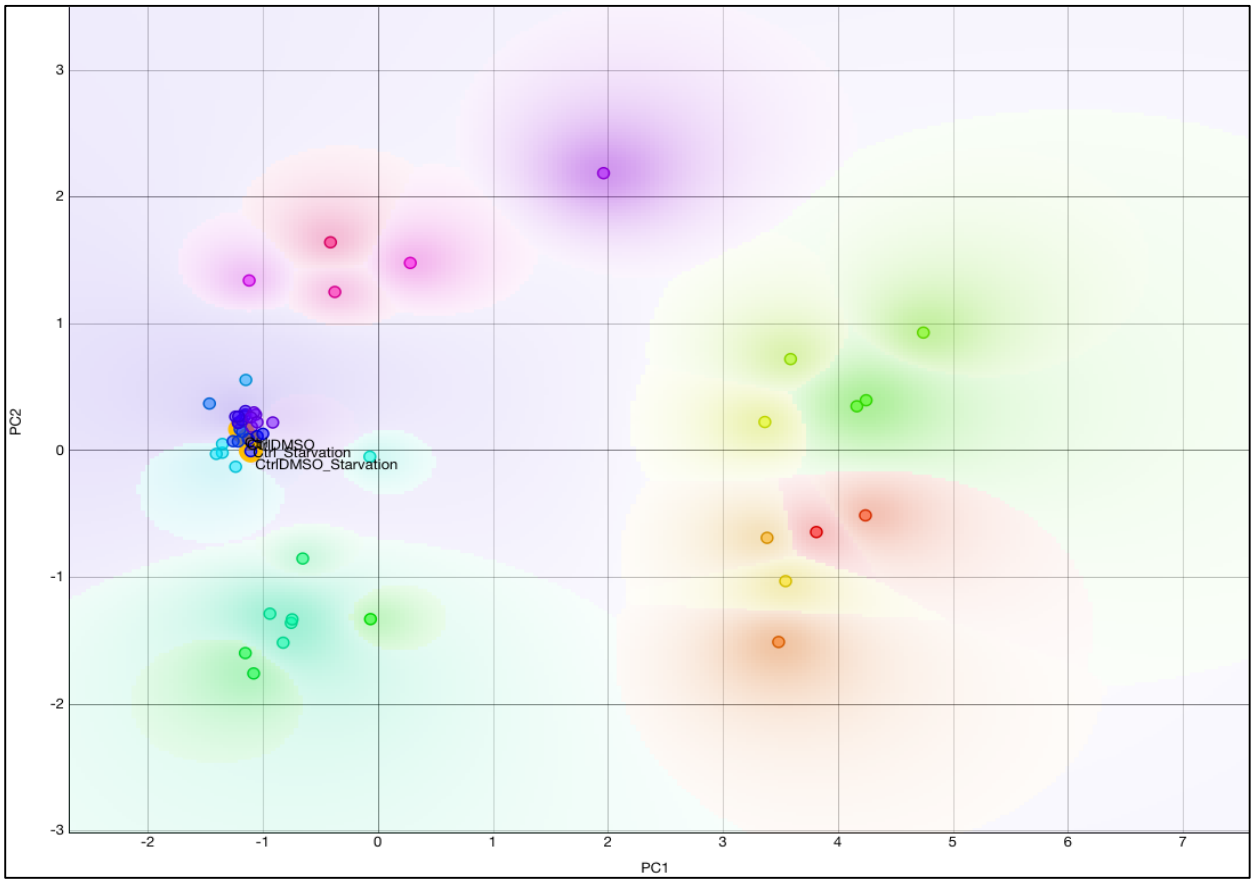


Figure 58. Spatial distribution on a 2-dimensional plane of the samples administrated for 2 hours on HeLa cells, displayed in a scatter plot in which the x- and the y-axes are defined by the PC1 and PC2, respectively.

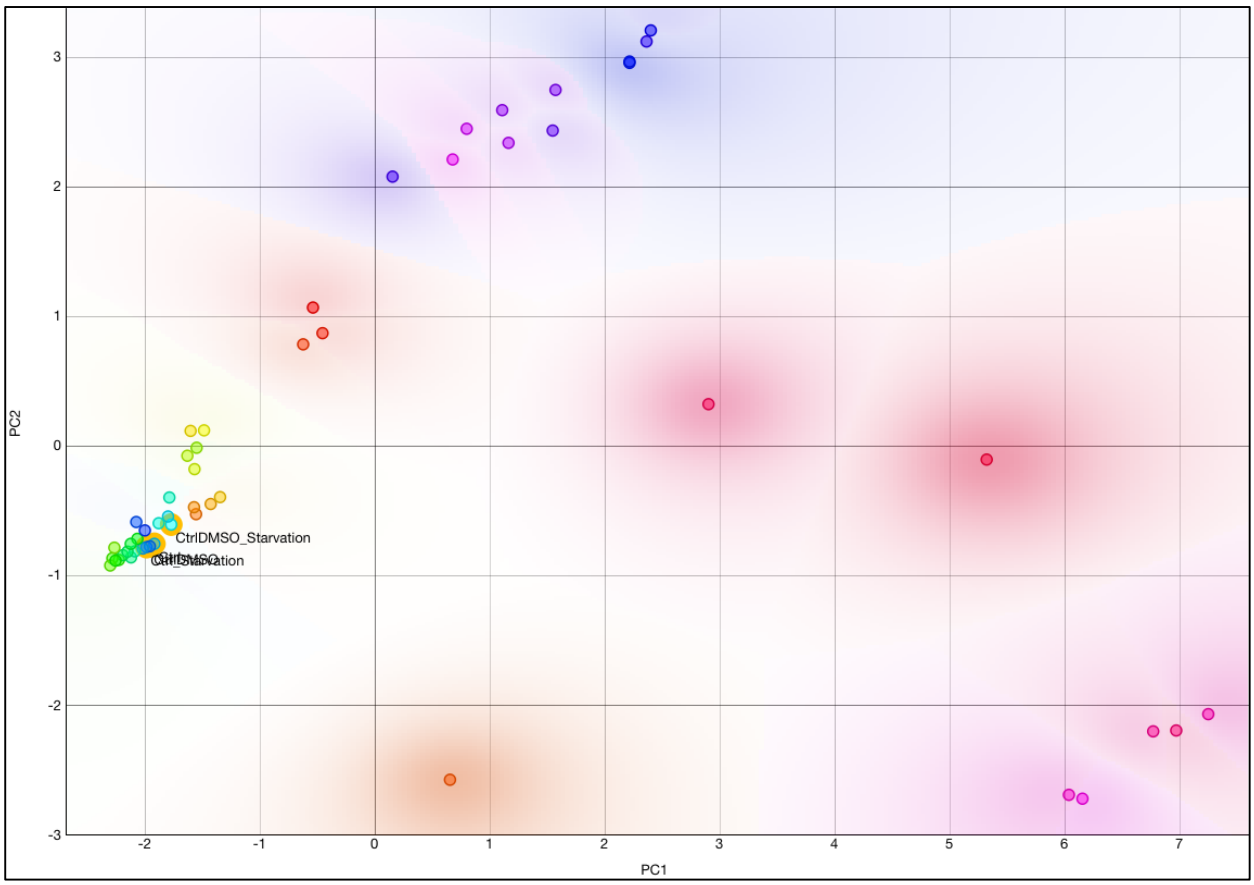


Figure 59. Spatial distribution on a 2-dimensional plane of the samples administrated for 20 hours on HeLa cells, displayed in a scatter plot in which the x- and the y-axes are defined by the PC1 and PC2, respectively.

5. Discussion

The present thesis work has been focussed on the investigation of the effects produced by 47 extracts on the autophagy pathway. Such extracts, prepared in different physical states (solid and semi-solid), have been received from researchers who have harvested the source organisms in different Countries in the world, as reported in paragraph 3.3: particularly, 13 came from Uzbekistan, 29 from Kazakhstan and the last 5 from Chile, among which one was obtained from an aquatic organism (ascidian).

Obviously, the evaluation of the effects of this large number of extracts following the classical approaches was supposed to be a very long and tedious procedure, which would have consisted in the application of different protocols in a huge number of serial steps, and therefore with a highly exposure to the possibility of mistakes and artifacts with consequent not reliable results, as already described within the "Introduction" chapter. For these reasons, efforts have been focussed on the development of a specific High Content Analysis (HCA) approach based on fluorescence and immunofluorescence assays, in order to select the extracts affecting the autophagy pathway in an easier and quicker way and also obtaining more reliable results. The High Content Analysis allowed to evaluate the effects of the extracts on the basis of the simultaneous evaluation of different parameters that were measured considering a corresponding number of cellular features determined by the markers, in turn chosen because able to describe and identify autophagic phenotypes: seen in this way, a great importance and responsibility can be attributed to the choose of the markers descriptive of the autophagy, as well as of their features, because it can determine the reliability and the accuracy of the results.

In the present work, two different cellular markers have been chosen in agreement to literature to identify and describe autophagic phenotypes: the first one was the Lysotracker, who selectively stains the lysosomes directly inside living cells, thus allowing to analyze features regarding the acidic compartments, which is one of the two cellular pathway involved in autophagy (De Vorkin & Gorski 2014; Klionsky et al. 2007; Klionsky et al. 2016; Liu et al. 2005; Pierzyńska-Mach et al. 2014; Yoshii & Mizushima 2017); the second one was the LC3B protein, present on the membrane of each single cellular vesicle/element involved in autophagic compartment, the other cellular pathway involved in the autophagy (Kabeya et al. 2000; Klionsky et al. 2016; Mizushima 2004; Mizushima et al. 2010).

Before to perform the High Content Analysis some important preliminary assays have been carried out, as reported in the following paragraph.

5.1 Preliminary analyses

Before to start to analyze the effects of the 47 extracts on autophagy some preliminary investigations were needed: it was necessary to choose the best cell model and then the best assay conditions, as for example the highest concentration of the solvent tolerated by the cells, the treatment dilutions and incubation timing for carrying out such investigations.

Concerning the choose of the best cell model, the three cell lines initially available (reported in paragraph 3.1) were evaluated: wild-type HeLa, LC3B-GFP HeLa and SH-SY5Y cells. The first step consisted in the evaluation of the best HeLa cell model to investigate autophagy between the two available: for this purpose, fluorescence and immunofluorescence assays were carried out in order to evaluate the better visualization of the LC3B protein, considered, a marker protein for autophagy investigation, as mentioned before. From a technical point of view, HeLa cells have been chosen as possible model because of their relatively easiness usage and their large dimensions, an important feature to perform fluorescence and immunofluorescence investigations for clear and easier data

interpretation. On the other hand, transfected HeLa cells added to these features the possibility to detect the LC3B protein avoiding the needing to use antibodies on fixed cells, thus reducing and simplifying the realization of the protocol steps. Looking at the results reported in paragraph 4.1.1, from the comparison of the images which displayed the LC3B protein in both cell lines, wild-type HeLa cells were considered to be the best cell model to investigate autophagy: in fact, despite the transfected HeLa cells ensured to detect the LC3B protein spots with a better resolution, the amount of the LC3B protein detected into the cells after different incubation times resulted always the same, conversely to what expected based on literature, with the highest peak of the LC3B protein amount approximately occurring two hours after the autophagy induction (Klionsky et al. 2016; Mizushima & Yoshimori 2007; Mizushima et al. 2010).

As reported in figure 14, LC3B protein spots into LC3B-GFP HeLa cells occurred approximately with comparable amounts after 1, 2, 4 and 6 hours since autophagy induction. Such unexpected results were attributed to the use of the LC3-GFP transfectants: the possible not perfectly stable transfection of the fluorescent protein, which could results in a not good production yield of the transfected protein (Kimura et al. 2007; Klionsky et al. 2016); the relatively weak bond between the GFP and the LC3B protein, besides its physiological cleavage due to the lysosomal activity after autophagosomes-lysosomes fusion event, which caused the dissociation of these two molecules and the subsequent presence of free GFP proteins into the cell cytosol (Gao et al. 2008; Hosokawa et al. 2006), an event well documented in literature and also exploited in some autophagy assays mostly based on immunoblotting analyses (Klionsky et al. 2106; Mizushima et al. 2010); further, also the quenching of the GFP fluorescence caused by the acidic pH environment of the inner of the lysosomes deserved to be considered (Mizushima et al. 2010); lastly, the possible formation of protein aggregates caused by the strong tendency of the LC3 protein to aggregate when overexpressed or co-expressed with other aggregates-prone proteins like the GFP (Kimura et al. 2007; Kuma et al. 2007), which can influence the final detection of the LC3B spots by altering their real number, as well as their area (Klionsky et al. 2106; Mizushima et al. 2010).

As reported in figure 15 it appeared evident that the amount of LC3B protein spots in the wild-type HeLa cells reached the highest peak 2 hours after autophagy induction and decreased progressively after longer incubation times.

After such evaluations and considerations, and because the investigation of the effects of the extracts will be performed using an automated confocal microscope producing high resolution images which allow to overcome possible problems joined to the low resolution of the images, wild-type HeLa cells were chosen as the best model between the two investigated to carry out further investigations of the autophagic pathway.

Wild-type HeLa and SH-SY5Y cells were further analyzed in order to select the best cell model based on the evaluation of their level of tolerance and the other effects caused by the treatment with several dilutions of DMSO. This preliminary analysis was very important for the next investigations because all the treatments have been diluted in a solution consisting in H_2O and DMSO before their administration to the cells: because DMSO was the only solvent able to dilute all the available extracts, it was very important to establish the highest dilution to administrate without causing any unpleasant effect on the two cell lines. For this purpose MTT assays were carried out on both cell lines after the administration of several DMSO dilutions for 2 and 20 hours to evaluate the possible adverse effects on the base of the percentage of actively growing cells produced by each treatment compared with a control sample. Further, the analysis has been performed by adding treatments in both physiological and autophagy-induced culturing conditions, as well as considering the effects produced after shorter and longer incubation timing to simulate the same conditions that will be used to perform the analysis of the effects of the extracts on autophagy pathway. Due to the different characteristics of the investigated cell lines, like for example genetic and metabolic characteristics or cellular membrane thickness and

permeability (ATCC n.d. a,b), two different series of solutions containing serial DMSO percentages have been chosen and the desired dilutions have been then deduced by comparing the percentages of living cells detected after both time durations for each treatment. From the results reported in bar plots contained in figures 16 to 19, it resulted that HeLa and SH-SY5Y cells were differently responsive to the DMSO dilutions tested as expected: more specifically, 0,25% was considered as the highest DMSO percentage useful for administration of the extracts on HeLa cells (figures 16 and 17), while 0,1 % was the DMSO percentage chosen for the administration on SH-SY5Y cells (figures 18 and 19). Such percentages have been determined considering the highest dilutions of DMSO administrated to the cells which produced a mortality rate considered as not too high (lower than 10-15%) if compared to the specific reference control sample. In this manner, the DMSO dilutions were chosen by selecting the percentages that were at the same time the lowest possible which allowed to dissolve the extracts and the highest possible which assured to limit the harmful effects.

A more careful analysis of the results, however, revealed an important effect caused by the induction of the autophagy through the starvation conditions: as evident from the observation of the bars related to the control samples, the incubation of the HeLa cells in starvation conditions produces a mortality rate considered as too high (higher than 10-15%) if compared to the normal cultured cells, especially after 20 hours, conversely to the SH-SY5Y cells, whose mortality rates after both 2 and 20 hours resulted to be more similar to the control for physiological conditions. Such evidences have led to plan another experiment to check for the effects caused by the starvation conditions, with and without the DMSO percentages previously established (0,25% for HeLa and 0,1% for SH-SY5Y) and after the incubation for different time durations (1, 2, 3, 4 and 20 hours), on both the cell lines. Observing the results reported in figures 20 and 21 it has been possible to confirm that HeLa cells appeared to be more susceptible than SH-SY5Y to the negative effects caused by the starvation, especially when treatments were administrated on cells in presence of DMSO for time durations longer than 2 hours. Based on this evidence, SH-SY5Y cells were considered as the best model and thus chosen to investigate about the ability of extracts in interfering with the autophagy pathway.

5.2 Extracts effects investigations

The effects produced by the extracts on the autophagy pathway have been investigated by evaluating phenotypes produced by different dilutions of each treatment, administrated on SH-SY5Y cells in two different culturing conditions for shorter and longer time durations to evaluate their effects over time. Particularly, treatments for each extracts were diluted in complete (10% FBS) and incomplete (1%FBS) medium as reported in paragraph 3.4, to simulate respectively physiological and autophagy-induced conditions, and then administrated next to control samples for both culturing conditions on the cells for both time durations chosen as check-points for autophagic activity on the base of the information suggested in literature (Klionsky et al. 2016; Mizushima et al. 2010).

As already introduced, the evaluation of the effects produced on autophagy pathway in SH-SY5Y cells have been performed by considering simultaneously data regarding seven different phenotypes, defined by the two autophagic marker previously chosen (LC3B and Lysotracker), deduced from the observation of different cellular features that have been listed below: 1) the percentage of living cells detected in each sample; 2-3) the percentage of cells which acidic and/or autophagic compartments resulted to be activated; 4-5) the number of lysosome and/or LC3B protein spots detected per cells; 6-7) the area occupied by the lysosome and/or LC3B protein spots. Such features, which were referred to the effects produced by each single treatment, described the status of the nuclei, which can be used to speculate about the toxicity effects, as well as lysosomal (or acidic) and autophagosomal compartments, which

constitute the autophagic pathway, as already well explained in paragraph 4.2, and were taken in consideration also thanks to the information found in literature (Badr et al. 2011; Balgi et al. 2009; Chu et al. 2009; Frankel et al. 2011; Kabeya et al. 2000; Klionsky et al. 2007; Klionsky et al. 2016; Liu et al. 2005; Mizushima 2004; Mizushima et al. 2010; Petibone et al. 2016; Qadir et al. 2008; Tang et al. 2010; Yoshii & Mizushima 2017).

However, before to analyze the effects of the treatments based on the phenotypes defined by these seven features, samples were firstly selected based on their cytotoxic potential: treatments associated to mortality rates considered too high (greater then 50%) were discarded, thus avoiding to waste time and resources in the following analysis of those producing phenotypes resulting at the same time interesting as well as dangerous (depending on the cytotoxic activity of natural products contained inside). For this purpose, the cytotoxic potential of the treatments has been evaluated based on the data regarding the living cells detected in each sample. This kind of features, already reported before among those considered for the analysis of the effects on autophagy pathway, has been therefore exploited to deduce two different parameters: such data were expressed as percentages of the amount of living cells detected for the control sample (assumed as the 100%) and this parameter, considered as descriptive of the toxicity rate produced by each treatment, allowed to discard "toxic" treatments as explained above; in the second case the amount of living cells detected for each sample has been compared to the total amount of cells, living and dead, detected for the same sample and considered as the 100%, thus evaluating the mortality rate produced by each treatments as a phenotypes associated to the autophagy. The reason behind the choose to consider the cytotoxic potential of the treatments as a phenotypes descriptive of their effects on autophagy, even after discarding those considered to have a too high cytotoxic potential, was due to the observation that autophagy and cell death are cellular processes closely related each other (Debnath et al. 2005; Fitzwalter & Thorbun 2015; Galluzzi et al. 2009; Gozuacik & Kimchi 2007; Lin & Baehrecke 2015; Petibone et al. 2016; Xia 2011; Yonekawa & Thorbun 2013) and the evaluation of both these aspects is needed in order to perform a complete analysis. The strategy developed to perform the present analysis was focussed on the assumption of the toxicity as one of the effects involved in the range of the phenotypes describing the autophagic activity, but at the same time avoiding to waste time and resources in the analysis of the treatments which have showed a too high cytotoxic potential.

However, all the features described above were detected and quantitatively measured by using an image analysis software (see paragraph 3.10), and the resulting data (with the exception of the percentage of living cells produced by each treatment and referred to the control samples) have been analyzed simultaneously to evaluate the phenotypes produced and find out those whose have produced interesting effects on autophagy.

Based on the measurements described above, a strategy to evaluate the effects of the extracts has been developed, and some important preliminary analyses were needed, as reported in paragraph 3.11. An important preliminary step was the normalization of the numerical values, which was required in order to make all the measurements comparable to each other. Obviously, all the measurements coming from different kind of features and obtained in distinct ways resulted to be very diverse, especially for the way in which they were scaled, and such difference made their comparison practically impossible. In this view all the measurements for each features were normalized by calculating the Z-Score, which means that all the numerical values were transformed (by using the mean and the standard deviation calculated for each dataset) in equivalent values which were distributed on the same numerical scale (around the value "0") and thus comparable to each other. After the normalization, each different treatment, originally represented by a dataset of values, have been better defined in an easier form by calculating the median values for each sample: the median value has been preferred (with respect the mean value) in order to reduce the possible drift of the measurements caused by the eventual presence of outlier values (results reported in table A1 and A2). After the calculation of the median values,

from the seven different datasets for each analysis (each containing a huge amount of numbers) data regarding phenotypes produced by the treatments with the extracts were included in just one dataset for each analysis, containing seven series of the median values resulting from each original dataset. More precisely, in the present analysis two datasets (consisting in values regarding treatments administrated for 2 and 20 hours), each containing 380 values (consisting in four control samples in addition to four different concentrations of each sample diluted in complete and incomplete medium) were produced and then used to carry out further analyses.
After that, another important preliminary step consisted in a further reduction of the dimension of the datasets, in order to make the investigation of the measured parameters easier and thus allowing the interpretation of the phenotypes produced by the treatments. Both the datasets previously obtained, containing data referred to the analysis of the effects produced by the treatments after 2 and 20 hours since their administration, were submitted to Principal Component Analysis (PCA), which allows to reduce their dimensions by discarding the amount of variance contained within data and considered not important for the analysis, as explained in paragraph 4.3.1: from the seven series of measurements obtained from each dataset, directly reflecting the seven features initially analyzed, just two new series of new values (whose factor scores reported in table A3 and A4) containing a part of the total starting variance, which consisted respectively in the 63 and the 68,5% of the total variance for 2 and 20 hours investigations, were obtained and then used to distinguish the autophagic phenotypes produced by the treatments. These two series of data, named "principal components", consisted in completely new values disposed in a virtual space created by the analysis and reproduced the most important fraction of the variance associated to the original seven series of data. The possibility to describe data using just 2 features proved to be very important because it has allowed to arrange the treatments in a space defined by two dimensions, defined by the Principal Components, positioning them according to their similarity, and thus making the interpretation of the results very easier and better.

After the preliminary steps, data were ready to perform the analysis of the autophagic phenotypes induced by the treatments through two "Hierarchical Cluster Analysis" (HCA), one for each dataset (2 and 20 hour treatments), carried out considering the factor scores obtained from the PCA. All the samples were linked each other based on the effects produced on autophagy using the "Ward linkage-method", which means that they have been aligned considering the smallest possible variance among them, and then clusters of treatments were defined by cutting dendograms to obtain the smallest number of clusters needed to sort out the two sets of control samples (Control and Control DMSO; Control Starvation and Control Starvation DMSO) in different groups, as evidently reported in the dendograms contained in figures 26 and 28. Following this approach, samples were divided based on their distances, which reflect their similarity, then grouped considering the control samples as reference and identifying them on the basis of their effects on the autophagy: one cluster contained treatments producing phenotypes similar to those produced by the control samples for normal conditions, in another there were treatments producing phenotypes similar to those produced by the control samples for autophagy-induced conditions and, lastly, two different clusters included treatments producing a phenotypes different form both control samples. In a such context, a deeper analysis of these groups has allowed to find out which phenotypes have produced the most interesting effects. The reasons why such treatments were considered interesting are evident: i) treatments administrated in physiological conditions and included into the cluster containing control samples reproducing the autophagy-induced conditions were interesting because considered as containing natural products able to induce autophagy, or at least to increase the autophagy rate; ii) conversely, natural products contained into treatments administrated in autophagy-induced conditions and included into the same cluster with controls for physiological conditions were considered able to inhibit or at least reduce the rate of the autophagy process; iii) treatments fallen into clusters that didn't contain any control samples were instead considered as interesting because

able to produce phenotypes different from both physiological and autophagy-induced conditions, thus resulting potentially interesting for autophagy as well as for investigations of possible effects in other fields of study. Moreover, because each sample has been defined by just two principal components, it has been possible to plot data on a bi-dimensional plane allowing to evaluate the effects of the treatments on autophagy also based on their spatial distribution, as previously introduced, leading to even more interesting observations as displayed in scatter plots as in figures 27 and 29. Observing the results based on their spatial distribution, it clearly appeared that, with the exception of one, most of the phenotypes produced by treatments administrated for 2 hours were placed very closed to those produced by the normal and starvation control samples, even if some of them were included in a cluster not containing any control samples. Such graphical analysis has proved that the distances among samples were short and hence, although included in different clusters the phenotypes produced by most of the treatments were similar each other. The treatment that produced the phenotypes found to be farthest from everyone else on the scatter plot (extract of Schrophullaria sp diluted at 0,1 mg/ml in autophagy-inducing medium), instead, was considered as the only one worthy of attention, even if such long distance could possibly reflect a cytotoxic activity. The picture of the situation was very similar for treatments evaluated after 20 hours since their administration: most of the phenotypes were still distributed around those produced by the control samples, but in this case the phenotypes different from those produced by the control samples (and hence included in different clusters) seemed to be shifted away towards the opposite part of the graph, occupying larger areas where they seemed quite sparse. Observing the graphical analysis of the results it was clear that treatments administrated for longer times produced better defined phenotypes, that the samples included in clusters without control samples resulted to produce phenotypes increasingly more different and that those included in the clusters containing at least one of the control samples maintained their strict similarity to the respective reference phenotypes.

The analysis resulted more interesting after the evaluation of the mortality rate associated to each treatment: as previously introduced in paragraph 4.3.3, the threshold value to evaluate toxicity has been set at the 50% and, making a very rough selection, all the treatments producing a mortality rate greater then 50% have been considered as toxic and discarded before the final analyses because of their harmful effects which could somehow influence also effects observed on the autophagic phenotypes. After this selection, by which 29 treatments administrated for 2 hours and 81 for 20 hours were discarded, all the phenotypes have been displayed again in scatter plots (reported in figures 30 and 31) for further analysing their distribution. As it was possible to expect, despite the discarded phenotypes resulted to be almost equally distributed into the different clusters, they constituted the larger part of the samples which were farthest to the control sample, confirming the presentiment that the cytotoxic activity could be a very influencing factor for autophagy (Debnath et al. 2005; Fitzwalter & Thorbun 2015; Galluzzi et al. 2009; Gozuacik & Kimchi 2007; Lin & Baehrecke 2015; Xia 2011; Yonekawa & Thorbun 2013). Therefore it was clear that the results of the former analysis wouldn't be trustworthy without considering the mortality rate associated to each treatment, as particularly confirmed by the case of the extract of *Schrophullaria sp* diluted at 0,1 mg/ml in autophagy-inducing medium that was initially taken in consideration and then discarded after the last selection.

After the described analyses, treatments were further investigated and sorted out based on their effects over time, which were evaluated by comparing the effects produced by each sample 2 and 20 hours since their administration. Through this analysis, treatments were sorted out in different categories depending on their activity: i) those showing the same effect after shorter as well as longer time durations, ii) those having an effect only after shorter or longer time durations and iii) those not producing any effects after both analysed time durations. Such analyses have been carried out for both type of effects at different times, with the cytotoxic effects analyzed before and the effects produced on autophagy after, considering just the treatments found to be not toxic.

Through the analysis of the cytotoxic effects, treatments have been divided in different categories depending on their toxicity and some evidences have been immediately emerged. As expected, the number of the treatments showing a toxic effect have increased over time and approximately half of those toxic after 2 hours have maintained such activity after 20 hours, as reported in paragraph 4.3.4.1. Strangely, the rest of the treatments resulting toxic after 2 hours seemed to lose such activity over time and this observation can be explained by making various assumptions: as most of such samples showed a wide gap between the percentage of living cells after 2 and 20 hours, it is possible to suppose that a huge number of cells were detached during the treatments administration, or that the samples contained molecules able to keep the toxic activity just for short time durations or, moreover, that the cells initially influenced by such toxic effect acquired the ability to counteract to them, thus saving themselves. Obviously, the rest of the treatments resulting to be toxic only after 20 hours have been considered as containing natural products able to influence the cell viability only over time. Because of their cytotoxicity and of their potential application in searches of new agents against cancer, further analyses will be needed to clarify about the toxic activity of the investigated treatments. Most of the treatments (285/376), however, have not shown any toxic activity after both 2 as well as 20 hours since their administration, thus resulting the most interesting for further analyses concerning the effects of the treatments on the autophagic activity.

All the treatments found to produce toxic effects have been reported in table 16, which contains also the original measurements of the features describing the autophagic activity: in order to help to distinguish between those producing toxic activity only after one or both treatments time durations, toxic treatments have been included within cells with a grey background.

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																			
#			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS		
					%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area			%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
1	Controls	Ctrl	100	100	79	2	38	100	27	26	100	100	95	16	66	100	84	24
2		Ctrl DMSO	107	94	100	7	42	100	53	25	93	100	100	31	66	100	115	23
3		Ctrl Starvation	100	100	76	2	38	100	52	25	100	100	93	17	67	100	98	24
4		Ctrl DMSO Starvation	87	93	100	8	42	100	81	24	97	100	100	32	68	100	121	23
5	EAGRC	0,1mg/ml Starvation	94	100	100	9	42	100	56	25	28	100	100	22	56	100	66	24
6	ERAAlop	0,1mg/ml	65	95	100	7	46	100	120	25	47	100	100	17	52	100	143	24
7	EAGAAsia	0,02mg/ml Starvation	64	87	100	14	45	100	80	24	49	100	100	20	51	100	147	22
8		0,05mg/ml Starvation	45	83	100	13	45	97	35	26	23	100	100	21	50	100	2	22
9		0,1mg/ml Starvation	42	77	100	9	43	100	71	26	40	100	100	21	54	100	108	23
10	EAGMA	0,01mg/ml Starvation	36	67	100	13	46	100	115	25	88	94	100	19	47	100	293	21
11	EAGMD	0,1mg/ml Starvation	74	92	100	13	49	100	102	25	44	94	100	28	54	100	164	23
12	EAGKL	0,1mg/ml Starvation	74	91	100	15	45	100	53	26	31	100	100	27	50	0	0	23
13	EAGAT	0,01mg/ml Starvation	32	80	100	7	51	100	80	27	81	100	100	17	51	100	107	24
14	ERPS	0,1mg/ml	48	91	100	8	40	100	85	24	5	50	100	9	74	0	0	/
15		0,01mg/ml Starvation	43	81	100	14	46	100	122	25	58	100	100	16	48	100	130	23
16		0,02mg/ml Starvation	53	87	100	16	45	100	65	27	18	98	100	10	54	0	0	25
17		0,05mg/ml Starvation	30	69	100	17	58	100	68	25	16	47	100	11	77	58	1	50
18		0,1mg/ml Starvation	5	48	0	0	79	100	77	23	13	30	100	16	71	25	0	56
19	ERHA	0,05mg/ml	80	100	84	3	37	100	93	25	48	100	100	15	78	100	225	22
20		0,02mg/ml Starvation	47	64	94	5	39	100	113	25	59	100	100	40	71	100	205	23
21		0,05mg/ml Starvation	138	100	96	5	39	100	92	25	44	100	100	38	76	100	182	22
22		0,1mg/ml Starvation	72	95	95	6	41	100	110	24	39	100	100	38	74	100	178	22
23	EAGAF	0,05mg/ml Starvation	108	92	95	5	37	100	92	25	42	100	100	44	77	100	147	22
24		0,1mg/ml Starvation	60	98	100	5	40	100	112	24	29	100	100	30	81	100	108	24
25	EAGAA	0,02mg/ml	96	100	76	2	36	100	61	26	48	100	92	9	83	100	176	21
26		0,05mg/ml Starvation	36	100	100	5	37	100	100	25	59	100	100	33	80	100	173	22
27		0,1mg/ml Starvation	83	94	100	4	38	100	91	25	29	100	100	25	87	100	161	22
28	EAGFO	0,05mg/ml	67	100	79	2	36	100	83	25	40	100	82	7	81	100	274	20
29		0,1mg/ml	88	100	88	3	39	100	84	25	3	100	0	0	242	50	1	80
30		0,02mg/ml Starvation	86	97	92	4	38	100	105	25	14	100	100	20	85	100	94	22
31		0,05mg/ml Starvation	24	100	50	1	41	100	51	26	4	100	0	0	89	67	1	63
32		0,1mg/ml Starvation	3	100	0	0	52	100	17	20	5	100	0	0	113	78	2	62
33	EAGPD	0,05mg/ml	71	100	91	3	38	100	75	26	40	100	100	21	79	100	7	24
34		0,1mg/ml	97	100	87	3	37	100	48	26	22	100	100	21	83	100	38	24
35		0,05mg/ml Starvation	111	97	96	6	39	87	8	26	39	100	100	42	78	100	34	25
36		0,1mg/ml Starvation	100	95	100	6	39	100	52	25	6	100	100	27	82	0	0	28
37	EAGTM	0,01mg/ml Starvation	130	96	100	5	38	100	106	25	47	100	100	46	76	100	108	23
38		0,1mg/ml Starvation	110	100	100	6	38	100	99	24	44	100	100	39	78	100	33	25

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																		
#			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS		
					%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area			%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
39	EAGC	0,01mg/ml	94	100	83	2	36	100	81	26	43	100	100	19	75	100	119	23
40		0,02mg/ml	96	100	83	2	36	100	51	26	26	100	100	14	81	100	160	23
41		0,05mg/ml	108	100	81	2	35	100	61	25	33	100	100	20	79	100	104	23
42		0,1mg/ml	91	100	83	2	36	100	50	25	24	100	100	31	77	100	174	22
43		0,01mg/ml Starvation	95	90	94	5	39	100	74	25	48	100	100	42	76	100	113	24
44		0,02mg/ml Starvation	52	64	83	5	38	100	74	27	39	100	100	50	75	100	127	24
45		0,05mg/ml Starvation	69	94	97	5	38	100	70	26	23	100	100	50	78	100	113	24
46		0,1mg/ml Starvation	79	94	94	5	39	100	89	26	13	100	100	26	80	100	69	26
47	EFHA	0,01mg/ml Starvation	35	67	100	4	36	100	90	26	90	96	100	41	74	100	82	25
48		0,1mg/ml Starvation	100	100	96	4	36	100	59	26	7	100	100	17	99	100	43	22
49	EAGSS	0,02mg/ml	87	100	83	2	36	100	91	25	39	100	100	21	74	100	138	24
50		0,1mg/ml	109	100	84	2	36	100	52	26	39	100	100	26	76	100	155	23
51		0,02mg/ml Starvation	98	82	100	5	40	100	97	25	47	100	100	50	75	100	167	23
52	EFIPS	0,02mg/ml	80	98	82	3	37	100	79	26	47	100	100	12	76	100	191	21
53		0,1mg/ml	50	100	89	2	36	100	101	26	27	100	100	16	81	100	204	21
54		0,02mg/ml Starvation	40	91	100	5	45	100	27	29	93	88	100	43	78	100	136	22
55		0,05mg/ml Starvation	88	89	100	5	40	100	91	25	30	87	100	42	82	100	143	22
56		0,1mg/ml Starvation	59	100	95	5	39	100	71	27	6	100	75	4	125	0	0	31
57	EAGCB	0,01mg/ml	49	100	89	3	38	100	64	27	80	100	93	19	75	100	49	26
58		0,02mg/ml	59	98	89	3	36	100	68	26	45	100	87	10	80	100	191	23
59		0,01mg/ml Starvation	84	100	100	5	38	100	40	28	41	100	100	44	74	100	118	24
60		0,02mg/ml Starvation	41	100	100	6	43	100	16	29	57	100	100	51	75	100	128	23
61		0,05mg/ml Starvation	68	98	95	5	39	100	15	28	48	100	100	44	77	100	114	24
62	EAGCT	0,1mg/ml Starvation	64	94	97	6	40	100	90	23	49	98	83	7	82	100	166	21
63	ERKL	0,1mg/ml	82	97	98	5	37	100	87	22	2	100	0	0	105	0	0	24
64		0,05mg/ml Starvation	96	98	100	13	43	100	91	23	3	90	75	9	77	33	0	32
65		0,1mg/ml Starvation	47	67	100	14	50	100	113	22	2	55	57	3	125	0	0	32
66	*Cryptomenia sp.*	0,1mg/ml Starvation	71	99	90	9	43	1	0	28	34	95	93	20	82	100	126	23
67	*C. intestinalis*	0,05mg/ml	73	100	77	2	37	100	26	26	49	100	94	10	80	100	124	21
68		0,1mg/ml	45	100	65	1	37	100	23	26	14	100	73	8	90	100	101	21
69		0,05mg/ml Starvation	71	96	96	6	39	71	2	29	15	100	100	16	91	100	110	22
70		0,1mg/ml Starvation	37	73	95	4	39	60	1	30	1	100	0	0	221	0	0	38
71	*Heliotropium sp. (T-1B)*	0,02mg/ml	67	98	92	4	39	100	32	26	26	100	100	14	84	100	200	21
72		0,05mg/ml	79	97	90	3	39	100	32	26	2	50	0	0	110	0	0	28
73		0,1mg/ml	51	89	85	3	43	100	47	25	3	100	40	0	91	0	0	/
74		0,01mg/ml Starvation	70	96	100	9	44	100	26	26	39	98	100	30	78	100	180	22
75		0,02mg/ml Starvation	65	91	98	7	43	87	4	28	6	53	95	9	93	77	25	24
76		0,05mg/ml Starvation	59	88	100	7	44	100	35	27	3	100	25	0	110	0	0	33
77		0,1mg/ml Starvation	26	71	94	6	47	96	14	26	2	100	0	0	141	0	0	89
78	*Heliotropium sp. (T-1C)*	0,1mg/ml	64	98	88	4	38	100	34	26	10	80	100	19	68	100	136	21
79		0,1mg/ml Starvation	91	99	100	11	45	91	5	28	27	100	100	27	74	100	74	22
80	*V. blattaria*	0,1mg/ml Starvation	43	44	100	10	38	100	65	26	23	100	100	12	97	100	82	23
81	*S. hissarica*	0,1mg/ml	91	100	68	1	36	100	47	27	45	100	76	4	79	100	150	24
82		0,1mg/ml Starvation	24	54	100	10	42	100	69	27	60	94	100	33	77	100	88	25
83	*V. songoricum*	0,1mg/ml	85	93	83	2	38	100	54	26	39	100	86	7	86	100	168	23
84		0,1mg/ml Starvation	42	70	100	11	45	100	72	26	15	100	100	31	82	100	80	28
85	*S. betoniciflora*	0,05mg/ml Starvation	46	59	100	9	42	100	65	25	165	93	100	24	74	100	58	24
86	*P. sewertzovii*	0,1mg/ml	43	97	90	3	41	100	80	26	87	100	82	6	74	100	103	24
87	*S. oreina*	0,1mg/ml Starvation	84	71	100	17	46	100	155	25	49	78	100	51	76	100	74	26
88	*C. umbrosa*	0,05mg/ml	56	95	92	5	43	100	143	27	36	100	93	13	82	100	108	25
89		0,01mg/ml Starvation	91	76	100	12	46	100	92	24	48	100	100	37	80	100	130	25
90		0,1mg/ml Starvation	120	93	100	11	46	100	419	24	32	82	100	32	77	100	121	24
91	*S. scharistanica*	0,02mg/ml	53	94	100	6	41	100	92	27	41	100	94	9	75	100	95	26
92		0,05mg/ml	48	94	100	6	40	100	123	26	59	100	100	14	72	100	79	26
93		0,1mg/ml	50	91	96	6	43	100	22	27	29	100	98	17	75	100	116	25
94	*Schrophullaria sp.*	0,02mg/ml	57	97	100	7	42	100	54	27	49	100	100	9	76	100	54	26
95		0,1mg/ml	92	98	100	6	39	100	48	27	46	100	100	13	80	100	109	25
96		0,05mg/ml Starvation	24	50	100	40	47	100	108	26	3	100	100	6	112	100	9	37
97		0,1mg/ml Starvation	2	0	75	39	100	0	0	124	3	100	33	1	107	0	0	/
98	*L. panzeroides*	0,05mg/ml	49	93	100	7	44	100	79	26	52	100	92	8	80	100	50	26
99		0,1mg/ml Starvation	93	85	100	11	45	100	61	27	34	81	100	23	80	100	44	27

Table 16. List of the treatments showing cytotoxic effect after 2 and 20 hours since their administration, reported on a grey background, and real values of the measurements of the features considered as descriptive of the cytotoxic and the autophagic activity.

The analysis of the effects produced on the autophagic pathway has been carried out following the same scheme used for the analysis of the cytotoxic activity previously described, but in this case treatments were analyzed also considering the culturing conditions present when they showed their activities. As already mentioned, the selection of the most

interesting treatments was performed based on few simple parameters: treatments administrated in physiological conditions and grouped along with control samples, which reproduce an autophagy-induced phenotype, were considered as interesting because able to induce autophagy, or at least increase the rate of its flux; treatments administrated in autophagy-induced conditions and included in the same groups with control samples for physiological conditions were considered as interesting because able to inhibit or to reduce the rate of the autophagy process; lastly, also treatments producing effects different from both control sample types were considered as interesting because able to induce particular phenotypes dependent from a perturbation of the features considered descriptive of the autophagy, even if not comparable with any of the previously hypothesized activity and taken as references. Based on such categories of interest, in which all the phenotypes produced after 2 and 20 hours since the administration have been included, treatments were then sorted considering their effects over time. More specifically, treatments were firstly analyzed considering those having the same effects after both investigated time durations and then the results have been crossed in order to find out treatments who acquired or lost their activity over time. Following this approach, treatments were divided in six different groups, as already reported in paragraphs 4.3.4.2 and 4.3.4.3:

- First group: treatments able to decrease the autophagic activity after both 2 and 20 hours since their administration;
- Second group: treatments found to increase the autophagic activity after both 2 and 20 hours since their administration;
- Third group: treatments producing effects different from both the phenotypes taken as reference, but however considered as interesting because of their ability to perturb parameters defining the autophagic activity after both the investigated time durations;
- Fourth group: treatments administrated in normal conditions which have produced not interesting effects after 2 hours but have shown the ability to induce autophagy, or to increase the autophagic rate, as well as those resulted different from both the phenotypes considered as references, after 20 hours since their administration;
- Fifth group: treatments administrated in autophagy-induced conditions which have produced not interesting effects after 2 hours but able to inhibit the autophagy or to decrease its rate, as well as those that produced phenotypes not comparable with none of the two phenotypes taken as reference, 20 hours after their administration;
- Sixth group: treatments which have produced effects considered as not interesting after 2 hours and that have changed their activity over time, becoming as interesting or showing a cytotoxic activity after 20 hours since their administration.

Practically, this last step of the investigation has allowed to define the treatments able to produce effects potentially interesting for the autophagy process, considering both shorter and longer incubation timings and dividing them depending on the type of the activity produced: those which have demonstrate the ability to increase the rate of the autophagic flux, those which have allowed to reduce the autophagic flux rate and those resulted able to perturb the physiological state of the autophagic flux without any similarity with the reference phenotypes considered, as well as those which have acquired one of these activity and those that were found to become toxic for the cells over time. The final results have been summarized in table 17, in which all the treatments able to produce an interesting effects after 2 and 20 hours since the administration have been reported along with the original measurements of the features used to carry out the investigations and better defined by using a colours system: the treatments increasing the autophagic activity have been reported on a green background, those able to decrease the autophagic activity on a red background, while the background of those found to produce a perturbation of the parameters describing autophagy in a different way from both the phenotypes taken as reference were blue colored and, lastly, treatments that acquired a cytotoxic

potential over time were distinguished using a grey background. As evident, all the treatments producing an interesting effect only after 2 hours since their administration have been excluded from the final list of the interesting treatments. Such decision has been taken considering that such treatments were able to affect the autophagy pathway for a limited time and as a consequence the related extracts have been considered as not interesting for the further deeper analyses. The interesting treatments reported within table 17, with the exception of those acquiring the cytotoxic potential after 20 hours since their administration, were considered as interesting because believed to contain natural products able to interfere with the autophagy pathway, and hence potentially considered for further investigations in order to detected molecules with pharmaceutical activities.

Concluding, many evidences can be taken out by looking at this final table. It is clear that, thanks to the analyses carried out and previously described into the present thesis work, the number of the treatments considered as interesting have been reduced by more than one fourth if compared to the number of the those considered at the beginning of the investigations (87/376), and such number falls down even more reaching one fifth of the initial amount (73/376) by excluding all the treatments which acquired a cytotoxic potential over time. Further, most of the interesting treatments resulted able to increase the autophagic rate after both 2 and 20 hours since their administration (44 treatments), while the other previously reported categories were represented by a significantly lower number of treatments. More specifically, 7 treatments were found to increase the autophagic activity just after 20 hours but didn't show any interesting effects after 2 hours, while other 7 treatments which increased the autophagy after 20 hours showed an effect interesting but not comparable with any reference phenotype after 2 hours since the administration. By the way of the effects interesting but not comparable with any reference phenotype, just 2 treatments were able to produce such phenotypes after both 2 and 20 hours, while other 7 treatments resulted to produce them only after 20 hours without showing any interesting effect after 2 hours since their administration, 6 of which were administrated in autophagy-induced conditions and just 1 in physiological conditions. Other 11 interesting treatments resulted able to decrease the autophagic rate: 1 of them produced such effects after both 2 and 20 hours, 4 of them only after 20 hours without any interesting effect showed after 2 hours, and just 1 of them reduced the autophagy after 2 hours but showing an effects different from both the reference phenotypes after 20 hours since their administration. Concluding, some treatments whose effects were considered as interesting after 2 hours were found to produce a cytotoxic effect after 20 hours since their administration, 5 of which were able to increase the autophagic rate, 5 were able to reduce it and other 4 produced effects interesting but not comparable with any reference phenotype after 2 hours since their administration.

Concerning the extracts which have provided the interesting treatments, their number was fallen approximately at the half of the initial amount shifting from 47 to 25, 17 of which taken into account because producing an increment of the autophagic rate (EAGRC, ERAAlop, EAGAAsia, EAGAAlop, EAGMA, EAGMD, EAGKL, EAGAS, EAGAT, EAGAU, ERPS, EAGPD, ERKL, ERKS, *Silene oreina*, *Scutellaria scharistanica*, *Schrophullaria sp.*), just 6 because involved in the reduction of such flux (EFIPS, EAGCT, *Cryptomenia sp.*, *Ciona intestinalis*, *Stachys betoniciflora*, *Cousina umbrosa*) and, surprisingly, the remaining 2 apparently involved in the onset of both these activities (EAGPS, EAGCB). Obviously, the real interest concerning these 2 extracts producing such ambiguous effects will need to be verified, while the other extracts can be immediately considered as interesting, especially as in the case of those observed for all the 4 dilutions investigated: after selected, such extracts can be addressed to further investigations specific for the effects showed in order to better define them and find out molecules, or molecular complexes, involved in such activities.

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																		
#			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS		
					%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area			%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
1	Controls	Ctrl	100	100	79	2	38	100	27	26	100	100	95	16	66	100	84	24
2		Ctrl DMSO	107	94	100	7	42	100	53	25	93	100	100	31	66	100	115	23
3		Ctrl Starvation	100	100	76	2	38	100	52	25	100	100	93	17	67	100	98	24
4		Ctrl DMSO Starvation	87	93	100	8	42	100	81	24	97	100	100	32	68	100	121	23
5	EAGRC	0,01mg/ml	102	100	88	3	41	100	54	25	104	100	100	20	50	100	85	24
6		0,02mg/ml	90	97	87	3	42	100	65	25	98	100	100	19	51	100	91	24
7		0,05mg/ml	69	100	96	6	43	100	79	26	106	100	100	18	50	100	93	24
8		0,1mg/ml	88	100	94	5	40	100	87	25	96	100	100	18	47	100	105	24
9	ERAAlop	0,01mg/ml	88	96	95	6	43	100	29	27	85	100	100	21	50	100	24	24
10		0,02mg/ml	90	97	94	6	40	93	18	27	73	100	100	20	52	100	13	24
11		0,05mg/ml	75	97	100	6	43	100	77	25	62	100	100	19	51	100	19	24
12		0,1mg/ml	65	95	100	7	46	100	120	25	47	100	100	17	52	100	143	24
13	EAGPS	0,01mg/ml	98	100	93	4	41	100	31	27	104	100	100	25	53	100	36	24
14		0,02mg/ml	95	100	92	4	40	100	50	26	91	100	100	33	51	100	47	25
15		0,05mg/ml	90	100	88	4	40	100	29	26	84	100	100	35	53	100	61	25
16		0,1mg/ml	84	96	80	2	39	100	62	26	88	100	100	32	52	100	58	25
17		0,01mg/ml Starvation	100	91	100	11	41	100	36	26	180	93	100	34	55	100	217	22
18		0,02mg/ml Starvation	100	90	96	7	39	100	19	26	122	88	100	31	56	100	131	23
19		0,05mg/ml Starvation	55	78	90	6	38	100	25	27	127	79	100	34	55	100	156	24
20		0,1mg/ml Starvation	66	70	88	3	37	87	10	27	72	76	100	30	57	0	0	25
21	EAGAAsia	0,01mg/ml	83	100	92	5	42	100	60	25	75	100	100	18	51	100	61	24
22		0,02mg/ml	78	97	100	6	44	100	56	26	93	100	100	17	51	100	39	25
23		0,05mg/ml	72	96	100	5	41	100	70	25	69	100	100	15	52	100	41	24
24		0,1mg/ml	72	98	96	6	42	100	60	25	58	100	100	15	54	100	148	22
25		0,02mg/ml Starvation	64	87	100	14	45	100	80	24	49	100	100	20	51	100	147	22
26	EAGAAlop	0,01mg/ml	101	97	92	4	41	100	60	25	105	100	100	19	50	100	67	25
27		0,02mg/ml	77	100	97	6	41	100	22	26	88	100	100	19	51	100	36	25
28		0,05mg/ml	80	100	100	6	42	100	29	26	98	100	100	18	51	100	59	25
29		0,1mg/ml	79	100	97	6	41	94	26	26	109	100	100	19	51	100	82	25
30		0,1mg/ml Starvation	83	75	100	8	44	87	7	28	51	75	100	27	57	100	75	25
31	EAGMA	0,01mg/ml	109	100	93	5	42	100	72	26	62	100	100	18	49	100	140	24
32		0,02mg/ml	83	100	100	7	44	100	109	25	68	100	100	20	50	100	125	23
33		0,05mg/ml	74	100	100	6	41	100	114	24	84	100	100	18	50	100	155	23
34		0,1mg/ml	73	100	100	8	45	100	87	26	81	100	100	19	48	100	196	21
35	EAGMD	0,01mg/ml	96	100	94	5	40	100	65	26	84	100	100	21	50	100	135	24
36		0,02mg/ml	84	100	96	5	42	100	76	26	81	100	100	20	50	100	119	24
37		0,05mg/ml	81	97	100	7	41	100	93	25	77	100	100	19	51	100	117	24
38		0,1mg/ml	83	100	100	6	41	100	115	24	67	100	100	19	51	100	128	24
39		0,1mg/ml Starvation	74	92	100	13	49	100	102	25	44	94	100	28	54	100	164	23
40	EAGKL	0,01mg/ml	76	100	100	7	43	100	91	25	86	99	100	20	51	100	66	25
41		0,02mg/ml	79	100	100	8	43	100	49	26	80	100	100	18	49	100	34	26
42		0,05mg/ml	92	100	100	7	43	100	108	25	74	100	100	20	51	100	35	27
43		0,1mg/ml	63	100	100	8	45	100	85	25	78	100	100	18	51	100	64	25
44		0,05mg/ml Starvation	83	94	100	15	49	100	43	26	74	98	100	30	52	100	94	24
45		0,1mg/ml Starvation	74	91	100	15	45	100	53	26	31	100	100	27	50	0	0	23
46	EAGAS	0,01mg/ml	99	100	95	5	40	100	28	27	92	100	100	19	49	100	79	24
47		0,02mg/ml	79	100	100	6	43	88	9	27	85	100	100	19	50	100	19	25
48		0,05mg/ml	76	100	100	5	41	100	66	26	88	100	100	20	49	100	29	25
49		0,1mg/ml	83	100	95	6	43	100	83	26	72	100	100	23	51	100	71	25
50	EAGAT	0,01mg/ml	91	97	100	15	44	100	69	26	81	100	100	16	50	100	86	24
51		0,02mg/ml	74	100	100	18	46	100	55	26	87	100	100	16	48	100	32	25
52		0,05mg/ml	60	100	100	16	45	100	184	23	80	100	100	18	50	100	27	26
53		0,1mg/ml	78	100	100	16	44	100	66	26	91	100	100	18	51	100	74	25
54	EAGAU	0,01mg/ml	85	100	100	17	42	100	30	27	88	100	100	19	48	100	76	25
55		0,02mg/ml	81	98	95	15	42	100	39	26	83	100	100	18	49	100	61	25
56		0,05mg/ml	77	100	97	16	43	100	103	26	118	100	100	15	49	100	38	26
57		0,1mg/ml	70	100	100	14	42	100	120	25	68	100	100	17	49	100	97	25
58	ERPS	0,01mg/ml	72	100	100	6	43	100	53	26	91	100	100	18	47	98	20	25
59		0,02mg/ml	69	100	100	7	45	100	57	26	70	100	100	17	45	100	90	25
60		0,05mg/ml	60	100	98	6	43	100	93	25	78	100	100	19	44	100	27	26
61		0,02mg/ml Starvation	53	87	100	16	45	100	65	27	18	98	100	10	54	0	0	25
62	EAGPD	0,01mg/ml	108	100	87	3	37	100	34	26	70	100	100	22	75	100	70	24
63		0,01mg/ml Starvation	135	98	97	6	37	93	13	27	68	100	100	44	76	15	0	26
64	EFIPS	0,1mg/ml Starvation	59	100	95	5	39	100	71	27	6	100	75	4	125	0	0	31
65	EAGCB	0,05mg/ml	69	100	92	4	38	100	62	26	50	100	100	20	73	100	94	24
66		0,05mg/ml Starvation	68	98	95	5	39	100	15	28	48	100	100	44	77	100	114	24
67	EAGCT	0,02mg/ml Starvation	92	96	96	6	40	100	45	24	82	99	100	20	76	100	144	21
68		0,05mg/ml Starvation	72	94	97	7	41	100	102	24	55	97	94	15	80	100	153	21
69	ERKL	0,01mg/ml	98	100	81	2	35	100	38	25	95	100	100	15	60	100	107	21
70		0,02mg/ml	88	98	93	4	37	100	87	23	94	100	100	15	62	100	62	23
71		0,05mg/ml	87	98	93	4	36	100	103	22	53	98	100	18	68	100	146	21
72		0,1mg/ml	82	97	98	5	37	100	87	22	2	100	0	0	105	0	0	24
73		0,05mg/ml Starvation	96	98	100	13	43	100	91	23	3	90	75	9	77	33	0	32
74	ERKS	0,1mg/ml	81	99	80	3	38	100	57	24	84	100	100	20	66	98	15	23

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																		
#			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS		
					%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area			%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
75		0,02mg/ml Starvation	84	95	100	7	42	100	67	25	103	100	100	31	67	50	1	23
76	*Cryptomenia sp.*	0,02mg/ml Starvation	82	97	96	8	41	37	0	26	70	100	92	12	73	100	55	24
77		0,05mg/ml Starvation	76	98	97	9	42	65	4	26	57	100	88	11	75	100	64	24
78		0,1mg/ml Starvation	71	99	90	9	43	1	0	28	34	95	93	20	82	100	126	23
79	*C. intestinalis*	0,05mg/ml Starvation	71	96	96	6	39	71	2	29	15	100	100	16	91	100	110	22
80	*S. betoniciflora*	0,1mg/ml Starvation	107	69	100	7	41	95	21	26	56	90	93	15	82	100	65	29
81	*Silene oreina*	0,01mg/ml	54	95	96	6	42	100	96	26	72	100	100	24	71	100	109	24
82		0,02mg/ml	74	96	100	6	42	100	58	26	79	100	100	21	70	100	100	25
83		0,05mg/ml	56	90	100	6	42	100	83	25	76	100	100	22	69	100	104	24
84		0,1mg/ml	88	97	96	6	41	100	59	26	61	100	100	26	69	100	143	23
85		0,1mg/ml Starvation	84	71	100	17	46	100	155	25	49	78	100	51	76	100	74	26
86	*C. umbrosa*	0,01mg/ml Starvation	91	76	100	12	46	100	92	24	48	100	100	37	80	100	130	25
87		0,1mg/ml Starvation	120	93	100	11	46	100	419	24	32	82	100	32	77	100	121	24
88	*S. scharistanica*	0,02mg/ml	53	94	100	6	41	100	92	27	41	100	94	9	75	100	95	26
89		0,1mg/ml	50	91	96	6	43	100	22	27	29	100	98	17	75	100	116	25
90	*Schrophullaria*	0,02mg/ml	57	97	100	7	42	100	54	27	49	100	100	9	76	100	54	26
91		0,1mg/ml	92	98	100	6	39	100	48	27	46	100	100	13	80	100	109	25

Table 17. List of the treatments resulted able to perturb the physiological conditions of the autophagic flux after 2 and 20 hours since their administration, reported along with the real values of the measurements of the features considered as descriptive of the autophagic activity and described through a colours system: treatments increasing the autophagic activity have been reported on a green background, treatments decreasing the autophagic activity on a faint red background, treatments producing phenotypes different from both those chosen as reference on a light blue background and treatments showing a cytotoxic effects on a grey background.

A final and complete picture concerning the effects produced by the investigated treatments has been reported in table A20, contained in section supplementary data. Such table consists in the complete list of the treatments, reported along with the original measurements of the analyzed features detected after 2 and 20 hours since the administration, in which the most interesting treatments, even including those excluded in table 17 for the reasons explained above, have been highlighted using a colours system, thus distinguishing them also on the base of the different types of activities. Specifically, the names of the most interesting treatments were reported on a faint orange background while the associated measurements were reported using different colours depending on the activity and following the same scheme already used for tables 16 and 17: the green background for the treatments increasing the autophagic activity, the red one for those decreasing such activity, the blue one for treatments whose activity were not comparable with the reference phenotypes and the grey one for treatments considered as toxic. This arrangement has allowed to better evaluate the effects of the most interesting treatments, as well as has demonstrated the ability of the analytical approach developed in making the selection of such treatments easier and faster, thus allowing then to avoid to waste time, material and money in the investigation of treatments completely devoid of interest.

5.3 Comparative High Content Analysis

In addition to the analysis of the effects produced by the extracts on autophagy in SH-SY5Y cells, other High Content Analyses have been carried out in parallel using also HeLa as cellular model. Despite they were put aside because of the negative effects produced by inducing autophagy through starvation (as clearly observed during the preliminary assays) HeLa cells have been then resumed and used, along with SH-SY5Y, in a kind of comparative High Content Analysis carried out to further evaluate the possibility to screen the effects produced on autophagy pathway by administrating the extracts also on a different cell model. Moreover, the results concerning such analysis would also allow to clarify some other issues, such as the possibility to use the same High Content Analysis approach to investigate extract effects using

another cell model, as well as to verify if the effects produced by such extracts were different depending on the used cell line. For these purposes, a comparative High Content Analysis has been performed by using only 6 extracts, randomly selected among the 47 previously reported in table 2, without knowing anything about their effects: *Verbascum blattaria, Stachys betoniciflora, Silene oreina*, EAGPD, EAGTM and ERKL. The results concerning such comparative High Content Analyses between HeLa and SH-SY5Y cells have been reported in figures 48 to 59 in which the analyses of the phenotypes produced after 2 and 20 hours of treatments have been displayed through diagrams, dendograms and scatter plots, as already done for the principal analysis. As evident looking at the graphs, the analysis of the effects produced on SH-SY5Y cells have led to results comparable to those resulting from the main analysis while the analysis of the phenotypes induced by the effects of the extracts investigated on HeLa cells have produced completely different and meaningless results. More specifically, as it appeared immediately clear looking at the dendograms and the scatter plot in figures 56, 57, 58 and 59, most of the samples look to occupy positions very closed to the control samples after both 2 and 20 hours after their administration with the tendency to shift away from them over time, as observed also in the analysis on SH-SY5Y cells, although they appeared sparser in the plane reported in the scatter plot already after 2 hours. Moreover, it was not possible to identify clusters to distinguish them on the basis of their activity: in fact, in order to sort the control samples in different clusters, the phenotypes were divided in 26 clusters after 2 hours and, surprisingly, 51 clusters were needed to sort out them after 20 hours since their administration.

In conclusion, some important findings have been taken out from the results of the comparative HCA, thus allowing to clarify some different issues concerning the developed method, the effects of the extracts and the used cell models. The distribution as well as the well-defined distances among the phenotypes produced by the treatments on both SH-SY5 and HeLa cells, which were reported within the scatter plots in figures 52-53 and 58-59, suggested that the method previously developed for investigations using SH-SY5Y cells seems to be suitable for analysing the effects of the extracts administrated also on different cell lines, with the only precaution of adapting the parameters to be entered in the image analysis software for identifying of the cellular elements belonging to different cell types. On the other hand, although the effects seemed to be well-defined for both cell models, it hasn't been possible to distinguish the autophagic phenotypes observed on HeLa cells in clusters reflecting their differences, thus confirming and supporting the choice to discard such cell line. This evidence has highlighted the importance of the choice of the best cell model, that represents a very critical step in order to take out interesting and reliable results from the analyses of the effects of the extracts on the autophagy pathway. As previously introduced, in the present work the SH-SY5Y cells have been preferred to the HeLa cells as model, and such choice has been already justified at the beginning of the discussion by considering only the results concerning the cytotoxic potential associated with two of the main analysis conditions, such as the effects of the starvation and the DMSO. Such important choice has been taken also considering the results of the comparative HCA, which was performed during the fine-tuning steps of the analytical method, but that has been then reported only in the end of the manuscript due to the choices made about the logical run and the organization of the different explanations. In this context, however, the comparative HCA has allowed to evaluate the reliability of the results concerning the investigation of the effects produced by the extracts on autophagy after administration on both the available cell lines, leading to select the SH-SY5Y cells as the best model for the investigation performed by applying the developed HCA method and discarding HeLa cells.

Such unexpected "failure" of the HeLa cells can be addressed to different possible reasons. Firstly, an important evidence is that different cell lines have shown very different and specific reactions after the administration of the same drugs or extracts as in this case, as well as after the cultivation in starvation conditions, as demonstrated and described

by Panchal et al. (2013, p. 75) and Warnes (2015) and also observed during the investigations reported and commented above. However, also the "particular nature" attributed to the HeLa cells at our days deserves to be taken into a deeper consideration. The HeLa cell line 'was born' in 1951 as the first human cell line in culture (Gey et al. 1952), and since that moment it became the most widely human cell line used in biological research all over the world. But the massive use of such cell line have rapidly lead to the contamination of other cell lines: surprisingly, the first interspecies cross-contaminations had been already described in the early 1960s (Brand & Syverton 1962; Defendi et al. 1960), but at the end of the same decade also some intraspecies contaminations had been then discovered and, despite they were more difficult to detect, 19 other human cell lines were found to be contaminated with HeLa cell line by the PhD Stanley Gartler (Gartler 1967). Unfortunately, almost 60 years after the detection of the contamination problem, and despite the huge technological advances, the cell lines contamination remains a very important problem (Lucey et al. 2006; Masters 2002; Nardone 2007; Stacey et al. 2000). Moreover, during this period HeLa cells have accumulated a huge amount of mutations which have almost completely changed its original genetic pattern (Adey et al. 2013; Chen 1988; Francke et al. 1973; Heneen 1976; Kraemer et al. 1974; Landry et al. 2013; Mincheva et al. 1987; Nelson-Rees et al. 1980; Popescu & Dipaolo 1989; Rueß et al. 1993; Stanbridge et al. 1981), and today there are many different genomic variants of the original cell line, sometimes unconsciously used, which present evident aberrations of the genetic expression with a consequent very important impact on the cellular functions, that may result to be very often strongly alterated (Landry et al 2013). Therefore, on the base of the results obtained from the comparative HCA, supported by these not still resolved issues concerning HeLa cells, this cell line has been discarded in order to avoid to base the evaluation of the effects produced by the extracts on cellular autophagic activity on strongly aberrant and not reliable results.

6. Conclusions and future perspectives

In conclusion, in the present thesis work multiple achievements have been reached. First of all, the primary screening of the effects produced by the extracts, performed by applying a High Content Analysis approach specifically developed for this purpose, has allowed to select those considered as interesting for their ability to perturb the physiological state of the autophagic process. The analysis has allowed to reduce the number of the extracts considered as interesting approximately at the half of the total and, considering the different dilutions tested, the total number has been reduced till one fourth. Moreover, treatments have been also sorted depending on the type of their activity on the autophagy process, evaluated on the basis of the phenotype produced: most of them have been selected as interesting because able to increase the physiological rate of the autophagy (EAGRC, ERAAlop, EAGAAsia, EAGAAlop, EAGMA, EAGMD, EAGKL, EAGAS, EAGAT, EAGAU, ERPS, EAGPD, ERKL, ERKS, *Silene oreina*, *Scutellaria scharistanica*, *Schrophullaria sp.*) while just few of them were considered as interesting because able to decrease such rate (EFIPS, EAGCT, *Cryptomenia sp.*, *Ciona intestinalis*, *Stachys betoniciflora*, *Cousina umbrosa*). Moreover, during the first steps of the analysis, treatments and hence the extracts have been also selected on the basis of their cytotoxic activity in order to discard from the analysis of the effects on autophagy those samples having an activity considered as toxic. Hence, the analysis has allowed to select the most interesting extracts and to sort them depending on what kind of activity produced on the autophagic pathway. The obtained results can help the design of following deeper analyses that will allow to save time and resources by setting up investigations in the wrong direction. Further analyses will need to define the molecules or the molecular complexes responsible of the activities.

Another important achievement reached during the present work, beside and in order to perform the primary screening, was the design and the fine-tuning of an analytical approach based on a High Content Analysis of the data measured from the images displaying the autophagic phenotypes produced by each treatment administrated in the analytical conditions. Two different autophagic markers, Lysosomes (Lysotracker) and LC3B protein (anti-LC3B antibody), respectively detected from living and fixed cells, and one other marker needed for defining the cells through the identification of nuclei (Hoechst 33342) at the beginning of the image analysis (it allowed also to investigate the potential cytotoxic activity of the extracts) were used to measure different parameters considered as descriptive of the autophagic activity and then analyzed simultaneously to detect and extrapolate the most interesting samples: the first parameter was the mortality rate produced by each treatment, considered as indirectly descriptive of the autophagic activity; the other parameters were the percentage of cell "autophagically" active, the number of the spots per cells as well as the size of such spots detected and measured from both lysosomes or LC3 proteins. The analytical approach can be further developed by working on different important factors such as: the markers describing the autophagic activity, which can be changed or also added adjusting the method to preserve the reliability of the results; the features measured and then used to define the autophagic phenotypes, which can also be chanced or added to carry out more accurated analyses; the search for the best cellular model, as just introduced during the present work. In this view the developed approach can represent a good tool to carry out the analysis of the effects of the extracts and also, at the same time, it can be viewed as a base from which start to build a more accurated and powerful tool to carry on the primary screening of the effects of natural crude extracts and/or single compounds.

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A. Supplementary data

FEATURES MEASUREMENTS AFTER 2 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					%Active Cells		# Per Cell		Area		%Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
Controls	Ctrl	100	100	0.593	79	-0.656	2	-0.810	38	-0.422	100	0.413	27	-0.788	26	-0.163
	Ctrl DMSO	107	94	0.236	100	0.544	7	0.012	42	0.027	100	0.413	53	-0.636	25	-0.309
	Ctrl Starvation	100	100	0.593	76	-0.869	2	-0.810	38	-0.422	100	0.413	52	-0.594	25	-0.309
	Ctrl DMSO Starvation	87	93	0.188	100	0.648	8	0.177	42	0.027	100	0.413	81	0.121	24	-0.309
EAGRC	0,01mg/ml	102	100	0.593	88	-0.103	3	-0.646	41	-0.085	100	0.413	54	-0.266	25	-0.163
	0,02mg/ml	90	97	0.420	87	-0.186	3	-0.646	42	0.027	100	0.413	65	-0.073	25	-0.126
	0,05mg/ml	69	100	0.593	96	0.387	6	-0.152	43	0.139	100	0.413	79	0.154	26	-0.090
	0,1mg/ml	88	100	0.593	94	0.257	5	-0.317	40	-0.198	100	0.413	87	0.297	25	-0.163
	0,01mg/ml Starvation	99	94	0.257	100	0.648	13	0.999	44	0.252	100	0.413	88	0.310	25	-0.163
	0,02mg/ml Starvation	106	97	0.396	100	0.648	10	0.505	42	0.027	100	0.413	111	0.701	24	-0.309
	0,05mg/ml Starvation	94	94	0.257	100	0.648	11	0.670	42	0.027	100	0.413	59	-0.174	26	-0.016
	0,1mg/ml Starvation	94	100	0.593	100	0.648	9	0.341	42	0.027	100	0.413	56	-0.233	25	-0.163
ERAAlop	0,01mg/ml	88	96	0.381	95	0.335	6	-0.152	43	0.139	100	0.413	29	-0.687	27	0.130
	0,02mg/ml	90	97	0.442	94	0.280	6	-0.152	40	-0.198	93	0.140	18	-0.864	27	0.130
	0,05mg/ml	75	97	0.430	100	0.648	6	-0.152	43	0.139	100	0.413	77	0.121	25	-0.163
	0,1mg/ml	65	95	0.333	100	0.648	7	0.012	46	0.476	100	0.413	120	0.852	25	-0.163
	0,01mg/ml Starvation	60	81	-0.478	100	0.648	13	0.999	44	0.252	100	0.413	58	-0.199	26	-0.016
	0,02mg/ml Starvation	67	86	-0.179	100	0.648	13	0.999	46	0.476	100	0.413	41	-0.477	26	-0.016
	0,05mg/ml Starvation	67	79	-0.609	100	0.648	15	1.328	48	0.701	100	0.174	22	-0.805	26	-0.016
	0,1mg/ml Starvation	51	78	-0.676	100	0.648	11	0.670	47	0.588	100	0.413	60	-0.157	26	-0.016
EAGPS	0,01mg/ml	98	100	0.593	93	0.217	4	-0.481	41	-0.085	100	0.413	31	-0.645	27	0.057
	0,02mg/ml	95	100	0.593	92	0.127	4	-0.481	40	-0.198	100	0.413	50	-0.334	26	-0.016
	0,05mg/ml	90	100	0.593	88	-0.088	4	-0.481	40	-0.198	100	0.413	29	-0.679	26	-0.016
	0,1mg/ml	84	96	0.364	80	-0.603	2	-0.810	39	-0.310	100	0.413	62	-0.123	26	-0.016
	0,01mg/ml Starvation	100	91	0.074	100	0.648	11	0.670	41	-0.085	100	0.413	36	-0.557	26	-0.016
	0,02mg/ml Starvation	100	90	0.022	96	0.387	7	0.012	39	-0.310	100	0.413	19	-0.847	26	-0.016
	0,05mg/ml Starvation	55	78	-0.649	90	0.022	6	-0.234	38	-0.422	100	0.413	25	-0.754	27	0.130
	0,1mg/ml Starvation	66	70	-1.120	88	-0.134	3	-0.646	37	-0.534	87	-0.134	10	-1.007	27	0.130
EAGAAsia	0,01mg/ml	83	100	0.593	92	0.154	5	-0.317	42	0.027	100	0.413	60	-0.157	25	-0.163
	0,02mg/ml	78	97	0.438	100	0.648	6	-0.152	44	0.195	100	0.413	56	-0.224	26	-0.016
	0,05mg/ml	72	96	0.381	100	0.648	5	-0.317	41	-0.141	100	0.413	70	0.003	25	-0.163
	0,1mg/ml	72	98	0.450	96	0.376	6	-0.152	42	0.027	100	0.413	60	-0.161	25	-0.163
	0,01mg/ml Starvation	61	87	-0.169	100	0.648	11	0.629	46	0.476	100	0.413	26	-0.729	26	-0.016
	0,02mg/ml Starvation	64	87	-0.169	100	0.648	14	1.081	45	0.364	100	0.413	80	0.171	24	-0.309
	0,05mg/ml Starvation	45	83	-0.359	100	0.648	13	0.999	45	0.364	97	0.188	35	-0.578	26	-0.016
	0,1mg/ml Starvation	42	77	-0.725	100	0.648	9	0.259	43	0.139	100	0.413	71	0.032	26	-0.090
EAGAAlop	0,01mg/ml	101	97	0.430	92	0.141	4	-0.481	41	-0.085	100	0.413	60	-0.161	25	-0.163
	0,02mg/ml	77	100	0.593	97	0.483	6	-0.152	41	-0.085	100	0.290	22	-0.796	26	-0.016
	0,05mg/ml	80	100	0.593	100	0.648	6	-0.152	42	0.027	100	0.304	29	-0.679	26	-0.016
	0,1mg/ml	79	100	0.593	97	0.464	6	-0.152	41	-0.085	94	0.174	26	-0.737	26	-0.016
	0,01mg/ml Starvation	83	89	-0.066	100	0.648	13	0.917	47	0.532	97	0.119	21	-0.813	26	-0.016
	0,02mg/ml Starvation	85	87	-0.152	100	0.648	12	0.834	43	0.139	100	0.413	29	-0.679	26	-0.016
	0,05mg/ml Starvation	66	80	-0.549	100	0.648	13	0.917	46	0.420	86	-0.544	10	-0.998	26	-0.016
	0,1mg/ml Starvation	83	75	-0.835	100	0.648	8	0.177	44	0.195	87	-0.353	7	-1.044	28	0.276
EAGMA	0,01mg/ml	109	100	0.593	93	0.231	5	-0.317	42	0.027	100	0.413	72	0.049	26	-0.016
	0,02mg/ml	83	100	0.593	100	0.648	7	0.012	44	0.252	100	0.413	109	0.667	25	-0.163
	0,05mg/ml	74	100	0.593	100	0.648	6	-0.152	41	-0.085	100	0.413	114	0.752	24	-0.309
	0,1mg/ml	73	100	0.593	100	0.648	8	0.177	45	0.364	100	0.413	87	0.297	26	-0.090
	0,01mg/ml Starvation	36	67	-1.311	100	0.648	13	0.999	46	0.476	100	0.413	115	0.760	25	-0.236
	0,02mg/ml Starvation	68	92	0.130	100	0.648	18	1.862	50	0.925	100	0.413	96	0.449	25	-0.163
	0,05mg/ml Starvation	57	90	0.022	100	0.648	18	1.821	48	0.701	100	0.413	115	0.760	25	-0.163
	0,1mg/ml Starvation	58	86	-0.223	100	0.648	15	1.245	46	0.476	100	0.413	119	0.840	24	-0.309
EAGMD	0,01mg/ml	96	100	0.593	94	0.280	5	-0.317	40	-0.198	100	0.413	65	-0.081	26	-0.016
	0,02mg/ml	84	100	0.593	96	0.376	5	-0.317	42	0.027	100	0.413	76	0.108	26	-0.016
	0,05mg/ml	81	97	0.442	100	0.648	7	0.012	41	-0.085	100	0.413	93	0.402	25	-0.163
	0,1mg/ml	83	100	0.593	100	0.648	6	-0.152	41	-0.085	100	0.413	115	0.764	24	-0.309
	0,01mg/ml Starvation	94	96	0.364	100	0.648	15	1.245	47	0.588	100	0.413	121	0.869	24	-0.309
	0,02mg/ml Starvation	109	97	0.396	100	0.648	14	1.081	46	0.420	100	0.413	109	0.667	25	-0.163
	0,05mg/ml Starvation	77	93	0.212	100	0.648	13	0.999	47	0.588	100	0.413	93	0.398	25	-0.163
	0,1mg/ml Starvation	74	92	0.153	100	0.648	13	0.999	49	0.785	100	0.413	102	0.550	25	-0.163
EAGKL	0,01mg/ml	76	100	0.593	100	0.648	7	-0.070	43	0.139	100	0.413	91	0.356	25	-0.163
	0,02mg/ml	79	100	0.593	100	0.648	8	0.177	43	0.139	100	0.413	49	-0.342	26	-0.016
	0,05mg/ml	92	100	0.593	100	0.648	7	0.012	43	0.139	100	0.413	108	0.651	25	-0.163
	0,1mg/ml	63	100	0.593	100	0.648	8	0.177	45	0.308	100	0.413	85	0.264	25	-0.163
	0,01mg/ml Starvation	51	86	-0.223	100	0.648	13	0.999	46	0.504	100	0.413	93	0.390	25	-0.236
	0,02mg/ml Starvation	79	96	0.373	100	0.648	16	1.451	48	0.701	100	0.413	57	-0.207	26	-0.016
	0,05mg/ml Starvation	83	94	0.224	100	0.648	15	1.328	49	0.813	100	0.413	43	-0.443	26	-0.016
	0,1mg/ml Starvation	74	91	0.074	100	0.648	15	1.328	45	0.308	100	0.413	53	-0.275	26	-0.016
EAGAS	0,01mg/ml	99	100	0.593	95	0.319	5	-0.317	40	-0.198	100	0.413	28	-0.695	27	0.130
	0,02mg/ml	79	100	0.593	100	0.648	6	-0.152	43	0.083	88	-0.037	9	-1.015	27	0.130
	0,05mg/ml	76	100	0.593	100	0.648	5	-0.317	41	-0.085	100	0.413	66	-0.056	26	-0.016

FEATURES MEASUREMENTS AFTER 2 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																	
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					%Active Cells		# Per Cell		Area		%Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
	0,1mg/ml	83	100	0.593	95	0.335	6	-0.152	43	0.139	100	0.413	83	0.230	26	-0.016
	0,01mg/ml Starvation	113	97	0.414	100	0.648	16	1.492	49	0.813	100	0.254	39	-0.510	26	-0.016
	0,02mg/ml Starvation	77	87	-0.139	100	0.648	13	0.999	46	0.476	91	-0.353	26	-0.737	25	-0.163
	0,05mg/ml Starvation	79	92	0.117	100	0.648	13	0.999	45	0.308	100	0.413	48	-0.367	26	-0.016
	0,1mg/ml Starvation	73	92	0.117	100	0.648	11	0.629	44	0.252	72	-0.863	5	-1.082	28	0.276
EAGAT	0,01mg/ml	91	97	0.438	100	0.648	15	1.328	44	0.252	100	0.413	69	-0.006	26	-0.016
	0,02mg/ml	74	100	0.593	100	0.648	18	1.821	46	0.476	100	0.413	55	-0.245	26	-0.016
	0,05mg/ml	60	100	0.593	100	0.648	16	1.492	45	0.364	100	0.413	184	1.921	23	-0.455
	0,1mg/ml	78	100	0.593	100	0.648	16	1.410	44	0.252	100	0.413	66	-0.056	26	-0.016
	0,01mg/ml Starvation	32	80	-0.676	100	0.648	7	-0.070	51	0.981	100	0.413	80	0.175	27	0.130
	0,02mg/ml Starvation	73	89	-0.042	100	0.648	8	0.094	50	0.925	100	0.413	35	-0.578	26	0.020
	0,05mg/ml Starvation	62	92	0.142	100	0.648	8	0.094	50	0.925	100	0.413	75	0.100	25	-0.163
	0,1mg/ml Starvation	69	85	-0.286	100	0.648	7	0.012	50	0.953	100	0.297	12	-0.965	27	0.130
EAGAU	0,01mg/ml	85	100	0.593	100	0.648	17	1.574	42	0.027	100	0.413	30	-0.658	27	0.130
	0,02mg/ml	81	98	0.463	95	0.350	15	1.245	42	0.027	100	0.413	39	-0.510	26	-0.016
	0,05mg/ml	77	100	0.593	97	0.459	16	1.492	43	0.139	100	0.413	103	0.562	26	-0.090
	0,1mg/ml	70	100	0.593	100	0.648	14	1.163	42	0.027	100	0.413	120	0.844	25	-0.236
	0,01mg/ml Starvation	93	89	-0.019	100	0.648	6	-0.152	49	0.813	100	0.266	30	-0.670	26	-0.016
	0,02mg/ml Starvation	73	89	-0.042	100	0.648	5	-0.317	49	0.813	100	0.413	38	-0.527	26	-0.016
	0,05mg/ml Starvation	78	93	0.212	100	0.648	6	-0.152	48	0.701	100	-0.134	12	-0.965	28	0.313
	0,1mg/ml Starvation	82	90	0.022	100	0.648	6	-0.152	47	0.588	100	0.413	30	-0.670	27	0.166
ERPS	0,01mg/ml	72	100	0.593	100	0.648	6	-0.152	43	0.167	100	0.413	53	-0.283	26	-0.016
	0,02mg/ml	69	100	0.593	100	0.648	7	0.012	45	0.364	100	0.413	57	-0.207	26	-0.016
	0,05mg/ml	60	100	0.593	98	0.335	6	-0.152	43	0.139	100	0.413	93	0.390	25	-0.163
	0,1mg/ml	48	91	-0.008	100	0.648	8	0.177	40	-0.198	100	0.413	85	0.264	24	-0.309
	0,01mg/ml Starvation	43	81	-0.549	100	0.648	14	1.081	46	0.448	100	0.413	122	0.886	25	-0.163
	0,02mg/ml Starvation	53	87	-0.169	100	0.648	16	1.492	45	0.364	100	0.413	65	-0.073	27	0.130
	0,05mg/ml Starvation	30	69	-1.192	100	0.648	17	1.698	58	1.823	100	0.413	68	-0.022	25	-0.126
	0,1mg/ml Starvation	5	48	-5.117	0	-5.609	0	-1.139	79	4.181	100	-3.416	77	0.129	23	-0.455
EFIAAlop	0,01mg/ml	102	100	0.593	71	-1.139	2	-0.810	41	-0.085	100	0.413	67	-0.039	25	-0.163
	0,02mg/ml	86	100	0.593	74	-0.991	2	-0.810	36	-0.647	100	0.413	49	-0.346	26	-0.016
	0,05mg/ml	85	100	0.593	73	-1.036	1	-0.974	37	-0.534	100	0.413	26	-0.733	26	-0.016
	0,1mg/ml	79	100	0.593	79	-0.640	2	-0.810	37	-0.534	100	0.413	100	0.516	24	-0.309
	0,01mg/ml Starvation	95	94	0.199	96	0.387	5	-0.317	39	-0.366	100	0.413	137	1.139	24	-0.309
	0,02mg/ml Starvation	97	96	0.389	92	0.167	5	-0.317	38	-0.422	100	0.413	76	0.112	25	-0.163
	0,05mg/ml Starvation	87	93	0.212	94	0.257	5	-0.317	39	-0.310	100	0.413	114	0.752	25	-0.163
	0,1mg/ml Starvation	123	95	0.257	93	0.201	5	-0.399	37	-0.534	100	0.413	95	0.432	24	-0.309
ERHA	0,01mg/ml	113	100	0.593	76	-0.824	2	-0.810	35	-0.759	100	0.413	92	0.373	25	-0.163
	0,02mg/ml	115	100	0.593	78	-0.742	2	-0.810	35	-0.759	100	0.413	77	0.121	25	-0.163
	0,05mg/ml	80	100	0.593	84	-0.353	3	-0.646	37	-0.534	100	0.413	93	0.390	25	-0.163
	0,1mg/ml	83	100	0.593	76	-0.862	1	-0.974	36	-0.647	100	0.413	46	-0.401	27	0.130
	0,01mg/ml Starvation	144	93	0.175	92	0.167	5	-0.317	40	-0.198	100	0.413	82	0.213	25	-0.163
	0,02mg/ml Starvation	47	64	-1.484	94	0.167	5	-0.317	39	-0.310	100	0.413	113	0.726	25	-0.163
	0,05mg/ml Starvation	138	100	0.593	96	0.407	5	-0.317	39	-0.310	100	0.413	92	0.373	25	-0.163
	0,1mg/ml Starvation	72	95	0.275	95	0.201	6	-0.152	41	-0.141	100	0.413	110	0.676	24	-0.309
EAGAF	0,01mg/ml	61	100	0.593	79	-0.655	2	-0.810	36	-0.647	100	0.413	58	-0.199	25	-0.163
	0,02mg/ml	114	100	0.593	71	-1.177	1	-0.974	35	-0.759	100	0.413	35	-0.582	27	0.130
	0,05mg/ml	86	100	0.593	75	-0.916	2	-0.810	37	-0.590	85	-0.283	9	-1.019	25	-0.163
	0,1mg/ml	67	100	0.593	81	-0.544	2	-0.810	34	-0.871	100	0.413	86	0.280	25	-0.163
	0,01mg/ml Starvation	95	73	-0.930	89	-0.395	4	-0.481	41	-0.141	100	0.413	89	0.331	25	-0.163
	0,02mg/ml Starvation	65	79	-0.609	92	0.127	6	-0.234	39	-0.310	100	0.413	86	0.272	25	-0.163
	0,05mg/ml Starvation	108	92	0.103	95	0.231	5	-0.399	37	-0.590	100	0.413	92	0.373	25	-0.163
	0,1mg/ml Starvation	60	98	0.460	100	0.648	5	-0.317	40	-0.198	100	0.413	112	0.718	24	-0.309
EAGAA	0,01mg/ml	105	100	0.593	80	-0.603	2	-0.810	37	-0.534	100	0.413	65	-0.073	26	-0.016
	0,02mg/ml	96	100	0.593	76	-0.842	2	-0.810	36	-0.647	100	0.413	61	-0.140	26	-0.090
	0,05mg/ml	76	100	0.593	79	-0.693	2	-0.810	36	-0.703	100	0.413	59	-0.182	25	-0.163
	0,1mg/ml	85	100	0.593	77	-0.774	2	-0.810	37	-0.534	100	0.413	44	-0.426	26	-0.016
	0,01mg/ml Starvation	77	93	0.185	100	0.648	5	-0.317	37	-0.534	100	0.413	90	0.348	26	-0.016
	0,02mg/ml Starvation	101	88	-0.066	96	0.387	5	-0.317	37	-0.534	100	0.413	90	0.352	25	-0.163
	0,05mg/ml Starvation	36	100	0.153	100	0.648	5	-0.317	37	-0.534	100	0.413	100	0.512	25	-0.163
	0,1mg/ml Starvation	83	94	0.236	100	0.648	4	-0.481	38	-0.478	100	0.413	91	0.365	25	-0.163
EAGFO	0,01mg/ml	102	100	0.593	75	-0.916	1	-0.974	34	-0.871	100	0.413	79	0.163	26	-0.090
	0,02mg/ml	71	100	0.593	72	-1.104	1	-0.974	38	-0.422	100	0.413	84	0.247	25	-0.163
	0,05mg/ml	67	100	0.593	79	-0.693	2	-0.810	36	-0.647	100	0.413	83	0.230	25	-0.163
	0,1mg/ml	88	100	0.593	88	-0.134	3	-0.646	39	-0.310	100	0.413	84	0.247	25	-0.163
	0,01mg/ml Starvation	54	100	0.333	100	0.648	4	-0.481	35	-0.759	100	0.413	125	0.928	25	-0.236
	0,02mg/ml Starvation	86	97	0.373	92	0.069	4	-0.481	38	-0.422	100	0.413	105	0.592	25	-0.163
	0,05mg/ml Starvation	24	100	0.257	50	-5.609	1	-0.974	41	-0.085	100	-3.416	51	-0.317	26	-0.090
	0,1mg/ml Starvation	3	100	0.593	0	-5.609	0	-1.139	52	1.150	100	-3.416	17	-0.880	20	-0.894
EAGPD	0,01mg/ml	108	100	0.593	87	-0.205	3	-0.646	37	-0.534	100	0.413	34	-0.594	26	-0.016
	0,02mg/ml	108	100	0.593	88	-0.134	3	-0.646	36	-0.647	100	0.413	56	-0.224	26	-0.016
	0,05mg/ml	71	100	0.593	91	-0.047	3	-0.646	38	-0.478	100	0.413	75	0.095	26	-0.016
	0,1mg/ml	97	100	0.593	87	-0.168	3	-0.646	37	-0.534	100	0.413	48	-0.359	26	-0.016
	0,01mg/ml Starvation	135	98	0.460	97	0.459	6	-0.152	37	-0.534	93	-0.086	13	-0.944	27	0.130
	0,02mg/ml Starvation	84	82	-0.445	100	0.648	6	-0.152	39	-0.310	100	0.413	77	0.121	26	-0.016

FEATURES MEASUREMENTS AFTER 2 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																	
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					%Active Cells		# Per Cell		Area		%Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
	0,05mg/ml Starvation	111	97	0.438	96	0.376	6	-0.152	39	-0.310	87	-0.353	8	-1.040	26	-0.016
	0,1mg/ml Starvation	100	95	0.307	100	0.648	6	-0.152	39	-0.310	100	0.413	52	-0.292	25	-0.163
EAGTM	0,01mg/ml	71	100	0.593	79	-0.669	2	-0.810	36	-0.647	100	0.413	81	0.192	26	-0.090
	0,02mg/ml	105	100	0.593	84	-0.340	2	-0.810	37	-0.534	100	0.413	31	-0.653	27	0.130
	0,05mg/ml	94	100	0.593	86	-0.211	2	-0.810	37	-0.534	100	0.413	51	-0.308	26	-0.016
	0,1mg/ml	62	100	0.593	84	-0.395	3	-0.728	40	-0.254	100	0.413	109	0.667	25	-0.163
	0,01mg/ml Starvation	130	96	0.389	100	0.648	5	-0.317	38	-0.422	100	0.413	106	0.621	25	-0.199
	0,02mg/ml Starvation	62	100	0.593	100	0.648	7	0.012	40	-0.198	100	0.413	93	0.398	25	-0.163
	0,05mg/ml Starvation	137	95	0.307	96	0.407	6	-0.152	40	-0.198	100	0.413	84	0.238	26	-0.016
	0,1mg/ml Starvation	110	100	0.593	100	0.648	6	-0.152	38	-0.422	100	0.413	99	0.499	24	-0.309
EAGC	0,01mg/ml	94	100	0.593	83	-0.440	2	-0.810	36	-0.647	100	0.413	81	0.188	26	-0.090
	0,02mg/ml	96	100	0.593	83	-0.395	2	-0.810	36	-0.647	100	0.413	51	-0.308	26	-0.016
	0,05mg/ml	108	100	0.593	81	-0.555	2	-0.810	35	-0.759	100	0.413	61	-0.140	25	-0.163
	0,1mg/ml	91	100	0.593	83	-0.440	2	-0.810	36	-0.647	100	0.413	50	-0.325	25	-0.163
	0,01mg/ml Starvation	95	90	0.022	94	0.257	5	-0.317	39	-0.366	100	0.413	74	0.079	25	-0.163
	0,02mg/ml Starvation	52	64	-1.484	83	-0.395	5	-0.317	38	-0.422	100	0.413	74	0.070	27	0.130
	0,05mg/ml Starvation	69	94	0.275	97	0.440	5	-0.317	38	-0.422	100	0.413	70	0.011	26	-0.016
	0,1mg/ml Starvation	79	94	0.257	94	0.257	5	-0.317	39	-0.310	100	0.413	89	0.335	26	-0.016
EFHA	0,01mg/ml	95	100	0.593	79	-0.669	2	-0.810	37	-0.534	100	0.413	44	-0.422	27	0.130
	0,02mg/ml	83	100	0.593	78	-0.742	2	-0.810	37	-0.534	100	0.413	60	-0.161	26	-0.016
	0,05mg/ml	83	100	0.593	85	-0.300	2	-0.810	37	-0.534	100	0.413	37	-0.544	26	-0.016
	0,1mg/ml	95	100	0.593	83	-0.395	2	-0.810	36	-0.647	100	0.413	65	-0.077	26	-0.016
	0,01mg/ml Starvation	35	67	-1.311	100	0.648	4	-0.481	36	-0.647	100	0.413	90	0.339	26	-0.016
	0,02mg/ml Starvation	100	80	-0.549	98	0.350	4	-0.481	37	-0.534	100	0.413	25	-0.746	26	-0.016
	0,05mg/ml Starvation	68	92	0.074	100	0.648	5	-0.317	35	-0.759	100	0.413	69	-0.006	26	-0.016
	0,1mg/ml Starvation	100	100	0.593	96	0.319	4	-0.481	36	-0.647	100	0.413	59	-0.182	26	-0.016
EAGSS	0,01mg/ml	113	100	0.593	86	-0.205	3	-0.646	36	-0.647	100	0.413	59	-0.174	26	-0.090
	0,02mg/ml	87	100	0.593	83	-0.431	2	-0.810	36	-0.647	100	0.413	91	0.365	25	-0.163
	0,05mg/ml	73	100	0.593	85	-0.300	2	-0.810	39	-0.310	100	0.413	80	0.175	25	-0.163
	0,1mg/ml	109	100	0.593	84	-0.395	2	-0.810	36	-0.647	100	0.413	52	-0.292	26	-0.016
	0,01mg/ml Starvation	76	93	0.185	100	0.648	7	-0.070	39	-0.310	100	0.413	58	-0.199	26	-0.016
	0,02mg/ml Starvation	98	82	-0.549	100	0.648	5	-0.317	40	-0.254	100	0.413	97	0.457	25	-0.163
	0,05mg/ml Starvation	99	96	0.333	96	0.319	6	-0.152	39	-0.310	100	0.413	100	0.508	26	-0.016
	0,1mg/ml Starvation	107	98	0.483	97	0.446	6	-0.152	40	-0.198	100	0.413	95	0.423	25	-0.163
EFIPS	0,01mg/ml	70	100	0.593	83	-0.395	2	-0.810	35	-0.759	100	0.413	52	-0.300	26	-0.016
	0,02mg/ml	80	98	0.414	82	-0.603	3	-0.728	37	-0.590	100	0.413	79	0.167	26	-0.090
	0,05mg/ml	75	100	0.593	85	-0.314	3	-0.646	37	-0.534	100	0.413	85	0.264	25	-0.163
	0,1mg/ml	50	100	0.593	89	-0.010	2	-0.810	36	-0.647	100	0.413	101	0.533	26	-0.016
	0,01mg/ml Starvation	119	97	0.355	95	0.335	5	-0.317	38	-0.422	100	0.413	58	-0.199	26	-0.016
	0,02mg/ml Starvation	40	91	0.022	100	0.648	5	-0.317	45	0.308	100	0.413	27	-0.721	29	0.422
	0,05mg/ml Starvation	88	89	-0.042	100	0.648	5	-0.317	40	-0.198	100	0.413	91	0.360	25	-0.199
	0,1mg/ml Starvation	59	100	0.593	95	-0.004	5	-0.317	39	-0.310	100	-1.085	71	0.020	27	0.057
EAGCB	0,01mg/ml	49	100	0.593	89	-0.074	3	-0.646	38	-0.422	100	0.413	64	-0.090	27	0.057
	0,02mg/ml	59	98	0.344	89	-0.047	3	-0.646	36	-0.647	100	0.413	68	-0.022	26	-0.016
	0,05mg/ml	69	100	0.593	92	-0.022	4	-0.563	38	-0.478	100	0.413	62	-0.119	26	-0.016
	0,1mg/ml	59	100	0.593	90	-0.134	3	-0.646	36	-0.647	100	0.413	66	-0.056	26	-0.090
	0,01mg/ml Starvation	84	100	0.593	100	0.398	5	-0.317	38	-0.422	100	0.413	40	-0.502	28	0.276
	0,02mg/ml Starvation	41	100	0.471	100	0.648	6	-0.152	43	0.139	100	-0.065	16	-0.897	29	0.422
	0,05mg/ml Starvation	68	98	0.321	95	0.273	5	-0.317	39	-0.310	100	-1.354	15	-0.914	28	0.276
	0,1mg/ml Starvation	56	96	0.117	100	0.648	6	-0.152	39	-0.366	100	0.065	51	-0.317	27	0.057
EAGCT	0,01mg/ml	104	98	0.498	68	-1.385	1	-0.974	37	-0.534	99	0.361	17	-0.889	25	-0.163
	0,02mg/ml	82	97	0.420	76	-0.881	2	-0.810	37	-0.534	100	0.413	76	0.112	24	-0.309
	0,05mg/ml	88	98	0.471	73	-1.046	1	-0.974	37	-0.534	100	0.413	77	0.129	24	-0.309
	0,1mg/ml	72	96	0.381	69	-1.294	1	-0.974	38	-0.422	100	0.413	66	-0.064	25	-0.163
	0,01mg/ml Starvation	91	97	0.402	98	0.503	7	0.012	40	-0.198	100	0.413	60	-0.157	25	-0.163
	0,02mg/ml Starvation	92	96	0.350	96	0.416	6	-0.152	40	-0.198	100	0.413	45	-0.418	24	-0.309
	0,05mg/ml Starvation	72	94	0.257	97	0.488	7	0.012	41	-0.085	100	0.413	102	0.541	24	-0.382
	0,1mg/ml Starvation	64	94	0.269	97	0.432	6	-0.152	40	-0.198	100	0.413	90	0.348	23	-0.455
EAGOT	0,01mg/ml	98	100	0.593	70	-1.259	2	-0.892	36	-0.647	100	0.413	70	0.003	24	-0.309
	0,02mg/ml	98	99	0.529	77	-0.789	2	-0.810	38	-0.478	100	0.413	70	0.011	24	-0.309
	0,05mg/ml	88	100	0.593	73	-1.046	2	-0.810	37	-0.534	100	0.413	56	-0.224	25	-0.163
	0,1mg/ml	89	98	0.503	77	-0.768	2	-0.810	37	-0.590	100	0.413	77	0.121	25	-0.163
	0,01mg/ml Starvation	102	98	0.487	98	0.528	8	0.177	41	-0.085	100	0.413	86	0.280	23	-0.455
	0,02mg/ml Starvation	103	98	0.503	97	0.488	7	0.012	41	-0.085	100	0.413	92	0.381	23	-0.455
	0,05mg/ml Starvation	97	98	0.489	98	0.518	8	0.177	42	0.027	100	0.413	63	-0.102	24	-0.309
	0,1mg/ml Starvation	100	99	0.507	98	0.499	7	0.012	41	-0.085	100	0.413	55	-0.250	25	-0.163
ERKL	0,01mg/ml	98	100	0.593	81	-0.555	2	-0.810	35	-0.759	100	0.413	38	-0.527	25	-0.163
	0,02mg/ml	88	98	0.505	93	0.231	4	-0.481	37	-0.534	100	0.413	87	0.297	23	-0.455
	0,05mg/ml	87	98	0.496	93	0.240	4	-0.481	36	-0.647	100	0.413	103	0.566	22	-0.601
	0,1mg/ml	82	97	0.411	98	0.515	5	-0.317	37	-0.534	100	0.413	87	0.297	22	-0.601
	0,01mg/ml Starvation	100	98	0.503	100	0.648	9	0.341	42	-0.029	100	0.413	32	-0.628	26	-0.016
	0,02mg/ml Starvation	98	97	0.414	100	0.648	10	0.505	41	-0.085	100	0.413	93	0.390	23	-0.455
	0,05mg/ml Starvation	96	98	0.493	100	0.648	13	0.999	43	0.139	100	0.413	91	0.365	23	-0.455
	0,1mg/ml Starvation	47	67	-1.311	100	0.648	14	1.081	50	0.925	100	0.413	113	0.735	22	-0.601
ERKS	0,01mg/ml	96	98	0.502	72	-1.082	2	-0.810	37	-0.534	100	0.413	104	0.583	22	-0.601

FEATURES MEASUREMENTS AFTER 2 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																	
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS						
					%Active Cells		# Per Cell		Area		%Active Cells		# Per Cell		Area		
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	
	0,02mg/ml	87	98	0.489	80	-0.603	2	-0.810	38	-0.422	100	0.413	107	0.625	22	-0.601	
	0,05mg/ml	77	99	0.511	79	-0.675	2	-0.810	38	-0.422	100	0.413	83	0.230	24	-0.309	
	0,1mg/ml	81	99	0.511	80	-0.603	3	-0.646	38	-0.422	100	0.413	57	-0.203	24	-0.309	
	0,01mg/ml Starvation	78	92	0.153	100	0.648	9	0.341	43	0.139	100	0.413	81	0.201	24	-0.309	
	0,02mg/ml Starvation	84	95	0.307	100	0.648	7	0.012	42	0.027	100	0.413	67	-0.043	25	-0.163	
	0,05mg/ml Starvation	75	88	-0.073	100	0.648	11	0.588	42	0.027	100	0.413	71	0.020	25	-0.163	
	0,1mg/ml Starvation	79	94	0.275	100	0.648	10	0.505	42	0.027	100	0.413	61	-0.140	25	-0.163	
ESHA	0,01mg/ml	98	99	0.521	71	-1.172	2	-0.892	37	-0.590	100	0.413	41	-0.472	25	-0.163	
	0,02mg/ml	83	98	0.491	77	-0.779	2	-0.810	38	-0.422	100	0.413	55	-0.241	25	-0.163	
	0,05mg/ml	92	98	0.505	77	-0.807	2	-0.810	38	-0.422	100	0.413	72	0.045	24	-0.309	
	0,1mg/ml	78	98	0.499	77	-0.786	2	-0.810	37	-0.590	100	0.413	41	-0.485	25	-0.163	
	0,01mg/ml Starvation	91	97	0.399	100	0.648	9	0.259	43	0.139	100	0.413	34	-0.594	25	-0.163	
	0,02mg/ml Starvation	84	96	0.342	100	0.648	8	0.177	41	-0.085	100	0.413	85	0.264	24	-0.309	
	0,05mg/ml Starvation	85	95	0.292	100	0.648	10	0.505	44	0.252	100	0.413	56	-0.224	25	-0.163	
	0,1mg/ml Starvation	93	98	0.493	100	0.648	9	0.341	44	0.252	100	0.413	48	-0.359	25	-0.163	
Ulva sp. (U-2C)	0,01mg/ml	78	98	0.503	79	-0.675	2	-0.810	36	-0.647	9	-3.284	0	-1.166	25	-0.163	
	0,02mg/ml	87	100	0.593	83	-0.435	3	-0.728	37	-0.506	83	-0.831	5	-1.082	26	-0.016	
	0,05mg/ml	90	100	0.593	79	-0.650	2	-0.810	37	-0.534	100	0.413	39	-0.510	26	-0.016	
	0,1mg/ml	85	100	0.593	80	-0.603	2	-0.810	37	-0.590	98	0.345	27	-0.708	26	-0.016	
	0,01mg/ml Starvation	108	100	0.593	100	0.648	8	0.177	41	-0.085	99	0.356	20	-0.838	26	-0.016	
	0,02mg/ml Starvation	94	98	0.485	98	0.534	8	0.177	41	-0.085	100	0.413	16	-0.897	27	0.130	
	0,05mg/ml Starvation	97	98	0.494	100	0.648	9	0.341	42	0.027	87	-0.101	4	-1.099	28	0.276	
	0,1mg/ml Starvation	94	99	0.513	100	0.648	9	0.341	43	0.139	100	0.413	26	-0.729	27	0.130	
Cryptomenia sp. (C-3B)	0,01mg/ml	63	100	0.593	80	-0.853	2	-0.810	36	-0.647	86	-0.134	6	-1.066	27	0.130	
	0,02mg/ml	65	98	0.487	82	-0.544	3	-0.728	37	-0.506	82	-0.820	4	-1.099	26	-0.016	
	0,05mg/ml	78	100	0.593	79	-0.669	2	-0.810	37	-0.534	95	0.201	9	-1.015	26	-0.016	
	0,1mg/ml	72	100	0.593	55	-2.196	2	-0.810	37	-0.590	100	0.335	27	-0.721	26	-0.016	
	0,01mg/ml Starvation	57	97	0.425	100	0.648	8	0.177	41	-0.085	22	-3.234	0	-1.166	28	0.276	
	0,02mg/ml Starvation	82	97	0.427	96	0.398	8	0.177	41	-0.085	37	-2.219	0	-1.166	26	-0.053	
	0,05mg/ml Starvation	76	98	0.503	97	0.453	9	0.341	42	0.027	65	-1.023	4	-1.095	26	-0.016	
	0,1mg/ml Starvation	71	99	0.499	90	0.006	9	0.341	43	0.139	1	-3.416	0	-1.166	28	0.203	
Ciona intestinalis (C-4B)	0,01mg/ml	59	100	0.593	81	-0.603	3	-0.646	37	-0.534	57	-1.741	1	-1.158	27	0.130	
	0,02mg/ml	63	98	0.502	82	-0.511	2	-0.810	39	-0.366	98	0.304	19	-0.855	26	-0.016	
	0,05mg/ml	73	100	0.593	77	-0.816	2	-0.810	37	-0.534	100	0.413	26	-0.733	26	-0.090	
	0,1mg/ml	45	100	0.593	65	-1.698	1	-0.974	37	-0.534	100	0.413	23	-0.788	26	-0.090	
	0,01mg/ml Starvation	71	98	0.499	98	0.496	6	-0.152	41	-0.057	91	-0.097	14	-0.939	26	-0.016	
	0,02mg/ml Starvation	68	98	0.450	92	0.154	4	-0.481	38	-0.422	81	-0.863	6	-1.061	28	0.203	
	0,05mg/ml Starvation	71	96	0.373	96	0.403	6	-0.234	39	-0.310	71	-1.905	2	-1.129	29	0.422	
	0,1mg/ml Starvation	37	73	-0.945	95	0.280	4	-0.481	39	-0.310	60	-1.135	1	-1.150	30	0.568	
Heliotropium sp. (T-1B)	0,01mg/ml	67	99	0.519	90	0.006	3	-0.646	40	-0.198	100	0.330	22	-0.796	26	-0.016	
	0,02mg/ml	67	98	0.483	92	0.089	4	-0.481	39	-0.310	100	0.294	32	-0.624	26	-0.016	
	0,05mg/ml	79	97	0.399	90	0.006	3	-0.646	39	-0.310	100	0.413	32	-0.624	26	-0.016	
	0,1mg/ml	51	89	-0.066	85	-0.329	3	-0.646	43	0.139	100	0.413	47	-0.376	25	-0.163	
	0,01mg/ml Starvation	70	96	0.369	100	0.648	9	0.341	44	0.195	100	0.344	26	-0.725	26	-0.016	
	0,02mg/ml Starvation	65	91	0.074	98	0.525	7	0.012	43	0.139	87	-0.965	4	-1.099	28	0.276	
	0,05mg/ml Starvation	59	88	-0.096	100	0.648	7	0.012	44	0.252	100	0.413	35	-0.578	27	0.130	
	0,1mg/ml Starvation	26	71	-1.039	94	0.257	6	-0.234	47	0.588	96	-0.457	14	-0.935	26	-0.016	
Heliotropium sp. (T-1C)	0,01mg/ml	69	100	0.593	90	-0.103	3	-0.646	38	-0.422	97	0.127	10	-0.998	28	0.276	
	0,02mg/ml	63	100	0.593	94	0.193	4	-0.481	40	-0.198	100	0.332	57	-0.207	24	-0.309	
	0,05mg/ml	88	100	0.593	86	-0.225	3	-0.646	37	-0.534	100	0.413	47	-0.371	26	-0.016	
	0,1mg/ml	64	98	0.498	88	-0.134	4	-0.481	38	-0.422	100	0.413	34	-0.603	26	-0.016	
	0,01mg/ml Starvation	85	98	0.505	100	0.648	10	0.423	43	0.139	100	0.239	23	-0.775	26	-0.016	
	0,02mg/ml Starvation	82	100	0.593	100	0.648	9	0.259	43	0.139	84	-0.544	4	-1.099	29	0.422	
	0,05mg/ml Starvation	86	100	0.593	100	0.648	10	0.505	44	0.252	100	0.413	20	-0.830	28	0.276	
	0,1mg/ml Starvation	91	99	0.511	100	0.648	11	0.670	45	0.364	91	-0.037	5	-1.082	28	0.276	
Verbascum blattaria	0,01mg/ml	81	98	0.494	68	-1.367	1	-0.974	40	-0.198	100	0.413	78	0.146	26	-0.016	
	0,02mg/ml	97	98	0.499	75	-0.916	2	-0.892	36	-0.647	100	0.413	44	-0.426	26	-0.016	
	0,05mg/ml	96	97	0.396	66	-1.509	1	-0.974	37	-0.534	100	0.413	44	-0.435	26	-0.016	
	0,1mg/ml	70	97	0.434	71	-1.139	1	-0.974	35	-0.759	100	0.413	52	-0.292	26	-0.016	
	0,01mg/ml Starvation	109	69	-1.164	100	0.648	10	0.423	40	-0.198	100	0.413	82	0.205	25	-0.163	
	0,02mg/ml Starvation	102	75	-0.835	100	0.648	11	0.588	40	-0.198	100	0.413	97	0.461	25	-0.163	
	0,05mg/ml Starvation	65	60	-1.691	100	0.648	9	0.341	41	-0.141	100	0.413	78	0.150	26	-0.016	
	0,1mg/ml Starvation	43	44	-2.619	100	0.648	10	0.505	38	-0.422	100	0.413	65	-0.081	26	-0.016	
Stachys hissarica	0,01mg/ml	91	100	0.593	79	-0.646	2	-0.810	39	-0.366	100	0.413	80	0.171	26	-0.053	
	0,02mg/ml	85	100	0.593	71	-1.139	2	-0.810	40	-0.254	100	0.413	41	-0.472	27	0.130	
	0,05mg/ml	51	93	0.185	71	-1.437	1	-0.974	40	-0.198	100	0.413	109	0.667	25	-0.163	
	0,1mg/ml	91	100	0.593	68	-1.328	1	-0.974	36	-0.647	100	0.413	47	-0.384	27	0.130	
	0,01mg/ml Starvation	106	77	-0.725	100	0.648	9	0.341	43	0.111	100	0.413	86	0.280	25	-0.163	
	0,02mg/ml Starvation	124	86	-0.223	100	0.648	9	0.341	42	0.027	100	0.413	55	-0.250	26	-0.016	
	0,05mg/ml Starvation	82	69	-1.311	100	0.648	11	0.670	42	0.027	100	0.413	101	0.524	25	-0.163	
	0,1mg/ml Starvation	24	54	-2.262	100	0.648	10	0.505	42	-0.029	100	0.413	69	-0.014	27	0.057	
Verbascum songoricum	0,01mg/ml	84	97	0.442	83	-0.469	3	-0.728	37	-0.562	100	0.413	63	-0.102	25	-0.163	
	0,02mg/ml	105	97	0.446	83	-0.395	3	-0.646	38	-0.422	100	0.413	55	-0.241	26	-0.016	
	0,05mg/ml	60	91	0.074	78	-0.742	2	-0.810	38	-0.478	100	0.413	65	-0.073	25	-0.163	
	0,1mg/ml	85	93	0.185	83	-0.431	2	-0.810	38	-0.422	100	0.413	54	-0.258	26	-0.016	

FEATURES MEASUREMENTS AFTER 2 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																	
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					%Active Cells		# Per Cell		Area		%Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
	0,01mg/ml Starvation	107	71	-1.039	100	0.648	10	0.423	44	0.223	100	0.413	144	1.256	25	-0.163
	0,02mg/ml Starvation	103	73	-0.945	100	0.648	9	0.341	43	0.083	100	0.413	132	1.054	25	-0.163
	0,05mg/ml Starvation	59	61	-1.691	100	0.648	10	0.505	45	0.364	100	0.413	99	0.499	26	-0.090
	0,1mg/ml Starvation	42	70	-1.120	100	0.648	11	0.670	45	0.364	100	0.413	72	0.045	26	-0.016
Stachys betoniciflora	**0,01mg/ml**	82	100	0.593	93	0.185	4	-0.563	39	-0.310	95	-0.134	22	-0.805	26	-0.016
	0,02mg/ml	88	98	0.498	93	0.222	4	-0.481	38	-0.422	90	0.049	7	-1.049	28	0.276
	0,05mg/ml	78	88	-0.066	81	-0.544	2	-0.810	42	0.027	100	0.413	60	-0.153	26	-0.016
	0,1mg/ml	79	95	0.292	83	-0.431	3	-0.728	39	-0.310	100	0.413	34	-0.599	27	0.057
	0,01mg/ml Starvation	142	79	-0.631	100	0.648	9	0.341	42	0.027	100	0.413	47	-0.384	26	-0.016
	0,02mg/ml Starvation	104	68	-1.243	100	0.648	10	0.505	43	0.139	63	-1.629	1	-1.150	26	-0.016
	0,05mg/ml Starvation	46	59	-1.786	100	0.648	9	0.341	42	-0.029	100	0.413	65	-0.073	25	-0.163
	0,1mg/ml Starvation	107	69	-1.179	100	0.648	7	0.012	41	-0.141	95	0.212	21	-0.813	26	-0.016
Phlomis sewertzovii	**0,01mg/ml**	71	95	0.333	93	0.231	4	-0.481	40	-0.198	100	0.413	62	-0.123	26	-0.016
	0,02mg/ml	63	94	0.275	93	0.179	4	-0.440	41	-0.113	100	0.413	69	-0.014	26	-0.016
	0,05mg/ml	76	98	0.457	89	-0.047	4	-0.481	39	-0.310	100	0.413	73	0.058	25	-0.163
	0,1mg/ml	43	97	0.402	90	-0.134	3	-0.687	41	-0.141	100	-0.954	80	0.175	26	-0.016
	0,01mg/ml Starvation	132	85	-0.272	100	0.648	12	0.834	45	0.364	100	0.413	113	0.726	25	-0.163
	0,02mg/ml Starvation	120	86	-0.223	100	0.648	12	0.834	45	0.308	100	0.413	171	1.702	25	-0.163
	0,05mg/ml Starvation	91	67	-1.311	100	0.648	13	0.958	47	0.588	100	0.413	102	0.554	26	-0.016
	0,1mg/ml Starvation	75	73	-0.965	100	0.648	10	0.505	45	0.392	100	0.413	131	1.029	25	-0.236
Phlomis salicifolia	**0,01mg/ml**	78	94	0.257	97	0.459	5	-0.317	41	-0.085	100	0.413	94	0.415	25	-0.163
	0,02mg/ml	70	96	0.344	92	0.167	5	-0.399	40	-0.198	100	0.413	50	-0.325	26	-0.016
	0,05mg/ml	83	94	0.266	93	0.185	4	-0.481	40	-0.198	100	0.413	95	0.432	24	-0.309
	0,1mg/ml	55	90	-0.019	93	0.167	4	-0.440	43	0.139	100	0.413	112	0.722	24	-0.309
	0,01mg/ml Starvation	114	85	-0.309	100	0.648	10	0.505	44	0.195	100	0.413	142	1.214	24	-0.309
	0,02mg/ml Starvation	139	82	-0.549	100	0.648	11	0.588	44	0.280	100	-0.353	5	-1.091	26	-0.016
	0,05mg/ml Starvation	59	70	-1.210	100	0.648	12	0.834	45	0.364	100	0.413	81	0.188	24	-0.309
	0,1mg/ml Starvation	55	80	-0.549	100	0.648	10	0.505	44	0.252	100	-3.416	50	-0.334	26	-0.016
Silene oreina	**0,01mg/ml**	54	95	0.307	96	0.364	6	-0.193	42	0.055	100	0.413	96	0.440	26	-0.016
	0,02mg/ml	74	96	0.373	100	0.648	6	-0.152	42	0.027	100	0.413	58	-0.199	26	-0.016
	0,05mg/ml	56	90	0.040	100	0.648	6	-0.152	42	0.027	100	0.413	83	0.230	25	-0.163
	0,1mg/ml	88	97	0.402	96	0.398	6	-0.152	41	-0.085	100	0.413	59	-0.182	26	-0.016
	0,01mg/ml Starvation	123	80	-0.618	100	0.648	13	0.999	46	0.476	100	0.413	142	1.223	25	-0.163
	0,02mg/ml Starvation	113	83	-0.386	100	0.648	16	1.410	47	0.588	100	0.413	108	0.655	26	-0.016
	0,05mg/ml Starvation	95	69	-1.164	100	0.648	16	1.492	48	0.701	100	0.413	141	1.197	25	-0.163
	0,1mg/ml Starvation	84	71	-1.065	100	0.648	17	1.574	46	0.476	100	0.413	155	1.433	25	-0.163
Phlomis tadschikistanica	**0,01mg/ml**	90	99	0.509	97	0.440	5	-0.317	41	-0.085	100	0.413	42	-0.460	26	-0.016
	0,02mg/ml	97	98	0.474	97	0.459	5	-0.317	40	-0.198	88	-0.037	4	-1.099	27	0.130
	0,05mg/ml	63	95	0.321	100	0.648	6	-0.152	42	-0.029	100	0.413	42	-0.456	26	-0.016
	0,1mg/ml	108	97	0.442	88	-0.088	3	-0.646	41	-0.085	100	0.413	64	-0.090	26	-0.016
	0,01mg/ml Starvation	128	83	-0.386	100	0.648	17	1.574	50	0.925	100	0.413	112	0.718	26	-0.016
	0,02mg/ml Starvation	103	81	-0.478	100	0.648	16	1.492	48	0.644	100	0.413	77	0.129	27	0.057
	0,05mg/ml Starvation	120	90	0.022	100	0.648	12	0.875	48	0.701	100	0.413	77	0.121	26	-0.016
	0,1mg/ml Starvation	85	83	-0.359	100	0.648	12	0.834	44	0.252	100	0.413	85	0.264	25	-0.126
Cousina umbrosa	**0,01mg/ml**	61	96	0.327	93	0.201	6	-0.234	41	-0.085	100	-3.416	63	-0.107	28	0.276
	0,02mg/ml	53	96	0.344	94	0.280	5	-0.399	41	-0.113	100	0.413	126	0.953	27	0.130
	0,05mg/ml	56	95	0.292	92	0.127	5	-0.399	43	0.139	100	-3.416	143	1.231	27	0.130
	0,1mg/ml	62	94	0.257	90	0.022	3	-0.646	41	-0.141	100	0.413	100	0.516	27	0.130
	0,01mg/ml Starvation	91	76	-0.835	100	0.648	12	0.834	46	0.476	100	-3.416	92	0.381	24	-0.309
	0,02mg/ml Starvation	111	80	-0.549	100	0.648	12	0.834	45	0.392	100	-3.416	138	1.155	25	-0.163
	0,05mg/ml Starvation	77	87	-0.169	100	0.648	11	0.711	47	0.532	/	/	/	/	/	/
	0,1mg/ml Starvation	120	93	0.170	100	0.648	11	0.670	46	0.476	100	-3.416	419	5.883	24	-0.309
Nepeta olgae	**0,01mg/ml**	51	86	-0.333	92	-0.047	5	-0.317	41	-0.085	100	0.413	103	0.558	26	-0.016
	0,02mg/ml	65	93	0.136	94	0.257	5	-0.317	42	0.027	100	0.413	89	0.327	26	-0.090
	0,05mg/ml	115	94	0.275	93	0.185	4	-0.481	43	0.139	100	0.413	68	-0.022	26	-0.016
	0,1mg/ml	74	95	0.247	90	-0.134	3	-0.646	42	0.027	100	0.413	84	0.247	26	-0.016
	0,01mg/ml Starvation	141	82	-0.445	100	0.648	12	0.834	45	0.364	100	0.413	112	0.714	25	-0.163
	0,02mg/ml Starvation	130	80	-0.549	100	0.648	13	0.999	48	0.701	100	0.413	56	-0.233	26	-0.016
	0,05mg/ml Starvation	94	71	-1.039	100	0.648	14	1.204	47	0.532	100	0.413	56	-0.224	26	-0.016
	0,1mg/ml Starvation	95	64	-1.522	100	0.648	13	0.999	45	0.392	100	0.413	77	0.129	26	-0.090
Scutellaria scharistanica	**0,01mg/ml**	57	95	0.321	93	0.201	4	-0.481	43	0.111	100	0.413	44	-0.426	27	0.057
	0,02mg/ml	53	94	0.212	100	0.648	6	-0.152	41	-0.057	100	0.413	92	0.377	27	0.130
	0,05mg/ml	48	94	0.257	100	0.648	6	-0.234	40	-0.198	100	0.413	123	0.895	26	-0.016
	0,1mg/ml	50	91	0.074	96	0.376	6	-0.152	43	0.111	100	0.413	22	-0.805	27	0.130
	0,01mg/ml Starvation	91	81	-0.478	100	0.648	12	0.834	49	0.813	100	0.413	100	0.516	26	-0.016
	0,02mg/ml Starvation	102	81	-0.495	100	0.648	14	1.081	47	0.588	100	0.413	41	-0.477	25	-0.126
	0,05mg/ml Starvation	105	66	-1.365	100	0.648	12	0.834	45	0.364	100	0.413	55	-0.241	26	-0.016
	0,1mg/ml Starvation	64	67	-1.311	100	0.648	12	0.834	44	0.195	100	0.413	115	0.768	26	-0.016
Schrophullaria sp.	**0,01mg/ml**	63	96	0.373	96	0.398	6	-0.152	43	0.083	100	0.413	94	0.411	26	-0.016
	0,02mg/ml	57	97	0.420	100	0.648	7	0.012	42	0.027	100	0.413	54	-0.258	27	0.057
	0,05mg/ml	73	96	0.350	100	0.648	7	0.012	40	-0.254	100	0.413	27	-0.716	28	0.276
	0,1mg/ml	92	98	0.457	100	0.648	6	-0.152	39	-0.310	100	0.413	48	-0.359	27	0.130
	0,01mg/ml Starvation	97	85	-0.286	100	0.648	43	5.850	48	0.701	88	-0.863	19	-0.855	27	0.130
	0,02mg/ml Starvation	115	78	-0.786	100	0.648	42	5.767	45	0.364	100	0.413	31	-0.645	27	0.130
	0,05mg/ml Starvation	24	50	-2.579	100	0.648	40	5.356	47	0.616	100	0.413	108	0.642	26	-0.016

FEATURES MEASUREMENTS AFTER 2 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					%Active Cells		# Per Cell		Area		%Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
	0,1mg/ml Starvation	2	0	-5.117	75	-5.609	39	5.274	100	6.538	0	-3.416	0	-1.166	124	14.315
Leonurus panzeroides	**0,01mg/ml**	59	95	0.284	96	0.364	6	-0.234	44	0.252	100	0.413	77	0.129	27	0.130
	0,02mg/ml	87	100	0.593	97	0.446	6	-0.234	42	0.027	100	0.413	87	0.297	26	-0.016
	0,05mg/ml	49	93	0.212	100	0.453	7	0.012	44	0.195	100	0.413	79	0.158	26	-0.090
	0,1mg/ml	85	97	0.408	96	0.416	6	-0.234	43	0.139	100	0.413	55	-0.241	25	-0.163
	0,01mg/ml Starvation	128	88	-0.092	100	0.648	14	1.163	47	0.616	100	0.413	131	1.029	25	-0.236
	0,02mg/ml Starvation	112	82	-0.415	100	0.648	14	1.163	45	0.364	100	0.413	13	-0.948	24	-0.309
	0,05mg/ml Starvation	152	89	-0.042	100	0.648	13	0.917	47	0.588	100	0.413	85	0.268	27	0.093
	0,1mg/ml Starvation	93	85	-0.286	100	0.648	11	0.670	45	0.364	100	0.413	61	-0.136	27	0.057

Table A1. Quantitative data (white columns) and correspondent Z-score values (grey columns) resulting from measurements of features selected for evaluating effects on autophagy after 2 hours of treatments: the percentage of living cells with respect to the control samples (written in red and reported in the first column) has been considered alone at the end of the analysis to consider the possible influence of the toxicity effects when occurring simultaneously with the most interesting observed autophagic phenotypes; the percentage of living cells referred to each single sample, the percentage of cells with acidic/autophagic compartments resulting activated after treatments, the number of lysosomes/LC3B proteins per cells and the area of lysosomes/LC3B proteins have been considered all together and used in the following multivariate analysis in order to interpret the effects produced by administrated extracts on autophagic phenotypes.

FEATURES MEASUREMENTS AFTER 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					Active Cells		# Per Cell		Area		Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
Controls	Ctrl	100	100	0.384	95	0.144	16	-0.806	66	-0.768	100	0.407	84	-0.480	24	-0.225
	Ctrl DMSO	93	100	0.384	100	0.419	31	0.424	66	-0.338	100	0.407	115	-0.348	23	-0.339
	Ctrl Starvation	100	100	0.384	93	0.049	17	-0.840	67	-0.864	100	0.407	98	-0.433	24	-0.282
	Ctrl DMSO Starvation	97	100	0.384	100	0.419	32	0.527	68	-0.243	100	0.407	121	-0.037	23	-0.225
EAGRC	0,01mg/ml	104	100	0.384	100	0.419	20	-0.123	50	-1.031	100	0.407	85	-0.244	24	-0.111
	0,02mg/ml	98	100	0.384	100	0.419	19	-0.174	51	-0.959	100	0.407	91	-0.170	24	-0.111
	0,05mg/ml	106	100	0.384	100	0.419	18	-0.259	50	-1.007	100	0.407	93	-0.147	24	-0.111
	0,1mg/ml	96	100	0.384	100	0.419	18	-0.259	47	-1.150	100	0.407	105	-0.014	24	-0.168
	0,01mg/ml Starvation	134	95	-0.129	100	0.419	16	-0.396	47	-1.150	100	0.407	158	0.619	23	-0.225
	0,02mg/ml Starvation	123	94	-0.008	100	0.419	17	-0.328	50	-1.031	100	0.407	107	0.019	24	-0.111
	0,05mg/ml Starvation	119	91	-0.450	100	0.419	18	-0.259	50	-1.007	100	0.407	119	0.152	24	-0.168
	0,1mg/ml Starvation	28	100	-0.569	100	0.419	22	0.014	56	-0.720	100	-2.900	66	-0.466	24	-0.111
ERAAlop	0,01mg/ml	85	100	0.384	100	0.419	21	-0.037	50	-1.007	100	0.407	24	-0.962	24	-0.111
	0,02mg/ml	73	100	0.384	100	0.419	20	-0.157	52	-0.935	100	0.407	13	-1.095	24	-0.111
	0,05mg/ml	62	100	0.384	100	0.419	19	-0.191	51	-0.959	100	0.107	19	-1.024	24	-0.111
	0,1mg/ml	47	100	0.384	100	0.419	17	-0.328	52	-0.935	100	0.407	143	0.444	24	-0.111
	0,01mg/ml Starvation	83	94	-0.129	100	0.419	21	-0.089	51	-0.983	100	0.407	88	-0.209	25	0.004
	0,02mg/ml Starvation	100	91	-0.386	100	0.419	17	-0.328	52	-0.911	100	0.407	152	0.542	23	-0.225
	0,05mg/ml Starvation	62	83	-0.950	100	0.419	17	-0.328	52	-0.935	100	0.407	131	0.300	25	0.004
	0,1mg/ml Starvation	68	100	0.384	100	0.419	17	-0.328	54	-0.840	100	0.407	117	0.134	24	-0.111
EAGPS	0,01mg/ml	104	100	0.384	100	0.419	25	0.219	53	-0.864	100	0.407	36	-0.823	24	-0.111
	0,02mg/ml	91	100	0.384	100	0.419	33	0.766	51	-0.959	100	0.407	47	-0.693	25	0.004
	0,05mg/ml	84	100	0.384	100	0.419	35	0.868	53	-0.864	100	0.407	61	-0.534	25	0.004
	0,1mg/ml	88	100	0.384	100	0.419	32	0.697	52	-0.911	100	-2.073	58	-0.563	25	-0.025
	0,01mg/ml Starvation	180	93	-0.092	100	0.419	34	0.834	55	-0.768	100	-2.900	217	1.310	22	-0.311
	0,02mg/ml Starvation	122	88	-0.569	100	0.419	31	0.646	56	-0.744	100	-2.900	131	0.300	23	-0.225
	0,05mg/ml Starvation	127	79	-1.045	100	0.419	34	0.817	55	-0.768	100	-2.900	156	0.595	24	-0.168
	0,1mg/ml Starvation	72	76	-1.284	100	0.419	30	0.527	57	-0.673	0	-2.900	0	-1.249	25	0.004
EAGAAsia	0,01mg/ml	75	100	0.384	100	0.419	18	-0.259	51	-0.983	100	0.407	61	-0.534	24	-0.111
	0,02mg/ml	93	100	0.384	100	0.419	17	-0.328	51	-0.947	100	0.407	39	-0.794	25	0.004
	0,05mg/ml	69	100	0.384	100	0.419	15	-0.447	52	-0.899	100	-0.328	41	-0.764	24	-0.111
	0,1mg/ml	58	100	0.384	100	0.419	15	-0.499	54	-0.816	100	0.407	148	0.500	22	-0.339
	0,01mg/ml Starvation	59	84	-0.950	100	0.419	23	0.048	50	-1.019	100	0.407	165	0.695	23	-0.225
	0,02mg/ml Starvation	49	100	0.384	100	0.419	20	-0.157	51	-0.947	100	-2.900	147	0.489	22	-0.339
	0,05mg/ml Starvation	23	100	0.384	100	0.419	21	-0.054	50	-0.995	100	-2.900	2	-1.225	22	-0.397
	0,1mg/ml Starvation	40	100	0.384	100	0.419	21	-0.054	54	-0.816	100	-2.900	108	0.028	23	-0.225
EAGAAlop	0,01mg/ml	105	100	0.384	100	0.419	19	-0.191	50	-1.007	100	0.407	67	-0.457	25	0.004
	0,02mg/ml	88	100	0.384	100	0.419	19	-0.191	51	-0.983	100	0.407	36	-0.823	25	0.004
	0,05mg/ml	98	100	0.384	100	0.419	18	-0.259	51	-0.959	100	0.407	59	-0.551	25	0.004
	0,1mg/ml	109	100	0.384	100	0.419	19	-0.191	51	-0.983	100	0.407	82	-0.285	25	0.004
	0,01mg/ml Starvation	133	89	-0.357	100	0.419	20	-0.157	49	-1.055	100	0.407	153	0.560	23	-0.225
	0,02mg/ml Starvation	222	96	0.146	100	0.419	17	-0.328	49	-1.055	100	0.407	124	0.217	25	-0.054
	0,05mg/ml Starvation	88	90	-0.283	100	0.419	24	0.151	51	-0.959	100	0.407	103	-0.037	26	0.089
	0,1mg/ml Starvation	51	75	-1.840	100	0.419	27	0.356	57	-0.673	100	-2.900	75	-0.362	25	-0.054
EAGMA	0,01mg/ml	62	100	0.384	100	0.419	18	-0.259	49	-1.055	100	0.407	140	0.406	24	-0.111
	0,02mg/ml	68	100	0.384	100	0.419	20	-0.123	50	-1.007	100	0.407	125	0.229	23	-0.225
	0,05mg/ml	84	100	0.384	100	0.419	18	-0.259	50	-1.031	100	0.407	155	0.583	23	-0.225
	0,1mg/ml	81	100	0.384	100	0.419	19	-0.191	48	-1.102	100	0.407	196	1.068	21	-0.454
	0,01mg/ml Starvation	88	94	-0.222	100	0.419	19	-0.191	47	-1.150	100	0.407	293	2.214	21	-0.454
	0,02mg/ml Starvation	85	96	-0.357	100	0.419	18	-0.259	48	-1.126	100	0.407	233	1.505	23	-0.282
	0,05mg/ml Starvation	74	93	-0.222	100	0.419	18	-0.259	51	-1.007	100	0.077	261	1.836	22	-0.339
	0,1mg/ml Starvation	84	100	0.384	100	0.419	19	-0.191	46	-1.162	100	0.407	221	1.363	22	-0.339
EAGMD	0,01mg/ml	84	100	0.384	100	0.419	21	-0.089	50	-1.007	100	0.407	135	0.347	24	-0.111
	0,02mg/ml	81	100	0.384	100	0.419	20	-0.123	50	-1.007	100	0.407	119	0.158	24	-0.111
	0,05mg/ml	77	100	0.384	100	0.419	19	-0.191	51	-0.959	100	0.407	117	0.134	24	-0.111
	0,1mg/ml	67	100	0.384	100	0.419	19	-0.225	51	-0.971	100	0.407	128	0.258	24	-0.111
	0,01mg/ml Starvation	95	93	-0.129	100	0.419	16	-0.430	48	-1.102	100	0.407	247	1.665	23	-0.225
	0,02mg/ml Starvation	107	97	-0.033	100	0.419	19	-0.191	47	-1.150	100	0.407	143	0.438	23	-0.225
	0,05mg/ml Starvation	75	83	-0.728	100	0.419	22	0.031	54	-0.816	100	0.407	201	1.127	23	-0.225
	0,1mg/ml Starvation	44	94	-0.450	100	0.419	28	0.390	54	-0.840	100	0.407	164	0.690	23	-0.225
EAGKL	0,01mg/ml	86	99	0.209	100	0.419	20	-0.123	51	-0.959	100	0.407	66	-0.469	25	0.004
	0,02mg/ml	80	100	0.384	100	0.419	18	-0.259	49	-1.055	100	0.407	34	-0.847	26	0.118
	0,05mg/ml	74	100	0.384	100	0.419	20	-0.157	51	-0.983	100	0.407	35	-0.835	27	0.232
	0,1mg/ml	78	100	0.384	100	0.419	18	-0.259	51	-0.959	100	0.407	64	-0.492	25	0.004
	0,01mg/ml Starvation	60	97	-0.092	100	0.419	19	-0.191	50	-1.007	100	0.407	159	0.630	24	-0.111
	0,02mg/ml Starvation	103	97	0.106	100	0.419	23	0.048	52	-0.935	100	0.407	129	0.270	23	-0.197
	0,05mg/ml Starvation	74	98	-0.061	100	0.419	30	0.527	52	-0.911	100	-2.900	94	-0.144	24	-0.111
	0,1mg/ml Starvation	31	100	0.384	100	0.419	27	0.373	50	-1.007	0	-2.900	0	-1.249	23	-0.225
EAGAS	0,01mg/ml	92	100	0.384	100	0.419	19	-0.191	49	-1.055	100	0.407	79	-0.321	24	-0.111
	0,02mg/ml	85	100	0.384	100	0.419	19	-0.191	50	-1.007	100	0.407	19	-1.030	25	0.004
	0,05mg/ml	88	100	0.384	100	0.419	20	-0.123	49	-1.055	100	0.407	29	-0.906	25	0.004
	0,1mg/ml	72	100	0.384	100	0.419	23	0.082	51	-0.959	100	0.407	71	-0.410	25	0.004
	0,01mg/ml Starvation	110	100	0.013	100	0.419	17	-0.362	48	-1.102	100	0.407	87	-0.226	25	0.004
	0,02mg/ml Starvation	121	94	-0.061	100	0.419	20	-0.157	47	-1.150	100	0.407	127	0.252	25	0.004
	0,05mg/ml Starvation	113	90	-0.283	100	0.419	23	0.048	52	-0.911	100	0.407	139	0.394	25	0.004

FEATURES MEASUREMENTS AFTER 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																	
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					Active Cells		# Per Cell		Area		Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
	0,1mg/ml Starvation	212	96	0.094	100	0.419	28	0.424	53	-0.864	100	0.407	88	-0.215	25	0.004
EAGAT	0,01mg/ml	81	100	0.384	100	0.419	16	-0.396	50	-1.007	100	0.407	86	-0.229	24	-0.111
	0,02mg/ml	87	100	0.384	100	0.419	16	-0.396	48	-1.102	100	0.407	32	-0.876	25	0.004
	0,05mg/ml	80	100	0.384	100	0.419	18	-0.242	50	-1.007	100	0.407	27	-0.935	26	0.118
	0,1mg/ml	91	100	0.384	100	0.419	18	-0.259	51	-0.983	100	0.407	74	-0.380	25	0.004
	0,01mg/ml Starvation	81	100	0.384	100	0.419	17	-0.328	51	-0.959	100	-0.695	107	0.016	24	-0.111
	0,02mg/ml Starvation	107	100	0.033	100	0.419	17	-0.362	48	-1.102	100	0.407	177	0.840	24	-0.111
	0,05mg/ml Starvation	81	93	-0.357	100	0.419	22	-0.003	49	-1.055	100	0.407	136	0.359	24	-0.111
	0,1mg/ml Starvation	113	100	0.384	100	0.419	17	-0.311	51	-0.959	100	0.407	122	0.193	25	0.004
EAGAU	0,01mg/ml	88	100	0.384	100	0.419	19	-0.191	48	-1.102	100	0.407	76	-0.353	25	0.004
	0,02mg/ml	83	100	0.384	100	0.419	18	-0.259	49	-1.079	100	0.407	61	-0.528	25	0.004
	0,05mg/ml	118	100	0.384	100	0.419	15	-0.447	49	-1.055	100	0.407	38	-0.800	26	0.118
	0,1mg/ml	68	100	0.384	100	0.419	17	-0.311	49	-1.043	100	0.407	97	-0.102	25	0.004
	0,01mg/ml Starvation	88	90	-0.283	100	0.419	19	-0.225	46	-1.198	100	0.407	130	0.288	24	-0.111
	0,02mg/ml Starvation	72	100	-0.222	100	0.419	16	-0.396	49	-1.043	100	0.407	150	0.524	24	-0.111
	0,05mg/ml Starvation	104	85	-0.683	100	0.419	17	-0.328	49	-1.055	100	0.407	109	0.042	24	-0.082
	0,1mg/ml Starvation	72	100	0.384	100	0.419	21	-0.089	53	-0.864	100	0.407	79	-0.315	25	0.004
ERPS	0,01mg/ml	91	100	0.384	100	0.419	18	-0.259	47	-1.150	98	0.233	20	-1.018	25	0.004
	0,02mg/ml	70	100	0.384	100	0.419	17	-0.311	45	-1.246	100	0.407	90	-0.191	25	0.004
	0,05mg/ml	78	100	0.384	100	0.419	19	-0.208	44	-1.317	100	0.407	27	-0.930	26	0.118
	0,1mg/ml	5	50	-4.063	100	-4.579	9	-0.875	74	0.139	0	-2.900	0	-1.249	/	/
	0,01mg/ml Starvation	58	100	-1.435	100	-4.579	16	-0.396	48	-1.102	100	-1.577	130	0.288	23	-0.282
	0,02mg/ml Starvation	18	98	-6.287	100	-4.579	10	-0.823	54	-0.816	0	-2.900	0	-1.249	25	-0.054
	0,05mg/ml Starvation	16	47	-3.322	100	-4.579	11	-0.772	77	0.270	58	-2.900	1	-1.240	50	2.805
	0,1mg/ml Starvation	13	30	-5.453	100	-4.579	16	-0.430	71	-0.028	25	-2.900	0	-1.249	56	3.548
EFIAAlop	0,01mg/ml	91	100	0.384	95	0.170	13	-0.601	73	0.079	100	0.407	172	0.778	22	-0.339
	0,02mg/ml	76	100	0.384	88	-0.205	8	-0.977	79	0.354	100	0.407	177	0.837	21	-0.454
	0,05mg/ml	93	100	0.384	94	0.125	14	-0.533	76	0.235	100	0.407	162	0.666	22	-0.339
	0,1mg/ml	60	100	0.384	100	0.419	16	-0.379	76	0.211	100	0.407	65	-0.480	25	0.004
	0,01mg/ml Starvation	86	96	0.094	100	0.419	45	1.552	74	0.139	100	0.407	145	0.465	23	-0.225
	0,02mg/ml Starvation	75	100	0.384	100	0.419	44	1.518	73	0.091	100	0.407	114	0.099	24	-0.168
	0,05mg/ml Starvation	69	100	0.384	100	0.419	40	1.244	73	0.091	100	0.407	170	0.760	23	-0.225
	0,1mg/ml Starvation	117	100	0.384	100	0.419	43	1.415	71	-0.004	100	0.407	69	-0.433	25	0.004
ERHA	0,01mg/ml	75	100	0.384	96	0.234	12	-0.669	79	0.378	100	0.407	206	1.186	22	-0.339
	0,02mg/ml	53	100	0.384	91	-0.035	11	-0.738	83	0.545	100	0.407	185	0.941	22	-0.339
	0,05mg/ml	48	100	0.384	100	0.419	15	-0.464	78	0.330	100	0.407	225	1.411	22	-0.339
	0,1mg/ml	53	100	0.384	100	0.419	19	-0.191	79	0.378	100	0.407	202	1.139	21	-0.454
	0,01mg/ml Starvation	57	100	0.033	100	0.419	34	0.800	72	0.032	100	0.407	47	-0.699	24	-0.111
	0,02mg/ml Starvation	59	100	0.384	100	0.419	40	1.244	71	-0.004	100	0.407	205	1.168	23	-0.225
	0,05mg/ml Starvation	44	100	0.384	100	0.419	38	1.125	76	0.235	100	0.407	182	0.902	22	-0.339
	0,1mg/ml Starvation	39	100	0.384	100	0.419	38	1.073	74	0.115	100	0.407	178	0.849	22	-0.339
EAGAF	0,01mg/ml	122	100	0.384	96	0.202	13	-0.601	76	0.235	100	0.407	174	0.808	21	-0.454
	0,02mg/ml	92	100	0.384	93	0.049	11	-0.755	77	0.270	100	0.407	167	0.725	21	-0.454
	0,05mg/ml	82	100	0.384	96	0.234	13	-0.601	77	0.282	100	0.407	199	1.103	22	-0.339
	0,1mg/ml	113	100	0.384	94	0.142	13	-0.635	78	0.330	100	0.407	116	0.116	23	-0.225
	0,01mg/ml Starvation	83	100	0.384	100	0.419	35	0.868	75	0.187	100	0.407	152	0.542	22	-0.339
	0,02mg/ml Starvation	111	99	0.229	100	0.419	36	0.971	74	0.115	100	0.407	121	0.181	24	-0.111
	0,05mg/ml Starvation	42	100	0.384	100	0.419	44	1.483	77	0.282	100	0.407	147	0.483	22	-0.339
	0,1mg/ml Starvation	29	100	0.384	100	0.419	30	0.527	81	0.485	100	0.407	108	0.028	24	-0.111
EAGAA	0,01mg/ml	66	100	0.384	94	0.049	13	-0.635	78	0.306	100	0.407	212	1.257	21	-0.454
	0,02mg/ml	48	100	0.384	92	-0.136	9	-0.875	83	0.557	100	0.407	176	0.831	21	-0.454
	0,05mg/ml	57	100	0.384	95	0.156	9	-0.909	81	0.473	100	0.407	206	1.186	21	-0.454
	0,1mg/ml	76	100	0.384	90	-0.080	8	-0.943	81	0.450	100	0.407	174	0.808	20	-0.568
	0,01mg/ml Starvation	80	100	0.384	100	0.419	40	1.210	77	0.282	100	0.407	65	-0.480	25	-0.025
	0,02mg/ml Starvation	58	96	0.094	100	0.419	41	1.278	77	0.282	100	0.407	169	0.743	22	-0.339
	0,05mg/ml Starvation	59	100	0.384	100	0.419	33	0.766	80	0.414	100	0.407	173	0.790	22	-0.339
	0,1mg/ml Starvation	29	100	0.384	100	0.419	25	0.185	87	0.760	100	0.407	161	0.648	22	-0.339
EAGFO	0,01mg/ml	88	100	0.384	96	0.202	18	-0.259	73	0.091	100	0.407	180	0.873	22	-0.339
	0,02mg/ml	50	100	0.384	86	-0.295	6	-1.080	82	0.521	100	0.407	229	1.452	21	-0.454
	0,05mg/ml	40	100	0.384	82	-0.489	7	-1.045	81	0.450	100	0.407	274	1.984	20	-0.625
	0,1mg/ml	3	100	0.384	0	-4.579	0	-1.490	242	8.137	50	-1.797	1	-1.243	80	6.292
	0,01mg/ml Starvation	58	100	0.384	100	0.419	42	1.381	75	0.163	100	0.407	151	0.536	22	-0.339
	0,02mg/ml Starvation	14	100	0.384	100	0.419	20	-0.123	85	0.664	100	0.407	94	-0.138	22	-0.397
	0,05mg/ml Starvation	4	100	0.384	0	-4.579	0	-1.490	89	0.855	67	-1.246	1	-1.237	63	4.348
	0,1mg/ml Starvation	5	100	0.384	0	-4.579	0	-1.490	113	2.001	78	-0.538	2	-1.225	62	4.263
EAGPD	0,01mg/ml	70	100	0.384	100	0.419	22	-0.003	75	0.187	100	0.407	70	-0.424	24	-0.111
	0,02mg/ml	70	100	0.384	100	0.419	15	-0.464	77	0.259	100	0.407	57	-0.572	24	-0.111
	0,05mg/ml	40	100	0.384	100	0.142	21	-0.054	79	0.378	100	0.407	7	-1.166	24	-0.111
	0,1mg/ml	22	100	0.384	100	0.419	21	-0.089	83	0.569	100	0.407	38	-0.805	24	-0.111
	0,01mg/ml Starvation	68	100	0.384	100	0.419	44	1.518	76	0.235	15	-2.599	0	-1.249	26	0.089
	0,02mg/ml Starvation	62	99	0.209	100	0.419	43	1.415	77	0.282	100	0.407	31	-0.888	24	-0.111
	0,05mg/ml Starvation	39	100	0.384	100	0.419	42	1.347	78	0.330	100	0.407	34	-0.853	25	0.004
	0,1mg/ml Starvation	6	100	-0.950	100	-4.579	27	0.356	82	0.509	0	-2.900	0	-1.249	28	0.347
EAGTM	0,01mg/ml	84	100	0.384	96	0.220	12	-0.669	76	0.235	100	0.407	131	0.300	23	-0.225
	0,02mg/ml	67	100	0.384	88	-0.205	8	-0.943	79	0.354	100	0.407	46	-0.702	24	-0.111

FEATURES MEASUREMENTS AFTER 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																	
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					Active Cells		# Per Cell		Area		Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
	0,05mg/ml	60	100	0.384	90	-0.080	22	0.014	78	0.330	100	0.407	64	-0.489	25	0.004
	0,1mg/ml	58	100	0.384	86	-0.295	9	-0.909	85	0.664	100	0.407	82	-0.283	23	-0.225
	0,01mg/ml Starvation	47	100	0.384	100	0.419	46	1.620	76	0.235	100	0.407	108	0.028	23	-0.225
	0,02mg/ml Starvation	105	100	0.384	100	0.419	44	1.518	72	0.044	100	0.407	38	-0.800	24	-0.111
	0,05mg/ml Starvation	70	100	0.384	100	0.419	38	1.125	75	0.187	100	0.407	81	-0.291	24	-0.111
	0,1mg/ml Starvation	44	100	0.384	100	0.419	39	1.142	78	0.306	100	0.407	33	-0.865	25	0.004
EAGC	0,01mg/ml	43	100	0.384	100	0.419	19	-0.225	75	0.187	100	0.407	119	0.158	23	-0.197
	0,02mg/ml	26	100	0.384	100	0.125	14	-0.533	81	0.485	100	0.407	160	0.636	23	-0.282
	0,05mg/ml	33	100	0.384	100	0.419	20	-0.140	79	0.378	100	0.407	104	-0.025	23	-0.225
	0,1mg/ml	24	100	0.384	100	0.419	31	0.629	77	0.282	100	0.407	174	0.808	22	-0.339
	0,01mg/ml Starvation	48	100	0.384	100	0.419	42	1.381	76	0.235	100	0.407	113	0.081	24	-0.111
	0,02mg/ml Starvation	39	100	0.162	100	0.419	50	1.928	75	0.187	100	0.407	127	0.246	24	-0.111
	0,05mg/ml Starvation	23	100	0.051	100	0.419	50	1.928	78	0.342	100	0.407	113	0.081	24	-0.139
	0,1mg/ml Starvation	13	100	-0.950	100	0.419	26	0.270	80	0.402	100	0.407	69	-0.433	26	0.061
EFHA	0,01mg/ml	75	100	0.384	100	0.419	17	-0.328	74	0.139	100	0.407	132	0.311	23	-0.225
	0,02mg/ml	61	100	0.384	100	0.419	14	-0.550	76	0.223	100	0.407	118	0.146	23	-0.225
	0,05mg/ml	95	100	0.384	100	0.419	18	-0.259	74	0.139	100	0.407	116	0.122	23	-0.225
	0,1mg/ml	65	100	0.384	97	0.220	13	-0.601	81	0.450	100	0.407	129	0.276	23	-0.225
	0,01mg/ml Starvation	90	96	0.117	100	0.419	41	1.313	74	0.115	100	0.407	82	-0.280	25	0.004
	0,02mg/ml Starvation	106	100	0.384	100	0.419	43	1.415	73	0.091	100	0.407	138	0.382	23	-0.225
	0,05mg/ml Starvation	103	95	0.023	100	0.419	43	1.415	77	0.282	100	0.407	60	-0.540	25	0.004
	0,1mg/ml Starvation	7	100	0.384	100	-4.579	17	-0.328	99	1.309	100	-1.797	43	-0.746	22	-0.397
EAGSS	0,01mg/ml	68	100	0.384	100	0.419	19	-0.191	76	0.235	100	0.407	131	0.300	24	-0.111
	0,02mg/ml	39	100	0.384	100	0.419	21	-0.071	74	0.139	100	0.407	138	0.382	24	-0.111
	0,05mg/ml	59	100	0.384	100	0.419	19	-0.191	78	0.330	100	0.407	152	0.548	23	-0.225
	0,1mg/ml	39	100	0.384	100	0.419	26	0.253	76	0.211	100	0.407	155	0.580	23	-0.225
	0,01mg/ml Starvation	82	100	0.384	100	0.419	49	1.859	75	0.163	100	0.407	177	0.837	22	-0.339
	0,02mg/ml Starvation	47	100	0.384	100	0.419	50	1.928	75	0.187	100	0.407	167	0.725	23	-0.225
	0,05mg/ml Starvation	66	100	0.384	100	0.419	44	1.483	76	0.211	100	0.407	64	-0.492	25	0.004
	0,1mg/ml Starvation	61	100	0.384	100	0.419	49	1.859	78	0.330	100	0.407	95	-0.132	25	0.004
EFIPS	0,01mg/ml	87	100	0.384	97	0.258	20	-0.123	74	0.139	100	0.407	153	0.560	23	-0.225
	0,02mg/ml	47	100	0.384	100	0.419	12	-0.669	76	0.235	100	0.407	191	1.009	21	-0.454
	0,05mg/ml	70	100	0.384	95	0.156	12	-0.704	85	0.641	100	0.407	185	0.932	22	-0.339
	0,1mg/ml	27	100	0.384	100	0.419	16	-0.396	81	0.450	100	0.407	204	1.159	21	-0.454
	0,01mg/ml Starvation	79	92	-0.143	100	0.419	45	1.552	77	0.282	100	0.407	111	0.057	23	-0.225
	0,02mg/ml Starvation	93	88	-0.477	100	0.419	43	1.449	78	0.318	100	0.407	136	0.359	22	-0.339
	0,05mg/ml Starvation	30	87	-0.569	100	0.419	42	1.381	82	0.521	100	0.407	143	0.435	22	-0.339
	0,1mg/ml Starvation	6	100	-1.840	75	-4.579	4	-1.216	125	2.551	0	-2.900	0	-1.249	31	0.690
EAGCB	0,01mg/ml	80	100	0.384	93	0.086	19	-0.191	75	0.163	100	0.407	49	-0.675	26	0.118
	0,02mg/ml	45	100	0.384	87	-0.270	10	-0.806	80	0.426	100	0.407	191	1.009	23	-0.225
	0,05mg/ml	50	100	0.384	100	0.419	20	-0.123	73	0.091	100	0.407	94	-0.138	24	-0.111
	0,1mg/ml	65	100	0.384	89	-0.338	15	-0.464	76	0.235	100	0.407	50	-0.661	25	0.004
	0,01mg/ml Starvation	41	100	0.384	100	0.419	44	1.535	74	0.139	100	0.407	118	0.146	24	-0.111
	0,02mg/ml Starvation	57	100	0.146	100	0.419	51	1.996	75	0.163	100	0.407	128	0.264	23	-0.225
	0,05mg/ml Starvation	48	100	0.384	100	0.419	44	1.483	77	0.282	100	0.407	114	0.099	24	-0.111
	0,1mg/ml Starvation	52	96	0.127	100	0.419	42	1.381	77	0.259	100	0.407	35	-0.841	25	0.004
EAGCT	0,01mg/ml	90	100	0.384	80	-0.562	5	-1.148	72	0.044	100	0.407	147	0.489	20	-0.568
	0,02mg/ml	95	100	0.384	72	-0.986	3	-1.319	75	0.163	100	0.407	116	0.122	21	-0.454
	0,05mg/ml	84	100	0.384	78	-0.680	4	-1.216	77	0.259	100	0.407	144	0.453	21	-0.454
	0,1mg/ml	83	98	0.269	70	-1.095	2	-1.353	82	0.497	100	0.407	163	0.672	20	-0.568
	0,01mg/ml Starvation	74	100	0.384	100	0.419	24	0.151	71	-0.004	100	0.407	147	0.483	20	-0.568
	0,02mg/ml Starvation	82	99	0.290	100	0.419	20	-0.123	76	0.235	100	0.407	144	0.453	21	-0.454
	0,05mg/ml Starvation	55	97	0.209	94	0.107	15	-0.464	80	0.426	100	0.407	153	0.560	21	-0.454
	0,1mg/ml Starvation	49	98	0.239	83	-0.414	7	-1.011	82	0.521	100	0.407	166	0.710	21	-0.454
EAGOT	0,01mg/ml	92	100	0.384	76	-0.776	3	-1.285	70	-0.052	100	0.407	31	-0.882	24	-0.111
	0,02mg/ml	99	100	0.384	60	-1.561	1	-1.421	77	0.282	100	0.407	21	-1.000	24	-0.111
	0,05mg/ml	101	100	0.384	61	-1.531	1	-1.421	76	0.235	100	0.407	87	-0.226	22	-0.339
	0,1mg/ml	89	100	0.384	67	-1.219	1	-1.421	76	0.235	100	0.407	59	-0.551	22	-0.339
	0,01mg/ml Starvation	106	100	0.384	100	0.419	25	0.219	66	-0.243	100	0.407	25	-0.953	23	-0.225
	0,02mg/ml Starvation	111	100	0.384	100	0.419	24	0.151	67	-0.195	100	0.407	73	-0.386	22	-0.339
	0,05mg/ml Starvation	93	100	0.384	100	0.419	24	0.151	68	-0.147	100	0.407	64	-0.492	23	-0.225
	0,1mg/ml Starvation	103	100	0.384	100	0.419	21	-0.054	69	-0.100	100	0.407	89	-0.203	22	-0.339
ERKL	0,01mg/ml	95	100	0.384	100	0.419	15	-0.464	60	-0.529	100	0.407	107	0.016	21	-0.454
	0,02mg/ml	94	100	0.384	100	0.419	15	-0.464	62	-0.434	100	0.407	62	-0.522	23	-0.225
	0,05mg/ml	53	98	0.265	100	0.419	18	-0.259	68	-0.147	100	0.407	146	0.477	21	-0.454
	0,1mg/ml	2	100	-2.952	0	-4.579	0	-1.490	105	1.596	0	-2.900	0	-1.249	24	-0.111
	0,01mg/ml Starvation	84	100	0.384	100	0.419	25	0.219	66	-0.243	100	0.407	126	0.240	21	-0.454
	0,02mg/ml Starvation	79	98	0.225	100	0.419	26	0.287	70	-0.052	100	0.407	121	0.181	21	-0.454
	0,05mg/ml Starvation	3	90	-0.950	75	-4.579	9	-0.875	77	0.282	33	-2.900	0	-1.249	32	0.804
	0,1mg/ml Starvation	2	55	-6.287	57	-4.579	3	-1.319	125	2.551	0	-2.900	0	-1.249	32	0.804
ERKS	0,01mg/ml	90	100	0.384	88	-0.174	5	-1.148	75	0.163	100	0.407	34	-0.847	23	-0.225
	0,02mg/ml	91	100	0.384	90	-0.089	7	-1.011	71	-0.004	100	0.407	55	-0.599	24	-0.111
	0,05mg/ml	86	100	0.384	93	0.086	13	-0.601	72	0.044	100	0.407	132	0.311	21	-0.454
	0,1mg/ml	84	100	0.384	100	0.419	20	-0.123	66	-0.243	98	0.325	15	-1.077	23	-0.225
	0,01mg/ml Starvation	108	100	0.384	100	0.419	30	0.561	66	-0.243	100	0.407	79	-0.315	23	-0.225

FEATURES MEASUREMENTS AFTER 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																	
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					Active Cells		# Per Cell		Area		Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
	0,02mg/ml Starvation	103	100	0.384	100	0.419	31	0.629	67	-0.195	50	-1.797	1	-1.243	23	-0.225
	0,05mg/ml Starvation	95	98	0.267	100	0.419	37	1.005	69	-0.100	100	0.407	73	-0.386	22	-0.339
	0,1mg/ml Starvation	85	98	0.221	100	0.419	39	1.176	69	-0.100	100	0.407	60	-0.540	22	-0.339
ESHA	**0,01mg/ml**	96	100	0.384	86	-0.278	5	-1.148	71	-0.004	100	0.407	149	0.512	20	-0.568
	0,02mg/ml	88	100	0.384	83	-0.455	5	-1.148	73	0.091	100	0.407	95	-0.126	22	-0.339
	0,05mg/ml	94	100	0.384	83	-0.431	4	-1.216	71	-0.028	100	0.407	127	0.246	22	-0.339
	0,1mg/ml	89	100	0.384	82	-0.499	5	-1.148	76	0.211	100	0.407	92	-0.161	23	-0.225
	0,01mg/ml Starvation	99	100	0.384	100	0.419	26	0.253	63	-0.386	100	0.407	137	0.370	21	-0.454
	0,02mg/ml Starvation	95	100	0.384	100	0.419	25	0.219	66	-0.243	100	0.407	116	0.122	21	-0.454
	0,05mg/ml Starvation	87	100	0.384	100	0.419	28	0.424	69	-0.100	100	0.407	130	0.288	22	-0.397
	0,1mg/ml Starvation	80	100	0.384	100	0.419	29	0.492	70	-0.052	100	0.407	125	0.229	21	-0.454
***Ulva sp.* (U-2C)**	**0,01mg/ml**	97	100	0.384	87	-0.228	5	-1.148	71	-0.004	100	0.407	79	-0.315	23	-0.225
	0,02mg/ml	82	100	0.384	89	-0.146	6	-1.080	72	0.044	100	0.407	74	-0.380	23	-0.225
	0,05mg/ml	77	100	0.384	87	-0.217	6	-1.114	74	0.139	100	0.407	107	0.010	22	-0.339
	0,1mg/ml	87	100	0.384	78	-0.678	4	-1.216	76	0.235	100	0.407	124	0.217	22	-0.339
	0,01mg/ml Starvation	90	100	0.384	100	0.419	26	0.287	68	-0.147	100	0.407	112	0.075	22	-0.339
	0,02mg/ml Starvation	80	100	0.384	100	0.419	22	0.014	70	-0.052	100	0.407	70	-0.421	24	-0.111
	0,05mg/ml Starvation	90	100	0.384	100	0.419	23	0.082	71	-0.004	100	0.407	79	-0.315	23	-0.225
	0,1mg/ml Starvation	70	98	0.267	100	0.419	27	0.322	75	0.187	100	0.407	77	-0.342	23	-0.225
***Cryptomenia sp.* (C-3B)**	**0,01mg/ml**	82	100	0.384	85	-0.325	4	-1.216	74	0.139	100	0.407	57	-0.581	23	-0.225
	0,02mg/ml	79	100	0.384	83	-0.440	4	-1.250	76	0.211	100	0.407	48	-0.681	24	-0.168
	0,05mg/ml	66	100	0.384	75	-0.855	2	-1.353	80	0.426	100	0.407	135	0.347	21	-0.454
	0,1mg/ml	66	100	0.384	61	-1.531	1	-1.421	81	0.473	100	0.407	51	-0.646	24	-0.111
	0,01mg/ml Starvation	82	100	0.384	100	0.419	22	0.014	70	-0.052	100	0.407	36	-0.823	25	-0.054
	0,02mg/ml Starvation	70	100	0.384	92	0.025	12	-0.669	73	0.091	100	0.407	55	-0.599	24	-0.111
	0,05mg/ml Starvation	57	100	0.384	88	-0.190	11	-0.738	75	0.187	100	0.407	64	-0.492	24	-0.168
	0,1mg/ml Starvation	34	95	0.051	93	0.062	20	-0.123	82	0.521	100	0.407	126	0.235	23	-0.225
***Ciona intestinalis* (C-4B)**	**0,01mg/ml**	88	100	0.384	96	0.208	10	-0.806	70	-0.052	100	0.407	83	-0.268	23	-0.225
	0,02mg/ml	80	100	0.384	96	0.215	9	-0.909	72	0.044	100	0.407	61	-0.528	23	-0.225
	0,05mg/ml	49	100	0.384	94	0.134	10	-0.806	80	0.426	100	0.407	124	0.211	21	-0.454
	0,1mg/ml	14	100	0.384	73	-1.143	8	-0.977	90	0.879	100	0.407	101	-0.061	21	-0.454
	0,01mg/ml Starvation	71	100	0.384	100	0.419	25	0.219	70	-0.052	100	0.407	124	0.214	22	-0.339
	0,02mg/ml Starvation	64	100	0.384	100	0.419	23	0.048	74	0.139	100	0.407	103	-0.031	22	-0.339
	0,05mg/ml Starvation	15	100	0.384	100	0.086	16	-0.396	91	0.927	100	0.407	110	0.045	22	-0.339
	0,1mg/ml Starvation	1	100	-2.952	0	-4.579	0	-1.490	221	7.159	0	-2.900	0	-1.249	38	1.519
***Heliotropium sp.* (T-1B)**	**0,01mg/ml**	58	100	0.384	95	0.181	9	-0.875	81	0.450	100	0.407	96	-0.120	22	-0.339
	0,02mg/ml	26	100	0.384	100	0.419	14	-0.533	84	0.617	100	0.407	200	1.112	21	-0.454
	0,05mg/ml	2	50	-3.619	0	-4.579	0	-1.490	110	1.858	0	-2.900	0	-1.249	28	0.347
	0,1mg/ml	3	100	-2.952	40	-4.579	0	-1.490	91	0.951	0	-2.900	0	-1.249	/	/
	0,01mg/ml Starvation	39	98	0.248	100	0.419	30	0.527	78	0.330	100	0.407	180	0.882	22	-0.339
	0,02mg/ml Starvation	6	53	-2.776	95	-1.247	9	-0.875	93	1.046	77	-2.900	25	-0.953	24	-0.111
	0,05mg/ml Starvation	3	100	-1.840	25	-4.579	0	-1.490	110	1.858	0	-2.900	0	-1.249	33	0.918
	0,1mg/ml Starvation	2	100	-3.428	0	-4.579	0	-1.490	141	3.315	0	-2.900	0	-1.249	89	7.321
***Heliotropium sp.* (T-1C)**	**0,01mg/ml**	77	100	0.384	92	0.003	8	-0.943	72	0.044	100	0.407	55	-0.599	24	-0.111
	0,02mg/ml	82	100	0.384	87	-0.253	6	-1.080	72	0.044	50	-1.246	1	-1.243	24	-0.168
	0,05mg/ml	70	100	0.384	94	0.131	10	-0.806	71	-0.004	100	0.407	79	-0.321	23	-0.225
	0,1mg/ml	10	80	-0.950	100	0.419	19	-0.191	68	-0.171	100	0.407	136	0.353	21	-0.454
	0,01mg/ml Starvation	98	100	0.384	100	0.419	28	0.424	67	-0.195	100	0.407	62	-0.516	21	-0.454
	0,02mg/ml Starvation	81	100	0.384	100	0.419	27	0.356	68	-0.147	100	0.407	15	-1.074	24	-0.168
	0,05mg/ml Starvation	84	100	0.384	100	0.419	26	0.287	67	-0.195	100	0.407	136	0.353	22	-0.339
	0,1mg/ml Starvation	27	100	0.384	100	0.419	27	0.356	74	0.115	100	0.407	74	-0.374	22	-0.339
Verbascum blattaria	**0,01mg/ml**	94	100	0.384	94	0.097	11	-0.738	73	0.091	100	0.407	123	0.199	24	-0.111
	0,02mg/ml	94	100	0.384	98	0.303	11	-0.772	70	-0.052	100	0.407	113	0.090	24	-0.111
	0,05mg/ml	81	100	0.384	98	0.311	11	-0.738	71	-0.028	100	0.407	166	0.713	23	-0.225
	0,1mg/ml	56	100	0.384	93	0.049	8	-0.943	80	0.426	100	0.407	140	0.403	23	-0.225
	0,01mg/ml Starvation	112	96	0.127	100	0.419	35	0.902	73	0.091	100	0.407	134	0.335	23	-0.225
	0,02mg/ml Starvation	103	100	0.384	100	0.419	41	1.313	71	-0.004	100	0.407	47	-0.693	26	0.118
	0,05mg/ml Starvation	82	93	-0.092	100	0.419	33	0.732	75	0.187	100	0.407	76	-0.350	24	-0.168
	0,1mg/ml Starvation	23	100	0.384	100	-4.579	12	-0.704	97	1.237	100	-2.900	82	-0.285	23	-0.282
Stachys hissarica	**0,01mg/ml**	112	100	0.384	71	-1.009	2	-1.353	74	0.127	100	0.407	83	-0.274	24	-0.111
	0,02mg/ml	82	100	0.384	75	-0.830	3	-1.285	78	0.330	100	0.407	114	0.093	24	-0.111
	0,05mg/ml	73	100	0.384	75	-0.830	2	-1.353	83	0.569	100	0.407	119	0.155	24	-0.111
	0,1mg/ml	45	100	0.384	76	-0.830	4	-1.216	79	0.378	100	0.407	150	0.518	24	-0.111
	0,01mg/ml Starvation	106	100	0.384	100	0.419	28	0.424	74	0.115	100	0.407	106	0.001	24	-0.111
	0,02mg/ml Starvation	84	97	0.127	100	0.419	27	0.339	76	0.235	100	0.407	71	-0.410	25	0.004
	0,05mg/ml Starvation	91	94	-0.008	100	0.419	35	0.902	73	0.091	100	0.407	35	-0.841	27	0.232
	0,1mg/ml Starvation	60	94	-0.092	100	0.419	33	0.783	77	0.282	100	0.407	88	-0.209	25	0.004
Verbascum songoricum	**0,01mg/ml**	91	100	0.384	79	-0.622	4	-1.216	76	0.235	100	0.407	108	0.025	24	-0.168
	0,02mg/ml	88	100	0.384	81	-0.580	4	-1.250	74	0.151	100	0.407	113	0.087	24	-0.111
	0,05mg/ml	84	100	0.384	77	-0.734	5	-1.182	74	0.139	100	0.407	155	0.583	22	-0.339
	0,1mg/ml	39	100	0.384	86	-0.349	7	-1.011	86	0.712	100	0.407	168	0.731	23	-0.225
	0,01mg/ml Starvation	154	100	0.384	99	0.288	23	0.065	76	0.235	100	0.407	121	0.175	23	-0.225
	0,02mg/ml Starvation	103	99	0.221	100	0.419	28	0.424	76	0.235	100	0.407	72	-0.395	25	0.004
	0,05mg/ml Starvation	58	83	-0.829	100	0.419	29	0.475	82	0.509	100	0.407	80	-0.303	25	0.004
	0,1mg/ml Starvation	15	100	-0.950	100	0.419	31	0.629	82	0.497	100	-2.900	80	-0.303	28	0.318

FEATURES MEASUREMENTS AFTER 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y																	
		% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
					Active Cells		# Per Cell		Area		Active Cells		# Per Cell		Area	
			Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
Stachys betoniciflora	**0,01mg/ml**	93	100	0.384	92	0.003	9	-0.875	73	0.091	100	0.407	64	-0.492	25	-0.025
	0,02mg/ml	104	100	0.384	89	-0.136	8	-0.943	75	0.187	100	0.407	75	-0.368	24	-0.111
	0,05mg/ml	90	100	0.384	89	-0.169	9	-0.875	73	0.091	100	0.407	89	-0.203	24	-0.111
	0,1mg/ml	111	100	0.384	84	-0.362	5	-1.148	75	0.187	100	0.407	50	-0.664	26	0.118
	0,01mg/ml Starvation	110	98	0.265	100	0.419	32	0.697	74	0.115	100	0.407	54	-0.610	25	0.004
	0,02mg/ml Starvation	133	96	0.122	100	0.419	30	0.561	73	0.068	100	0.407	77	-0.336	25	0.004
	0,05mg/ml Starvation	165	93	-0.092	100	0.419	24	0.116	74	0.139	100	0.407	58	-0.569	24	-0.111
	0,1mg/ml Starvation	56	90	-0.401	93	0.003	15	-0.464	82	0.497	100	0.407	65	-0.478	29	0.404
Phlomis sewertzovii	**0,01mg/ml**	113	100	0.384	89	-0.136	9	-0.875	74	0.139	100	0.407	115	0.105	25	0.004
	0,02mg/ml	102	100	0.384	86	-0.275	8	-0.977	73	0.091	100	0.407	58	-0.563	25	0.004
	0,05mg/ml	95	100	0.384	87	-0.247	5	-1.148	74	0.151	100	0.407	121	0.181	24	-0.111
	0,1mg/ml	87	100	0.384	82	-0.580	6	-1.114	74	0.139	100	0.407	103	-0.037	24	-0.111
	0,01mg/ml Starvation	92	93	-0.092	100	0.419	34	0.817	76	0.211	100	0.407	130	0.282	24	-0.111
	0,02mg/ml Starvation	126	98	0.248	100	0.419	34	0.851	73	0.103	100	0.407	56	-0.587	25	0.004
	0,05mg/ml Starvation	117	95	0.081	100	0.419	35	0.902	75	0.187	100	0.407	74	-0.374	24	-0.111
	0,1mg/ml Starvation	50	86	-0.569	100	0.419	37	1.039	79	0.354	100	0.407	85	-0.250	25	-0.054
Phlomis salicifolia	**0,01mg/ml**	94	100	0.384	91	-0.035	10	-0.806	74	0.139	100	0.407	79	-0.312	24	-0.111
	0,02mg/ml	99	100	0.384	93	0.062	10	-0.806	72	0.044	100	0.407	88	-0.212	25	0.004
	0,05mg/ml	96	100	0.384	85	-0.316	9	-0.875	72	0.020	100	0.407	93	-0.155	24	-0.111
	0,1mg/ml	67	100	0.384	74	-0.896	3	-1.285	77	0.282	100	0.407	62	-0.519	25	0.004
	0,01mg/ml Starvation	111	98	0.217	100	0.419	34	0.800	74	0.151	100	0.407	42	-0.752	25	0.004
	0,02mg/ml Starvation	115	97	0.188	100	0.419	37	1.056	71	-0.004	100	0.407	82	-0.285	25	0.004
	0,05mg/ml Starvation	122	94	-0.008	100	0.419	40	1.210	74	0.139	100	0.407	37	-0.814	26	0.118
	0,1mg/ml Starvation	95	91	-0.222	100	0.419	35	0.920	76	0.235	100	0.407	78	-0.327	25	0.004
Silene oreina	**0,01mg/ml**	72	100	0.384	100	0.419	24	0.151	71	-0.004	100	0.407	109	0.040	24	-0.111
	0,02mg/ml	79	100	0.384	100	0.419	21	-0.054	70	-0.052	100	0.407	100	-0.073	25	0.004
	0,05mg/ml	76	100	0.384	100	0.419	22	0.014	69	-0.123	100	0.407	104	-0.020	24	-0.111
	0,1mg/ml	61	100	0.384	100	0.419	26	0.287	69	-0.100	100	0.407	143	0.438	23	-0.197
	0,01mg/ml Starvation	101	97	0.182	100	0.419	43	1.449	71	-0.004	100	0.407	137	0.370	23	-0.225
	0,02mg/ml Starvation	118	96	0.117	100	0.419	44	1.518	71	-0.004	100	0.407	121	0.175	24	-0.111
	0,05mg/ml Starvation	78	87	-0.505	100	0.419	47	1.688	77	0.282	100	0.407	125	0.229	24	-0.111
	0,1mg/ml Starvation	49	78	-1.155	100	0.419	51	1.962	76	0.235	100	0.407	74	-0.371	26	0.089
Phlomis tadschikistanica	**0,01mg/ml**	90	100	0.384	91	-0.009	9	-0.875	78	0.330	100	0.407	78	-0.333	25	0.004
	0,02mg/ml	76	100	0.384	100	0.419	18	-0.259	72	0.020	100	0.407	134	0.329	24	-0.111
	0,05mg/ml	70	100	0.384	97	0.247	13	-0.635	74	0.151	100	0.407	113	0.087	23	-0.225
	0,1mg/ml	57	100	0.384	92	0.035	9	-0.875	73	0.091	100	0.407	121	0.181	24	-0.111
	0,01mg/ml Starvation	100	96	0.051	100	0.419	39	1.176	73	0.103	100	0.407	101	-0.061	25	0.004
	0,02mg/ml Starvation	116	100	0.384	100	0.419	42	1.381	70	-0.052	100	0.407	76	-0.350	25	0.004
	0,05mg/ml Starvation	109	94	-0.008	100	0.419	44	1.518	72	0.032	100	0.407	91	-0.173	25	0.004
	0,1mg/ml Starvation	76	95	0.033	100	0.419	43	1.449	75	0.187	100	0.407	82	-0.277	25	0.004
Cousina umbrosa	**0,01mg/ml**	62	100	0.384	100	0.419	19	-0.225	74	0.139	100	0.407	156	0.595	25	0.004
	0,02mg/ml	50	100	0.384	100	0.419	18	-0.259	76	0.211	100	0.407	109	0.040	24	-0.111
	0,05mg/ml	36	100	0.384	93	0.086	13	-0.618	82	0.521	100	0.407	108	0.028	25	0.004
	0,1mg/ml	51	100	0.384	95	0.170	13	-0.635	83	0.569	100	0.407	113	0.087	24	-0.111
	0,01mg/ml Starvation	48	100	0.384	100	0.419	37	1.039	80	0.426	100	0.407	130	0.282	25	0.004
	0,02mg/ml Starvation	62	100	0.384	100	0.419	41	1.295	75	0.187	100	0.407	41	-0.764	26	0.118
	0,05mg/ml Starvation	58	91	-0.222	100	0.419	41	1.313	77	0.282	100	0.407	89	-0.197	25	0.004
	0,1mg/ml Starvation	32	82	-1.284	100	0.419	32	0.663	77	0.282	100	0.407	121	0.181	24	-0.111
Nepeta olgae	**0,01mg/ml**	84	100	0.384	92	-0.035	10	-0.806	74	0.115	100	0.407	95	-0.126	25	0.004
	0,02mg/ml	78	100	0.384	93	0.054	10	-0.806	75	0.163	100	0.407	112	0.069	24	-0.111
	0,05mg/ml	64	100	0.384	93	0.075	13	-0.635	76	0.211	100	0.407	66	-0.475	25	0.004
	0,1mg/ml	82	100	0.384	100	0.419	13	-0.635	77	0.282	100	0.407	109	0.040	24	-0.111
	0,01mg/ml Starvation	103	100	0.384	100	0.419	38	1.073	69	-0.112	98	-0.176	31	-0.879	26	0.089
	0,02mg/ml Starvation	110	97	0.182	100	0.419	38	1.125	73	0.091	100	0.407	45	-0.720	26	0.118
	0,05mg/ml Starvation	97	100	0.384	100	0.419	39	1.176	72	0.044	100	0.407	75	-0.368	25	0.004
	0,1mg/ml Starvation	81	100	0.384	100	0.419	41	1.278	77	0.282	100	0.407	84	-0.256	25	0.004
Scutellaria scharistanica	**0,01mg/ml**	59	100	0.384	99	0.263	16	-0.379	76	0.247	100	0.407	93	-0.150	24	-0.111
	0,02mg/ml	41	100	0.384	94	0.035	9	-0.857	75	0.163	100	0.407	95	-0.132	26	0.118
	0,05mg/ml	59	100	0.384	100	0.419	14	-0.533	72	0.056	100	0.407	79	-0.315	26	0.118
	0,1mg/ml	29	100	0.384	98	0.062	17	-0.345	75	0.187	100	0.407	116	0.116	25	-0.054
	0,01mg/ml Starvation	107	97	0.199	100	0.419	35	0.868	72	0.044	100	0.407	68	-0.445	26	0.061
	0,02mg/ml Starvation	82	100	0.384	100	0.419	35	0.902	75	0.187	100	-0.006	48	-0.681	26	0.118
	0,05mg/ml Starvation	63	86	-0.569	100	0.419	41	1.278	74	0.139	100	0.407	83	-0.274	26	0.118
	0,1mg/ml Starvation	98	85	-0.604	100	0.419	35	0.902	78	0.330	100	0.407	74	-0.380	25	-0.054
Schrophullaria sp.	**0,01mg/ml**	54	100	0.384	100	0.419	10	-0.806	74	0.139	100	0.407	91	-0.173	25	0.004
	0,02mg/ml	49	100	0.384	100	0.419	9	-0.875	76	0.211	100	0.407	54	-0.610	26	0.118
	0,05mg/ml	64	100	0.384	100	0.419	10	-0.806	75	0.175	100	0.407	54	-0.616	26	0.118
	0,1mg/ml	46	100	0.384	100	0.419	13	-0.618	80	0.438	100	0.407	109	0.040	25	0.004
	0,01mg/ml Starvation	103	97	0.146	100	0.419	35	0.902	74	0.115	100	0.407	73	-0.386	25	0.004
	0,02mg/ml Starvation	70	98	0.236	100	0.419	36	0.971	76	0.235	100	0.407	98	-0.090	25	0.004
	0,05mg/ml Starvation	3	100	-6.287	100	-4.579	6	-1.080	112	1.954	100	-2.900	9	-1.148	37	1.376
	0,1mg/ml Starvation	3	100	-6.287	33	-4.579	1	-1.421	107	1.727	0	-2.900	0	-1.249	/	/
Leonurus panzeroides	**0,01mg/ml**	69	100	0.384	95	0.003	12	-0.669	78	0.330	100	0.407	43	-0.740	26	0.118
	0,02mg/ml	61	100	0.384	88	-0.330	9	-0.909	79	0.354	100	0.407	31	-0.882	27	0.232
	0,05mg/ml	52	100	0.384	92	-0.380	8	-0.943	80	0.426	100	0.407	50	-0.664	26	0.118

FEATURES MEASUREMENTS AFTER 20 HOURS TREATMENT ADMINISTRATION ON SH-SY5Y															
	% Living Cells	% Living Cells (per sample)		LYSOSOMES						LC3B II PROTEINS					
				Active Cells		# Per Cell		Area		Active Cells		# Per Cell		Area	
		Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score	Original	Z-score
0,1mg/ml	72	100	0.384	87	-0.295	7	-1.011	74	0.151	100	0.407	42	-0.749	27	0.232
0,01mg/ml Starvation	90	100	0.239	100	0.419	33	0.783	75	0.187	100	0.407	39	-0.788	26	0.118
0,02mg/ml Starvation	85	95	-0.092	100	0.419	33	0.732	75	0.187	100	0.407	38	-0.800	26	0.118
0,05mg/ml Starvation	61	94	-0.104	100	0.419	31	0.629	76	0.235	100	0.407	56	-0.587	26	0.118
0,1mg/ml Starvation	34	81	-1.155	100	0.419	23	0.082	80	0.426	100	0.407	44	-0.726	27	0.232

Table A2. Quantitative data (white columns) and correspondent Z-score values (grey columns) resulting from measurements of features selected for evaluating effects on autophagy after 20 hours of treatments: the percentage of living cells with respect to the control samples (written in red and reported in the first column) has been considered alone at the end of the analysis to consider the possible influence of the toxicity effects when occurring simultaneously with the most interesting observed autophagic phenotypes; the percentage of living cells referred to each single sample, the percentage of cells with acidic/autophagic compartments resulting activated after treatments, the number of lysosomes/LC3B proteins per cells and the area of lysosomes/LC3B proteins have been considered all together and used in the following multivariate analysis in order to interpret the effects produced by administrated extracts on autophagic phenotypes.

Factor scores associated to 2 hours treatments after Principal Component Analysis (PCA)						
2 hours' treatments		**PC1**	**PC2**	**2 hours' treatments**	**PC1**	**PC2**
Controls	Untreated	-1.04335413742	0.839364068725	Starvation	-0.165226692502	-0.371287249214
	0,1%DMSO	-1.10850318842	0.897022520147	0.1%DMSO_Starvation	-0.0135777168692	-0.679626063698
EAGRC	0,01mg/ml	-0.751420786794	0.278918480514	0,01mg/ml_Starvation	0.638209516558	-0.962456173198
	0,02mg/ml	-0.612062306209	0.317656244559	0,02mg/ml_Starvation	0.120390032864	-0.939934183768
	0,05mg/ml	-0.275357491314	-0.312774938657	0,05mg/ml_Starvation	0.379587912505	-0.69225111493
	0,1mg/ml	-0.5558911338	-0.234797674283	0,1mg/ml_Starvation	-0.0368437736605	-0.620041267277
ERAAlop	0,01mg/ml	-0.1201675203	0.00139901186479	0,01mg/ml_Starvation	1.00482657986	-0.771242791623
	0,02mg/ml	-0.233364995771	0.178485473511	0,02mg/ml_Starvation	0.962026997137	-0.705033193795
	0,05mg/ml	-0.220610542646	-0.512011330907	0,05mg/ml_Starvation	1.50266294383	-0.62241017582
	0,1mg/ml	0.0899019606709	-0.721103562743	0,1mg/ml_Starvation	1.02785262265	-0.638352648678
EAGPS	0,01mg/ml	-0.551846935979	0.153050798578	0,01mg/ml_Starvation	0.401235936176	-0.603513111783
	0,02mg/ml	-0.625470450879	0.114711885815	0,02mg/ml_Starvation	-0.110960328484	-0.120235413302
	0,05mg/ml	-0.646494242198	0.348646655413	0,05mg/ml_Starvation	0.0204122443841	0.275407806958
	0,1mg/ml	-0.815011566829	0.710707029355	0,1mg/ml_Starvation	0.0331223168076	0.835518866459
EAGAAsia	0,01mg/ml	-0.478221020828	-0.0444047363412	0,01mg/ml_Starvation	0.715842761975	-0.512643584635
	0,02mg/ml	-0.150914009914	-0.379391683332	0,02mg/ml_Starvation	0.861389838351	-0.991250870056
	0,05mg/ml	-0.426752584441	-0.43772649281	0,05mg/ml_Starvation	1.04375673658	-0.586523336297
	0,1mg/ml	-0.299749595646	-0.25785792765	0,1mg/ml_Starvation	0.572572632976	-0.579250667069
EAGAAlop	0,01mg/ml	-0.559833657737	0.0252942235056	0,01mg/ml_Starvation	0.945241162987	-0.473587299241
	0,02mg/ml	-0.333449446251	-0.0956542110293	0,02mg/ml_Starvation	0.696697664508	-0.616475752524
	0,05mg/ml	-0.276876493074	-0.241368267353	0,05mg/ml_Starvation	1.26145540033	-0.141264700735
	0,1mg/ml	-0.305271812764	-0.0464816708846	0,1mg/ml_Starvation	0.892830300118	0.155161112266
EAGMA	0,01mg/ml	-0.40919622174	-0.0970817668217	0,01mg/ml_Starvation	1.40291902948	-1.03100028641
	0,02mg/ml	-0.124620012922	-0.697780403124	0,02mg/ml_Starvation	1.53258733585	-1.26984083784
	0,05mg/ml	-0.429450947532	-0.724800248085	0,05mg/ml_Starvation	1.46666600921	-1.33494498957
	0,1mg/ml	0.0468738134012	-0.640971898884	0,1mg/ml_Starvation	1.05597415051	-1.19840844665
EAGMD	0,01mg/ml	-0.505991512849	-0.113452315959	0,01mg/ml_Starvation	0.847639474148	-1.21806900883
	0,02mg/ml	-0.399832887603	-0.214831104797	0,02mg/ml_Starvation	0.711307305331	-1.07095615945
	0,05mg/ml	-0.209898262171	-0.648704974465	0,05mg/ml_Starvation	0.803939843596	-0.964472464318
	0,1mg/ml	-0.429100045394	-0.727722345774	0,1mg/ml_Starvation	0.917591536626	-0.987759089639
EAGKL	0,01mg/ml	-0.233314241729	-0.601539215756	0,01mg/ml_Starvation	0.928590062632	-0.978158757229
	0,02mg/ml	-0.0370867767514	-0.479175208866	0,02mg/ml_Starvation	1.11051447454	-0.937535181955
	0,05mg/ml	-0.172979101179	-0.699559726521	0,05mg/ml_Starvation	1.13874598801	-0.82786584432
	0,1mg/ml	-0.00760842400915	-0.66111201292	0,1mg/ml_Starvation	0.993871408081	-0.887615056483
EAGAS	0,01mg/ml	-0.461803970823	0.0511446932001	0,01mg/ml_Starvation	1.19602331736	-0.81056181082
	0,02mg/ml	-0.121876190039	0.0336380559058	0,02mg/ml_Starvation	1.06059496091	-0.372632054353
	0,05mg/ml	-0.437799976345	-0.377730942404	0,05mg/ml_Starvation	0.763776267882	-0.748197309032
	0,1mg/ml	-0.246427645555	-0.267797310077	0,1mg/ml_Starvation	0.907586553829	0.188281220398
EAGAT	0,01mg/ml	0.81776753495	-0.964291813965	0,01mg/ml_Starvation	0.794587273339	-0.376703755874
	0,02mg/ml	1.15222560893	-1.08131622373	0,02mg/ml_Starvation	0.531926309597	-0.322511871364
	0,05mg/ml	0.778377560642	-1.61983340834	0,05mg/ml_Starvation	0.396377182889	-0.54785830215
	0,1mg/ml	0.800969583593	-0.987307439655	0,1mg/ml_Starvation	0.659816126694	-0.107300765666
EAGAU	0,01mg/ml	0.851957436835	-0.868858857675	0,01mg/ml_Starvation	0.335367653229	-0.168304110597
	0,02mg/ml	0.629190843568	-0.615891444509	0,02mg/ml_Starvation	0.209901134975	-0.203231882916
	0,05mg/ml	0.782722900701	-1.05504195187	0,05mg/ml_Starvation	0.406518417709	0.168674312446
	0,1mg/ml	0.484973226348	-1.19287056459	0,1mg/ml_Starvation	0.260671706291	-0.180405904784
ERPS	0,01mg/ml	-0.232066124701	-0.372039875966	0,01mg/ml_Starvation	1.14299750967	-1.0903882502
	0,02mg/ml	-0.0418562311044	-0.439316880901	0,02mg/ml_Starvation	1.29294818431	-0.933380251808
	0,05mg/ml	-0.301184663146	-0.35499984291	0,05mg/ml_Starvation	2.39144871392	-1.00145849005
	0,1mg/ml	-0.0192632788473	-0.717889897262	0,1mg/ml_Starvation	3.76570457366	6.23003564556
EFIAAlop	0,01mg/ml	-0.904611208563	1.03003246002	0,01mg/ml_Starvation	-0.484247167193	-0.569429439025
	0,02mg/ml	-1.0854714113	1.01604724431	0,02mg/ml_Starvation	-0.572373920324	-0.132103649225
	0,05mg/ml	-1.15507687995	1.20390296261	0,05mg/ml_Starvation	-0.424546850807	-0.333666374828
	0,1mg/ml	-1.11334018484	0.470134208102	0,1mg/ml_Starvation	-0.663143141344	-0.252665795672
ERHA	0,01mg/ml	-1.16369699196	0.674144645741	0,01mg/ml_Starvation	-0.380178907758	-0.137386087811
	0,02mg/ml	-1.16635980951	0.673895514039	0,02mg/ml_Starvation	0.312086686769	-0.209599810383
	0,05mg/ml	-0.938065739723	0.283862307666	0,05mg/ml_Starvation	-0.593667931792	-0.365935234267
	0,1mg/ml	-1.12524649543	1.04641885678	0,1mg/ml_Starvation	-0.340464310727	-0.379398637154
EAGAF	0,01mg/ml	-1.12273068481	0.691238164154	0,01mg/ml_Starvation	-0.00359823534878	0.33562792195
	0,02mg/ml	-1.19492178781	1.30841039732	0,02mg/ml_Starvation	-0.0331630580306	-0.13302634136
	0,05mg/ml	-0.969712248422	1.3651337605	0,05mg/ml_Starvation	-0.560736749619	-0.208216843445
	0,1mg/ml	-1.19922929229	0.488671329649	0,1mg/ml_Starvation	-0.524455419066	-0.658820140197
EAGAA	0,01mg/ml	-1.0093522106	0.680466369341	0,01mg/ml_Starvation	-0.439632742068	-0.481080308148
	0,02mg/ml	-1.10140816706	0.836323380991	0,02mg/ml_Starvation	-0.402751684902	-0.337252216569
	0,05mg/ml	-1.14819160758	0.711229712077	0,05mg/ml_Starvation	-0.480541773756	-0.568267675473
	0,1mg/ml	-1.028264054	0.884736051197	0,1mg/ml_Starvation	-0.601028794265	-0.474258991188
EAGFO	0,01mg/ml	-1.29703016701	0.867969401831	0,01mg/ml_Starvation	-0.777281465549	-0.647131398692
	0,02mg/ml	-1.14272207535	0.980791958657	0,02mg/ml_Starvation	-0.661689664417	-0.112618242095
	0,05mg/ml	-1.11278319502	0.618612247712	0,05mg/ml_Starvation	-0.166303689952	6.01396882371
	0,1mg/ml	-0.834599077622	0.171184940708	0,1mg/ml_Starvation	-0.232081208603	5.97996427696
EAGPD	0,01mg/ml	-0.898359289453	0.455851069204	0,01mg/ml_Starvation	-0.32360974184	0.146788774694
	0,02mg/ml	-0.932171302329	0.313439522265	0,02mg/ml_Starvation	0.0299969497648	-0.456934216606
	0,05mg/ml	-0.846817526584	0.185566949604	0,05mg/ml_Starvation	-0.221261902605	0.302690799582
	0,1mg/ml	-0.889833771958	0.374685474677	0,1mg/ml_Starvation	-0.370087248549	-0.435400081841
EAGTM	0,01mg/ml	-1.08298247585	0.635593985338	0,01mg/ml_Starvation	-0.547392486577	-0.607883377856
	0,02mg/ml	-0.952263521303	0.676025083115	0,02mg/ml_Starvation	-0.323670800163	-0.658144133682
	0,05mg/ml	-0.994997302797	0.453294982514	0,05mg/ml_Starvation	-0.261070774652	-0.329576203403
	0,1mg/ml	-0.86498643468	0.293535477566	0,1mg/ml_Starvation	-0.580142646517	-0.683384655245
EAGC	0,01mg/ml	-1.07090082614	0.472179256705	0,01mg/ml_Starvation	-0.383905728519	-0.174657756582
	0,02mg/ml	-1.05268777828	0.579674471222	0,02mg/ml_Starvation	0.334451816632	0.439989282088
	0,05mg/ml	-1.16364502163	0.599973241155	0,05mg/ml_Starvation	-0.451872124843	-0.250677664754
	0,1mg/ml	-1.11477173544	0.565916581695	0,1mg/ml_Starvation	-0.396525199218	-0.188459887398
EFHA	0,01mg/ml	-0.963365827501	0.858762136361	0,01mg/ml_Starvation	0.0624591393973	-0.377979459272
	0,02mg/ml	-1.01921240844	0.800695898504	0,02mg/ml_Starvation	-0.268898738565	0.0687950958306
	0,05mg/ml	-1.00629863083	0.571867444771	0,05mg/ml_Starvation	-0.496557737385	-0.407105323345
	0,1mg/ml	-1.04625457241	0.526102680248	0,1mg/ml_Starvation	-0.802595451465	-0.0809019268546
EAGSS	0,01mg/ml	-0.96415465925	0.32767771152	0,01mg/ml_Starvation	-0.202613791963	-0.432619861664
	0,02mg/ml	-1.09508957287	0.399443804993	0,02mg/ml_Starvation	-0.0547594879242	-0.519258581238
	0,05mg/ml	-0.949716549475	0.366469488336	0,05mg/ml_Starvation	-0.317556477649	-0.334786465776
	0,1mg/ml	-1.05221990876	0.575778340969	0,1mg/ml_Starvation	-0.389952850942	-0.456015220218
EFIPS	0,01mg/ml	-1.10034506226	0.572050952445	0,01mg/ml_Starvation	-0.497982369461	-0.129645375494
	0,02mg/ml	-0.925988976508	0.572801971384	0,02mg/ml_Starvation	0.138848314436	-0.0353984184583
	0,05mg/ml	-0.939524455081	0.285453327391	0,05mg/ml_Starvation	-0.270441939116	-0.522568455888

Factor scores associated to 2 hours treatments after Principal Component Analysis (PCA)						
2 hours' treatments		**PC1**	**PC2**	**2 hours' treatments**	**PC1**	**PC2**
	0,1mg/ml	-1.00883620497	0.109174306998	0,1mg/ml_Starvation	-0.17982940221	0.719007946889
EAGCB	0,01mg/ml	-0.799841416536	0.275544227182	0,01mg/ml_Starvation	-0.488795607937	-0.0115365391861
	0,02mg/ml	-0.813275294743	0.212154248241	0,02mg/ml_Starvation	0.0836229210081	0.12530344605
	0,05mg/ml	-0.799313317009	0.187587442315	0,05mg/ml_Starvation	0.0807563682876	0.934383100632
	0,1mg/ml	-0.957093496155	0.249383572022	0,1mg/ml_Starvation	-0.139748609602	-0.204086692826
EAGCT	0,01mg/ml	-1.18304754379	1.4653039026	0,01mg/ml_Starvation	-0.263557231399	-0.419155164561
	0,02mg/ml	-1.06168832006	0.742235613609	0,02mg/ml_Starvation	-0.415856546334	-0.28574221692
	0,05mg/ml	-1.19686487893	0.91487350932	0,05mg/ml_Starvation	-0.22214563042	-0.635154670615
	0,1mg/ml	-1.06893055518	1.19578256763	0,1mg/ml_Starvation	-0.41780619811	-0.522144228693
EAGOT	0,01mg/ml	-1.26063171203	1.05760656937	0,01mg/ml_Starvation	-0.253291964445	-0.695573241788
	0,02mg/ml	-1.08360883353	0.698927795714	0,02mg/ml_Starvation	-0.364146364816	-0.631013287087
	0,05mg/ml	-1.09636469093	0.983374565694	0,05mg/ml_Starvation	-0.158218362507	-0.543936845635
	0,1mg/ml	-1.05687235651	0.704047169045	0,1mg/ml_Starvation	-0.26442610487	-0.392878969942
ERKL	0,01mg/ml	-1.17440602054	0.689584236967	0,01mg/ml_Starvation	0.026465045235	-0.478317605717
	0,02mg/ml	-0.885204570184	-0.271048625314	0,02mg/ml_Starvation	-0.00343736043343	-0.9243025298
	0,05mg/ml	-0.980418943378	-0.395476843612	0,05mg/ml_Starvation	0.370275550086	-1.08860438712
	0,1mg/ml	-0.784100753804	-0.581764200173	0,1mg/ml_Starvation	1.49792507912	-1.15776574635
ERKS	0,01mg/ml	-1.2137647911	0.674522577126	0,01mg/ml_Starvation	0.156127566478	-0.751031690074
	0,02mg/ml	-1.13341433034	0.326968582117	0,02mg/ml_Starvation	-0.11521766897	-0.535518104803
	0,05mg/ml	-1.0395865722	0.570010972255	0,05mg/ml_Starvation	0.418171834377	-0.746993942724
	0,1mg/ml	-0.943495040514	0.558853693882	0,1mg/ml_Starvation	0.208860210618	-0.691144554747
ESHA	0,01mg/ml	-1.15480946763	1.16023091937	0,01mg/ml_Starvation	0.0335542288703	-0.494657932736
	0,02mg/ml	-0.990067927955	0.804160107399	0,02mg/ml_Starvation	-0.12458131942	-0.723251811561
	0,05mg/ml	-1.04899015872	0.7074418251	0,05mg/ml_Starvation	0.294996150297	-0.660839411531
	0,1mg/ml	-1.07271204054	0.856800400629	0,1mg/ml_Starvation	0.0992725436817	-0.576310890996
Ulva sp. (U-2C)	0,01mg/ml	-0.231390812552	2.49394264849	0,01mg/ml_Starvation	-0.133063893287	-0.351452359297
	0,02mg/ml	-0.668031367434	1.29053684034	0,02mg/ml_Starvation	-0.0480130532035	-0.226733689181
	0,05mg/ml	-1.02403134433	0.81565155244	0,05mg/ml_Starvation	0.282220514838	-0.0484774079022
	0,1mg/ml	-1.03467952632	0.853586850015	0,1mg/ml_Starvation	0.15035781381	-0.396550907406
Cryptomenia sp. (C-3B)	0,01mg/ml	-0.908729998453	1.36548094133	0,01mg/ml_Starvation	0.904396360569	1.34628680076
	0,02mg/ml	-0.630721811883	1.37109044999	0,02mg/ml_Starvation	0.515308009539	0.984502724946
	0,05mg/ml	-0.988409972393	1.03575811377	0,05mg/ml_Starvation	0.368903292157	0.379411211559
	0,1mg/ml	-1.11751277453	2.00351006176	0,1mg/ml_Starvation	1.0515819269	1.80803358382
Ciona intestinalis (C-4B)	0,01mg/ml	-0.363201578074	1.83197725291	0,01mg/ml_Starvation	-0.191693648683	0.0966506864717
	0,02mg/ml	-0.888619417594	0.852697591061	0,02mg/ml_Starvation	-0.28484794929	0.871041091238
	0,05mg/ml	-1.06865909663	0.961141405622	0,05mg/ml_Starvation	0.301213903815	1.1418797022
	0,1mg/ml	-1.22143797972	1.66636062708	0,1mg/ml_Starvation	0.590299544868	1.09095883113
Heliotropium sp. (T-1B)	0,01mg/ml	-0.697180295332	0.405341072522	0,01mg/ml_Starvation	0.195087802963	-0.410912454228
	0,02mg/ml	-0.606866392969	0.25697103558	0,02mg/ml_Starvation	0.504833125885	0.54281556171
	0,05mg/ml	-0.707421070694	0.328367606982	0,05mg/ml_Starvation	0.260770122885	-0.287359926278
	0,1mg/ml	-0.382405696135	0.499125110403	0,1mg/ml_Starvation	0.777642803394	0.529666011924
Heliotropium sp. (T-1C)	0,01mg/ml	-0.669804074408	0.70263124393	0,01mg/ml_Starvation	0.187195643589	-0.392083873116
	0,02mg/ml	-0.717433428926	-0.0280981271351	0,02mg/ml_Starvation	0.399537004271	0.221190373916
	0,05mg/ml	-0.893216597821	0.418465825513	0,05mg/ml_Starvation	0.324229477936	-0.379486312394
	0,1mg/ml	-0.700970324844	0.355777465047	0,1mg/ml_Starvation	0.612357044774	-0.18220989197
Verbascum blattaria	0,01mg/ml	-0.961462720502	1.25753963484	0,01mg/ml_Starvation	0.700622857611	-0.707134700519
	0,02mg/ml	-1.09490689276	1.0138926622	0,02mg/ml_Starvation	0.66787702124	-0.836613729303
	0,05mg/ml	-1.08578516672	1.48037445376	0,05mg/ml_Starvation	0.96078335626	-0.594988496005
	0,1mg/ml	-1.17465170675	1.16928839583	0,1mg/ml_Starvation	1.34501182434	-0.586026268895
Stachys hissarica	0,01mg/ml	-0.94782947574	0.650902944467	0,01mg/ml_Starvation	0.590047438662	-0.693070330768
	0,02mg/ml	-0.870092120736	1.22222322442	0,02mg/ml_Starvation	0.378896616434	-0.540236691748
	0,05mg/ml	-0.874508978023	1.14705881337	0,05mg/ml_Starvation	1.02581517433	-0.854708704384
	0,1mg/ml	-1.14957021194	1.37661454077	0,1mg/ml_Starvation	1.3879131213	-0.56790418247
Verbascum songoricum	0,01mg/ml	-0.956294330606	0.51436448722	0,01mg/ml_Starvation	0.854532698658	-0.933316682407
	0,02mg/ml	-0.786660928316	0.52038065249	0,02mg/ml_Starvation	0.695713761463	-0.866786799307
	0,05mg/ml	-0.824826319278	0.749256377789	0,05mg/ml_Starvation	1.26069609205	-0.735064767725
	0,1mg/ml	-0.778913288743	0.618000401998	0,1mg/ml_Starvation	1.13205481492	-0.682454470762
Stachys betoniciflora	0,01mg/ml	-0.605281545302	0.437427782048	0,01mg/ml_Starvation	0.553656901092	-0.496208074804
	0,02mg/ml	-0.489206299629	0.458320251422	0,02mg/ml_Starvation	1.43855008874	0.508882346812
	0,05mg/ml	-0.480594015472	0.705362767779	0,05mg/ml_Starvation	0.984924282062	-0.584878044458
	0,1mg/ml	-0.705722651803	0.694444810975	0,1mg/ml_Starvation	0.549340029167	-0.183605188635
Phlomis sewertzovii	0,01mg/ml	-0.500476198331	-0.000637510486784	0,01mg/ml_Starvation	0.825026227059	-0.976845832589
	0,02mg/ml	-0.412976493957	0.00260921018499	0,02mg/ml_Starvation	0.806580293268	-1.20721785042
	0,05mg/ml	-0.671439254662	0.0972671900029	0,05mg/ml_Starvation	1.50780780164	-0.887394178342
	0,1mg/ml	-0.322713757559	0.844952030047	0,1mg/ml_Starvation	0.910144688564	-0.929476727111
Phlomis salicifolia	0,01mg/ml	-0.346994942878	-0.390365039463	0,01mg/ml_Starvation	0.514909014195	-1.02803461788
	0,02mg/ml	-0.462299210114	0.0619719513041	0,02mg/ml_Starvation	0.94484336835	-0.0884393893274
	0,05mg/ml	-0.576698399997	-0.194225823834	0,05mg/ml_Starvation	1.1613816089	-0.872783988584
	0,1mg/ml	-0.274877229796	-0.23753347613	0,1mg/ml_Starvation	1.63113775218	1.05844632989
Silene oreina	0,01mg/ml	-0.17610470348	-0.317243201448	0,01mg/ml_Starvation	1.14247173978	-1.1348715296
	0,02mg/ml	-0.193473670873	-0.391690457796	0,02mg/ml_Starvation	1.39281850486	-1.10402960207
	0,05mg/ml	-0.0950188053986	-0.530732811274	0,05mg/ml_Starvation	1.7892734209	-1.27949976368
	0,1mg/ml	-0.267039626554	-0.222590571624	0,1mg/ml_Starvation	1.70878084469	-1.37836186815
Phlomis tadschikistanica	0,01mg/ml	-0.423384001861	-0.131913988791	0,01mg/ml_Starvation	1.64254151974	-1.16129019225
	0,02mg/ml	-0.306271191134	0.23841385236	0,02mg/ml_Starvation	1.52405473397	-0.981287984115
	0,05mg/ml	-0.201669259518	-0.333463534816	0,05mg/ml_Starvation	0.908393369693	-0.793563084088
	0,1mg/ml	-0.620758086432	0.282371751034	0,1mg/ml_Starvation	0.816933364887	-0.860102591812
Cousina umbrosa	0,01mg/ml	0.735484473829	1.65081661041	0,01mg/ml_Starvation	1.96194305265	0.69348188766
	0,02mg/ml	-0.325590622846	-0.260952642142	0,02mg/ml_Starvation	1.88179614587	0.551190898786
	0,05mg/ml	0.71629358149	1.41646493944	0,05mg/ml_Starvation	1.71253912611	0.918900540306
	0,1mg/ml	-0.481540744078	0.116082165173	0,1mg/ml_Starvation	1.57099466617	-0.552548899034
Nepeta olgae	0,01mg/ml	-0.0524915525723	0.00812924069275	0,01mg/ml_Starvation	0.900403972534	-0.96846815766
	0,02mg/ml	-0.229765405964	-0.190767623296	0,02mg/ml_Starvation	1.22669378292	-0.738498290905
	0,05mg/ml	-0.331220403428	0.0280888764733	0,05mg/ml_Starvation	1.4996633385	-0.808123624958
	0,1mg/ml	-0.48026634817	0.249311751571	0,1mg/ml_Starvation	1.50121452248	-0.832294769001
Scutellaria scharistanica	0,01mg/ml	-0.343773466332	0.131964987405	0,01mg/ml_Starvation	1.15987228009	-0.848774596425
	0,02mg/ml	-0.0836798808423	-0.474122330324	0,02mg/ml_Starvation	1.15596411454	-0.756874668799
	0,05mg/ml	-0.26010647444	-0.622785748503	0,05mg/ml_Starvation	1.33549782557	-0.668180974199
	0,1mg/ml	0.00138617392601	0.00767011611448	0,1mg/ml_Starvation	1.26793273643	-0.912176579825
Schrophullaria sp.	0,01mg/ml	-0.165894780652	-0.350492731023	0,01mg/ml_Starvation	4.53384205014	-1.77383700054
	0,02mg/ml	-0.0816039592562	-0.414104626138	0,02mg/ml_Starvation	4.25871846674	-2.33304789834
	0,05mg/ml	-0.0946650748407	-0.24463432161	0,05mg/ml_Starvation	4.86746752041	-2.46276780918
	0,1mg/ml	-0.319006358893	-0.324152147436	0,1mg/ml_Starvation	14.7822728643	9.39095899565

Factor scores associated to 2 hours treatments after Principal Component Analysis (PCA)						
2 hours' treatments		**PC1**	**PC2**	**2 hours' treatments**	**PC1**	**PC2**
Leonurus panzeroides	0,01mg/ml	-0.0576913257336	-0.169394469015	0,01mg/ml_Starvation	1.04143413397	-1.18432777984
	0,02mg/ml	-0.338683645657	-0.338878002495	0,02mg/ml_Starvation	0.990225896586	-0.754230264937
	0,05mg/ml	0.0230735282534	-0.405354895906	0,05mg/ml_Starvation	0.962838225529	-0.808606443225
	0,1mg/ml	-0.285943895564	-0.23145117024	0,1mg/ml_Starvation	0.791381013894	-0.641789091516

Table A3. Factor scores of Principal Components 1 and 2 for each samples of dataset containing measurements regarding samples treated for 2 hours with the tested extracts under analysis.

Factor scores associated to 20 hours treatments after Principal Component Analysis (PCA)						
20 hours' treatments		**PC1**	**PC2**	**20 hours' treatments**	**PC1**	**PC2**
Controls	Untreated	-0.462332915708	0.410375379506	Starvation	-0.810463832901	-0.319616223058
	0,1%DMSO	-0.443799259264	0.420230130368	0.1%DMSO_Starvation	-0.827515568979	-0.306822191736
EAGRC	0,01mg/ml	-0.867802947551	-0.156699534156	0,01mg/ml_Starvation	-0.799480592361	-0.134337884664
	0,02mg/ml	-0.848761939931	-0.0878975252566	0,02mg/ml_Starvation	-0.701568062172	-0.153725636843
	0,05mg/ml	-0.849704558553	-0.0436337442209	0,05mg/ml_Starvation	-0.553876265916	-0.369854819128
	0,1mg/ml	-0.928187373233	-0.0811237117396	0,1mg/ml_Starvation	1.04806967279	-1.96782369138
ERAAlop	0,01mg/ml	-0.757075783636	-0.297389822575	0,01mg/ml_Starvation	-0.617596258155	-0.370176370167
	0,02mg/ml	-0.689506788122	-0.208636836742	0,02mg/ml_Starvation	-0.618973912587	-0.218316284286
	0,05mg/ml	-0.574087040866	-0.310892470616	0,05mg/ml_Starvation	-0.286703275902	-0.48111706499
	0,1mg/ml	-0.914244288017	0.102382851295	0,1mg/ml_Starvation	-0.833799055733	0.096000307892
EAGPS	0,01mg/ml	-0.789549767346	-0.403541654985	0,01mg/ml_Starvation	0.316720694877	-2.12510934799
	0,02mg/ml	-0.921950149432	-0.780062263907	0,02mg/ml_Starvation	0.757789953469	-2.31609718775
	0,05mg/ml	-0.941146185427	-0.796110236404	0,05mg/ml_Starvation	0.886050571463	-2.60280853359
	0,1mg/ml	0.12943258018	-1.74370396264	0,1mg/ml_Starvation	1.42692373862	-2.69559401665
EAGAAsia	0,01mg/ml	-0.777510606885	-0.0845320384589	0,01mg/ml_Starvation	-0.508520167579	-0.733324074352
	0,02mg/ml	-0.681941540103	-0.049247941123	0,02mg/ml_Starvation	0.388512699419	-1.41832890854
	0,05mg/ml	-0.364490077562	-0.266099155727	0,05mg/ml_Starvation	0.628438765238	-1.72663061987
	0,1mg/ml	-0.909088926465	0.246904176388	0,1mg/ml_Starvation	0.511821871865	-1.49108300083
EAGAAlop	0,01mg/ml	-0.783719010703	-0.119538906314	0,01mg/ml_Starvation	-0.710923754809	-0.368841629972
	0,02mg/ml	-0.715005522091	-0.15781076992	0,02mg/ml_Starvation	-0.794706820739	-0.06556868374
	0,05mg/ml	-0.739965821899	-0.0688232050534	0,05mg/ml_Starvation	-0.600355958084	-0.560960148919
	0,1mg/ml	-0.805497561563	-0.0895235734856	0,1mg/ml_Starvation	1.53818337126	-2.71185673708
EAGMA	0,01mg/ml	-0.956801786283	0.0100225160541	0,01mg/ml_Starvation	-1.12389039762	-0.12998383913
	0,02mg/ml	-0.967798776566	-0.0981199356164	0,02mg/ml_Starvation	-0.884375295896	-0.209067032148
	0,05mg/ml	-1.00707671449	0.0310816769927	0,05mg/ml_Starvation	-0.836359176247	-0.211293432901
	0,1mg/ml	-1.17852498263	0.00222631306232	0,1mg/ml_Starvation	-1.21839285313	0.0288196337562
EAGMD	0,01mg/ml	-0.967046856225	-0.0965128215608	0,01mg/ml_Starvation	-0.954511759344	0.0380990641109
	0,02mg/ml	-0.928363801043	-0.0974182599989	0,02mg/ml_Starvation	-0.851835997404	-0.254417729066
	0,05mg/ml	-0.896543628433	-0.0376976537139	0,05mg/ml_Starvation	-0.61362145332	-0.501032262556
	0,1mg/ml	-0.914094513913	-0.00295816780436	0,1mg/ml_Starvation	-0.73907417841	-0.687691750786
EAGKL	0,01mg/ml	-0.705587571493	-0.226382454625	0,01mg/ml_Starvation	-0.788595380941	-0.196463663191
	0,02mg/ml	-0.690981248195	-0.129650978092	0,02mg/ml_Starvation	-0.861081430635	-0.300972675788
	0,05mg/ml	-0.664874446065	-0.163006067767	0,05mg/ml_Starvation	0.61516706707	-2.12058925857
	0,1mg/ml	-0.74991000206	-0.0613191175331	0,1mg/ml_Starvation	0.584368607191	-2.00804464376
EAGAS	0,01mg/ml	-0.848211727621	-0.128493043699	0,01mg/ml_Starvation	-0.65652125884	-0.167421306958
	0,02mg/ml	-0.687260463133	-0.192328555261	0,02mg/ml_Starvation	-0.76037634383	-0.29372553265
	0,05mg/ml	-0.735985742018	-0.239292212761	0,05mg/ml_Starvation	-0.658870763552	-0.427630362582
	0,1mg/ml	-0.832448640928	-0.281889811273	0,1mg/ml_Starvation	-0.780496020148	-0.580150014494
EAGAT	0,01mg/ml	-0.80833599167	0.038291099758	0,01mg/ml_Starvation	-0.381522813339	-0.422695834431
	0,02mg/ml	-0.700183546004	-0.0671859940258	0,02mg/ml_Starvation	-0.871931983121	-0.0332752850156
	0,05mg/ml	-0.665376674067	-0.135953972146	0,05mg/ml_Starvation	-0.680489136918	-0.48863333422
	0,1mg/ml	-0.775863515976	-0.0553148302643	0,1mg/ml_Starvation	-0.854969973941	0.0603897601422
EAGAU	0,01mg/ml	-0.82935961242	-0.139420669232	0,01mg/ml_Starvation	-0.698294628581	-0.365024915982
	0,02mg/ml	-0.779241352006	-0.107088965144	0,02mg/ml_Starvation	-0.684042822787	-0.139694494935
	0,05mg/ml	-0.661197359672	0.00344432087207	0,05mg/ml_Starvation	-0.414505582016	-0.447342879783
	0,1mg/ml	-0.829957573762	-0.00601785402816	0,1mg/ml_Starvation	-0.785812649432	-0.121331147028
ERPS	0,01mg/ml	-0.644038254629	-0.267147103177	0,01mg/ml_Starvation	3.90096847579	-0.581511286171
	0,02mg/ml	-0.875047662193	-0.0874285569761	0,02mg/ml_Starvation	7.03959639626	-3.01448175483
	0,05mg/ml	-0.765007201659	-0.265606432277	0,05mg/ml_Starvation	6.75729216179	-1.15243872047
	0,1mg/ml	6.37248382023	-1.69176118848	0,1mg/ml_Starvation	7.70016729145	-2.34283410911
EFIAAlop	0,01mg/ml	-0.50855957856	0.709917532922	0,01mg/ml_Starvation	-0.87979139902	-0.929172224152
	0,02mg/ml	-0.14642014422	1.13018774203	0,02mg/ml_Starvation	-0.936790882425	-0.839282401021
	0,05mg/ml	-0.42892271518	0.711724289432	0,05mg/ml_Starvation	-1.00702325059	-0.5752287781
	0,1mg/ml	-0.381963953947	0.425478917631	0,1mg/ml_Starvation	-0.813693572417	-0.855950308939
ERHA	0,01mg/ml	-0.517192943813	0.898397644868	0,01mg/ml_Starvation	-0.510853513076	-0.622376326794
	0,02mg/ml	-0.238294784784	1.02394916405	0,02mg/ml_Starvation	-1.10387638014	-0.556464490289
	0,05mg/ml	-0.730231542408	0.735526475519	0,05mg/ml_Starvation	-0.99201793733	-0.436527928881
	0,1mg/ml	-0.752764559088	0.522946917309	0,1mg/ml_Starvation	-1.00807351523	-0.449887540308
EAGAF	0,01mg/ml	-0.516270067733	0.751230293529	0,01mg/ml_Starvation	-0.89401499158	-0.325502508593
	0,02mg/ml	-0.36178267468	0.887021524172	0,02mg/ml_Starvation	-0.753176276917	-0.51278735039
	0,05mg/ml	-0.545232735349	0.808662723007	0,05mg/ml_Starvation	-0.979342740937	-0.715930229317
	0,1mg/ml	-0.270701396047	0.750782588789	0,1mg/ml_Starvation	-0.595635273766	-0.0371300047209
EAGAA	0,01mg/ml	-0.464706814047	0.88606178469	0,01mg/ml_Starvation	-0.686728786832	-0.626692626118
	0,02mg/ml	-0.150989965565	1.11668126976	0,02mg/ml_Starvation	-0.856780146145	-0.669227586315
	0,05mg/ml	-0.41791476131	1.09871484817	0,05mg/ml_Starvation	-0.848129581985	-0.14625436544
	0,1mg/ml	-0.228547417373	1.10217751354	0,1mg/ml_Starvation	-0.605251223983	0.348241177953
EAGFO	0,01mg/ml	-0.610680922875	0.488594080056	0,01mg/ml_Starvation	-1.00300532513	-0.681121020767
	0,02mg/ml	-0.121999000882	1.35279744349	0,02mg/ml_Starvation	-0.453227785953	0.418538382175
	0,05mg/ml	-0.154596689873	1.39602245587	0,05mg/ml_Starvation	5.14449807382	1.95680323127
	0,1mg/ml	8.00003801111	4.40716550835	0,1mg/ml_Starvation	5.16008708686	2.64446147407
EAGPD	0,01mg/ml	-0.501450921343	0.160463683218	0,01mg/ml_Starvation	0.669679827352	-2.20016193097
	0,02mg/ml	-0.362779094147	0.478417063439	0,02mg/ml_Starvation	-0.604163667909	-0.900031020842
	0,05mg/ml	-0.129940121279	0.221327520625	0,05mg/ml_Starvation	-0.63053253342	-0.747465340803
	0,1mg/ml	-0.307203616901	0.301887087095	0,1mg/ml_Starvation	4.98822831872	-1.02036834311
EAGTM	0,01mg/ml	-0.373288791806	0.748920750118	0,01mg/ml_Starvation	-0.916836452235	-0.872942647128
	0,02mg/ml	0.188269948318	0.940718362422	0,02mg/ml_Starvation	-0.786007507518	-0.964998199047
	0,05mg/ml	-0.100187726814	0.297714335031	0,05mg/ml_Starvation	-0.750260722621	-0.585204293546
	0,1mg/ml	0.232915641856	1.08594830204	0,1mg/ml_Starvation	-0.594433197011	-0.618573787566
EAGC	0,01mg/ml	-0.575426654117	0.377298680386	0,01mg/ml_Starvation	-0.850252504404	-0.69472889633
	0,02mg/ml	-0.336074143544	0.7994870657	0,02mg/ml_Starvation	-0.906056242134	-1.15575025576
	0,05mg/ml	-0.512152809239	0.359868092003	0,05mg/ml_Starvation	-0.791227827775	-1.17344252256
	0,1mg/ml	-0.862493765225	-0.0969667072852	0,1mg/ml_Starvation	0.125612171229	-0.511503363286
EFHA	0,01mg/ml	-0.601691119595	0.447198629619	0,01mg/ml_Starvation	-0.668530384525	-0.840813657907
	0,02mg/ml	-0.504538003195	0.605274031706	0,02mg/ml_Starvation	-0.977688890873	-0.738793146364
	0,05mg/ml	-0.583593100405	0.376970266301	0,05mg/ml_Starvation	-0.55549113169	-0.925773075672
	0,1mg/ml	-0.319498332592	0.773985143033	0,1mg/ml_Starvation	4.05530772238	0.756210061023
EAGSS	0,01mg/ml	-0.571408752455	0.395987742329	0,01mg/ml_Starvation	-1.14978414526	-0.966357157189
	0,02mg/ml	-0.63758476644	0.292602803084	0,02mg/ml_Starvation	-1.11005202934	-1.00887014707
	0,05mg/ml	-0.612437982029	0.450812245351	0,05mg/ml_Starvation	-0.753936605106	-0.835388368535
	0,1mg/ml	-0.742406961794	0.11327275724	0,1mg/ml_Starvation	-0.854776711355	-1.00253009746
EFIPS	0,01mg/ml	-0.580144152548	0.371628500391	0,01mg/ml_Starvation	-0.666725589907	-1.03337157885
	0,02mg/ml	-0.677181291086	0.780424033422	0,02mg/ml_Starvation	-0.569605177236	-1.06705352231
	0,05mg/ml	-0.339405898009	0.995282355848	0,05mg/ml_Starvation	-0.469009636593	-0.980662557472

Factor scores associated to 20 hours treatments after Principal Component Analysis (PCA)						
20 hours' treatments		**PC1**	**PC2**	**20 hours' treatments**	**PC1**	**PC2**
	0,1mg/ml	-0.693896235334	0.688979634761	0,1mg/ml_Starvation	6.37374866205	0.394161048167
EAGCB	0,01mg/ml	-0.157573878152	0.332044031894	0,01mg/ml_Starvation	-0.920306943004	-0.823472703458
	0,02mg/ml	-0.0915009170388	1.09323561287	0,02mg/ml_Starvation	-0.950471100979	-1.22451748531
	0,05mg/ml	-0.553902606238	0.244721337307	0,05mg/ml_Starvation	-0.859701651227	-0.745293636915
	0,1mg/ml	0.163331149728	0.616843600657	0,1mg/ml_Starvation	-0.549854876136	-0.904375194774
EAGCT	0,01mg/ml	0.0586368408872	1.15432967885	0,01mg/ml_Starvation	-0.851456050999	0.0668128846085
	0,02mg/ml	0.492132222482	1.35729860798	0,02mg/ml_Starvation	-0.652952306093	0.29968295861
	0,05mg/ml	0.245711735413	1.30309068688	0,05mg/ml_Starvation	-0.308117174495	0.636285241408
	0,1mg/ml	0.598017834427	1.52779081334	0,1mg/ml_Starvation	0.128768666938	1.17321848359
EAGOT	0,01mg/ml	0.53686773345	1.12025370086	0,01mg/ml_Starvation	-0.611625668495	-0.215166773659
	0,02mg/ml	1.19186448071	1.46691417479	0,02mg/ml_Starvation	-0.706749256831	-0.0901118728133
	0,05mg/ml	0.97309553673	1.52344481927	0,05mg/ml_Starvation	-0.647228629994	-0.0774056916685
	0,1mg/ml	0.825758206606	1.42114988207	0,1mg/ml_Starvation	-0.668167856914	0.104810564339
ERKL	0,01mg/ml	-0.777195427828	0.251598184689	0,01mg/ml_Starvation	-0.867502026421	-0.0829973670335
	0,02mg/ml	-0.603461183263	0.235738065618	0,02mg/ml_Starvation	-0.746271796029	-0.139120034519
	0,05mg/ml	-0.73157733332	0.25221653525	0,05mg/ml_Starvation	5.27819065766	-0.228074972664
	0,1mg/ml	6.43347535454	-0.298080946185	0,1mg/ml_Starvation	8.34075502867	-1.44288105465
ERKS	0,01mg/ml	0.149238116363	0.979026680246	0,01mg/ml_Starvation	-0.787639600883	-0.365199044708
	0,02mg/ml	0.00321316806138	0.853463896262	0,02mg/ml_Starvation	0.304251293773	-1.43756268836
	0,05mg/ml	-0.414109197573	0.644853694697	0,05mg/ml_Starvation	-0.799555058807	-0.685211934467
	0,1mg/ml	-0.487049673006	-0.0345304840971	0,1mg/ml_Starvation	-0.788302094656	-0.839938013885
ESHA	0,01mg/ml	-0.143736379703	1.08516439968	0,01mg/ml_Starvation	-0.938598331093	-0.139116890234
	0,02mg/ml	0.161773080723	1.09124339207	0,02mg/ml_Starvation	-0.847613666097	-0.098005542074
	0,05mg/ml	0.0620445831785	1.13878360806	0,05mg/ml_Starvation	-0.860519033219	-0.161265782339
	0,1mg/ml	0.258632430651	1.14620030173	0,1mg/ml_Starvation	-0.863930046832	-0.203331485394
Ulva sp. (U-2C)	0,01mg/ml	0.0459052941315	0.999536456268	0,01mg/ml_Starvation	-0.797641433533	-0.107503598623
	0,02mg/ml	0.00404920232512	0.945507662858	0,02mg/ml_Starvation	-0.575974685769	0.0667502765839
	0,05mg/ml	-0.0084278186583	1.05520979622	0,05mg/ml_Starvation	-0.6209803835	0.0408427282643
	0,1mg/ml	0.304350863065	1.27405021153	0,1mg/ml_Starvation	-0.557562802015	-0.108682707411
Cryptomenia sp. (C-3B)	0,01mg/ml	0.209268013543	1.08039210078	0,01mg/ml_Starvation	-0.494603281004	0.0205757717671
	0,02mg/ml	0.342262455813	1.14279792551	0,02mg/ml_Starvation	-0.110843737059	0.633147126576
	0,05mg/ml	0.453521046823	1.47393336569	0,05mg/ml_Starvation	0.0387551763615	0.763101711785
	0,1mg/ml	1.16929885062	1.57211374936	0,1mg/ml_Starvation	-0.141707339711	0.357027412728
Ciona intestinalis (C-4B)	0,01mg/ml	-0.327793741059	0.672474982034	0,01mg/ml_Starvation	-0.77904861315	-0.010639793618
	0,02mg/ml	-0.2398710424	0.740387808669	0,02mg/ml_Starvation	-0.64699529929	0.139784283465
	0,05mg/ml	-0.273928116119	0.893480972928	0,05mg/ml_Starvation	-0.121824349982	0.787524755466
	0,1mg/ml	0.767347057195	1.38113623916	0,1mg/ml_Starvation	8.47025845979	1.76329843956
Heliotropium sp. (T-1B)	0,01mg/ml	-0.20080306149	0.906316138669	0,01mg/ml_Starvation	-0.781477381484	-0.0604119682815
	0,02mg/ml	-0.609077175288	0.83318128439	0,02mg/ml_Starvation	3.86282029548	-1.44290376398
	0,05mg/ml	6.90897638525	-0.455877095651	0,05mg/ml_Starvation	6.27891843337	0.359084450894
	0,1mg/ml	6.25628962805	-0.516187450438	0,1mg/ml_Starvation	8.9349903708	0.721803898349
Heliotropium sp. (T-1C)	0,01mg/ml	-0.0558634840033	0.805792157435	0,01mg/ml_Starvation	-0.767267449117	-0.301188579014
	0,02mg/ml	0.933852961693	0.168114028625	0,02mg/ml_Starvation	-0.57669754772	-0.285113513183
	0,05mg/ml	-0.254719216351	0.697332259966	0,05mg/ml_Starvation	-0.85849822351	-0.0887413453173
	0,1mg/ml	-0.207201298544	-0.341508946666	0,1mg/ml_Starvation	-0.658133733936	-0.119961677814
Verbascum blattaria	0,01mg/ml	-0.278109025786	0.766549291351	0,01mg/ml_Starvation	-0.756101479977	-0.508724384767
	0,02mg/ml	-0.428848572871	0.685887905967	0,02mg/ml_Starvation	-0.721852184384	-0.809938788506
	0,05mg/ml	-0.565976217921	0.739011491606	0,05mg/ml_Starvation	-0.469559713419	-0.537117179287
	0,1mg/ml	-0.168956526032	1.04627705105	0,1mg/ml_Starvation	4.52723056704	0.591451572975
Stachys hissarica	0,01mg/ml	0.651996073746	1.35123586493	0,01mg/ml_Starvation	-0.679998716634	-0.0991128440301
	0,02mg/ml	0.520947998551	1.38676066949	0,02mg/ml_Starvation	-0.420225763672	-0.153065765277
	0,05mg/ml	0.594825682795	1.52339003484	0,05mg/ml_Starvation	-0.389594929133	-0.677751952976
	0,1mg/ml	0.449745687275	1.41108177442	0,1mg/ml_Starvation	-0.434227038376	-0.506195044637
Verbascum songoricum	0,01mg/ml	0.341714496184	1.2532853483	0,01mg/ml_Starvation	-0.544254721661	0.222960400993
	0,02mg/ml	0.300185927487	1.25208576939	0,02mg/ml_Starvation	-0.480368524936	-0.168516435468
	0,05mg/ml	0.2440580744	1.27546692487	0,05mg/ml_Starvation	0.0282478307916	-0.549096356825
	0,1mg/ml	0.132670103455	1.31122400177	0,1mg/ml_Starvation	1.52486226417	-2.07003260185
Stachys betoniciflora	0,01mg/ml	-0.0527407529089	0.796871125239	0,01mg/ml_Starvation	-0.553068295926	-0.40325804889
	0,02mg/ml	0.0376827671012	0.911754393476	0,02mg/ml_Starvation	-0.52450317818	-0.353827181409
	0,05mg/ml	-0.0109529048594	0.859930855811	0,05mg/ml_Starvation	-0.30962419442	-0.160598421547
	0,1mg/ml	0.329780735189	1.07645302538	0,1mg/ml_Starvation	0.42421095314	0.360033984514
Phlomis sewertzovii	0,01mg/ml	-0.0422112154099	0.918771432987	0,01mg/ml_Starvation	-0.572306086538	-0.501485777546
	0,02mg/ml	0.166595037278	0.913966631443	0,02mg/ml_Starvation	-0.584110504067	-0.515699447377
	0,05mg/ml	0.0477595054349	1.12954837512	0,05mg/ml_Starvation	-0.560902547398	-0.576151591523
	0,1mg/ml	0.290092205367	1.13976987958	0,1mg/ml_Starvation	-0.265702463014	-0.870141457972
Phlomis salicifolia	0,01mg/ml	-0.0787636067143	0.790181809824	0,01mg/ml_Starvation	-0.518638091711	-0.49873990395
	0,02mg/ml	-0.159501672569	0.760573336001	0,02mg/ml_Starvation	-0.681848189292	-0.679092424013
	0,05mg/ml	0.055176579333	0.869947663514	0,05mg/ml_Starvation	-0.469207734294	-0.875543510836
	0,1mg/ml	0.679870980219	1.31516555391	0,1mg/ml_Starvation	-0.399832216634	-0.686129849662
Silene oreina	0,01mg/ml	-0.66686686247	0.0493585507741	0,01mg/ml_Starvation	-0.923576412153	-0.883509685589
	0,02mg/ml	-0.593420032067	0.166946256849	0,02mg/ml_Starvation	-0.849070461127	-0.972652009503
	0,05mg/ml	-0.664773825695	0.0930176346626	0,05mg/ml_Starvation	-0.538965045308	-1.2506448158
	0,1mg/ml	-0.81030154915	-0.0327132765317	0,1mg/ml_Starvation	-0.178458313795	-1.79123411413
Phlomis tadschikistanica	0,01mg/ml	0.00559297720829	0.904422918666	0,01mg/ml_Starvation	-0.652623131702	-0.75348578121
	0,02mg/ml	-0.626193629685	0.371673758165	0,02mg/ml_Starvation	-0.834872889197	-0.838843902823
	0,05mg/ml	-0.386930049233	0.664513616069	0,05mg/ml_Starvation	-0.698127228594	-1.04894017211
	0,1mg/ml	-0.207493443346	0.868866536582	0,1mg/ml_Starvation	-0.638937121065	-0.944413097664
Cousina umbrosa	0,01mg/ml	-0.615004301706	0.43330848612	0,01mg/ml_Starvation	-0.731467398115	-0.362404169694
	0,02mg/ml	-0.520990574024	0.400931561473	0,02mg/ml_Starvation	-0.650011755989	-0.741362040559
	0,05mg/ml	-0.111747023977	0.824283798375	0,05mg/ml_Starvation	-0.48649957441	-0.918851777787
	0,1mg/ml	-0.185625855368	0.83383683175	0,1mg/ml_Starvation	0.0106515487209	-0.89873136847
Nepeta olgae	0,01mg/ml	-0.0896453865038	0.815272787135	0,01mg/ml_Starvation	-0.433541495296	-0.956201364712
	0,02mg/ml	-0.193297841354	0.829482947959	0,02mg/ml_Starvation	-0.564176176438	-0.740303846879
	0,05mg/ml	-0.10811919805	0.667006648335	0,05mg/ml_Starvation	-0.762481276729	-0.669435363239
	0,1mg/ml	-0.424333393893	0.679876056428	0,1mg/ml_Starvation	-0.731364538943	-0.641965731634
Scutellaria scharistanica	0,01mg/ml	-0.353346450174	0.5007477938	0,01mg/ml_Starvation	-0.594092781074	-0.546204929067
	0,02mg/ml	-0.0820374943374	0.861714650254	0,02mg/ml_Starvation	-0.409642151036	-0.638526153891
	0,05mg/ml	-0.397315360539	0.506533717022	0,05mg/ml_Starvation	-0.332039747082	-1.09461802458
	0,1mg/ml	-0.278718084445	0.534935443574	0,1mg/ml_Starvation	-0.20820860479	-0.817680280555
Schrophullaria sp.	0,01mg/ml	-0.369081422313	0.728585256012	0,01mg/ml_Starvation	-0.580626626509	-0.564711712725
	0,02mg/ml	-0.233090457633	0.753737309317	0,02mg/ml_Starvation	-0.647608299593	-0.493361523587
	0,05mg/ml	-0.256408754358	0.694391398782	0,05mg/ml_Starvation	8.23683867818	-1.74968166954
	0,1mg/ml	-0.35437505844	0.731676429884	0,1mg/ml_Starvation	7.91119187648	-1.73121837181

Factor scores associated to 20 hours treatments after Principal Component Analysis (PCA)						
20 hours' treatments		**PC1**	**PC2**	**20 hours' treatments**	**PC1**	**PC2**
Leonurus panzeroides	0,01mg/ml	0.0528278970335	0.721376123892	0,01mg/ml_Starvation	-0.480535446204	-0.460229647089
	0,02mg/ml	0.375217908809	0.948300874318	0,02mg/ml_Starvation	-0.325229045867	-0.569881792836
	0,05mg/ml	0.371352481042	1.0240106476	0,05mg/ml_Starvation	-0.32130931758	-0.462044350614
	0,1mg/ml	0.290286748857	0.957365459156	0,1mg/ml_Starvation	0.349453115729	-0.487177291812

Table A4. Factor scores of Principal Components 1 and 2 for each samples of dataset containing measurements regarding samples treated for 20 hours with the tested extracts under analysis.

2 Hours Treatments - Clusters and Toxicity									
#C.	Treatments	%l.c.	PC1	PC2	#C.	Treatments	%l.c.	PC1	PC2
1	*Schrophullaria_sp* 0,1mg/ml_Starvation	2	14.7822728643	9.39095899565		C-4B_0,01mg/ml	59	-0.363201578074	1.83197725291
	Ctrl	100	-1.04335413742	0.839364068725		C-4B_0,02mg/ml	63	-0.888619417594	0.852697591061
	CtrlDMSO	107	-1.10850318842	0.897022520147		C-4B_0,05mg/ml	73	-1.06865909663	0.961141405622
	EAGRC_0,01mg/ml	102	-0.751420786794	0.278918480514		C-4B_0,1mg/ml	45	-1.22143797972	1.66636062708
	EAGRC_0,02mg/ml	90	-0.612062306209	0.317656244559		C-4B_0,02mg/ml_Starvation	68	-0.28484794929	0.871041091238
	EAGPS_0,05mg/ml	90	-0.646494242198	0.348646655413		C-4B_0,05mg/ml_Starvation	71	0.301213903815	1.1418797022
	EAGPS_0,1mg/ml	84	-0.815011566829	0.710707029355		C-4B_0,1mg/ml_Starvation	37	0.590299544868	1.09095883113
	EAGPS_0,1mg/ml_Starvation	66	0.0331223168076	0.835518866459		T-1B_0,01mg/ml	67	-0.697180295332	0.405341072522
	ERPS_0,1mg/ml_Starvation	5	3.76570457366	6.23003564556		T-1B_0,02mg/ml	67	-0.606866392969	0.25697103558
	EFIAAlop_0,01mg/ml	102	-0.904611208563	1.03003246002		T-1B_0,05mg/ml	79	-0.707421070694	0.328367606982
	EFIAAlop_0,02mg/ml	86	-1.0854714113	1.01604724431		T-1B_0,1mg/ml	51	-0.382405696135	0.499125110403
	EFIAAlop_0,05mg/ml	85	-1.15507687995	1.20390296261		T-1C_0,01mg/ml	69	-0.669804074408	0.70263124393
	EFIAAlop_0,1mg/ml	79	-1.11334018484	0.470134208102		T-1C_0,05mg/ml	88	-0.893216597821	0.418465825513
	ERHA_0,01mg/ml	113	-1.16369699196	0.674144645741		T-1C_0,1mg/ml	64	-0.700970324844	0.355777465047
	ERHA_0,02mg/ml	115	-1.16635980951	0.673895514039		*Verbascum_blattaria*_0,01mg/ml	81	-0.961462720502	1.25753963484
	ERHA_0,05mg/ml	80	-0.938065739723	0.283862307666		*Verbascum_blattaria*_0,02mg/ml	97	-1.09490689276	1.0138926622
	ERHA_0,1mg/ml	83	-1.12524649543	1.04641885678		*Verbascum_blattaria*_0,05mg/ml	96	-1.08578516672	1.48037445376
	EAGAF_0,01mg/ml	61	-1.12273068481	0.691238164154		*Verbascum_blattaria*_0,1mg/ml	70	-1.17465170675	1.16928839583
	EAGAF_0,02mg/ml	114	-1.19492178781	1.30841039732		*Stachys_hissarica*_0,01mg/ml	91	-0.94782947574	0.650902944467
	EAGAF_0,05mg/ml	86	-0.969712248422	1.3651337605	2	*Stachys_hissarica*_0,02mg/ml	85	-0.870092120736	1.22222322442
	EAGAF_0,1mg/ml	67	-1.19922929229	0.488671329649		*Stachys_hissarica*_0,05mg/ml	51	-0.874508978023	1.14705881337
	EAGAA_0,01mg/ml	105	-1.0093522106	0.680466369341		*Stachys_hissarica*_0,1mg/ml	91	-1.14957021194	1.37661454077
	EAGAA_0,02mg/ml	96	-1.10140816706	0.836323380991		*Verbascum_songoricum*_0,01mg/ml	84	-0.956294330606	0.51436448722
	EAGAA_0,05mg/ml	76	-1.14819160758	0.711229712077		*Verbascum_songoricum*_0,02mg/ml	105	-0.786660928316	0.52038065249
	EAGAA_0,1mg/ml	85	-1.028264054	0.884736051197		*Verbascum_songoricum*_0,05mg/ml	60	-0.824826319278	0.749256377789
	EAGFO_0,01mg/ml	102	-1.29703016701	0.867969401831		*Verbascum_songoricum*_0,1mg/ml	85	-0.778913288743	0.618000401998
	EAGFO_0,02mg/ml	71	-1.14272207535	0.980791958657		*Stachys_betoniciflora*_0,01mg/ml	82	-0.605281545302	0.437427782048
	EAGFO_0,05mg/ml	67	-1.11278319502	0.618612247712		*Stachys_betoniciflora*_0,02mg/ml	88	-0.489206299629	0.458320251422
	EAGFO_0,1mg/ml	88	-0.834599077622	0.171184940708		*Stachys_betoniciflora*_0,05mg/ml	78	-0.480594015472	0.705362767779
	EAGFO_0,05mg/ml_Starvation	24	-0.166303689952	6.01396882371		*Stachys_betoniciflora*_0,1mg/ml	79	-0.705722651803	0.694444810975
	EAGFO_0,1mg/ml_Starvation	3	-0.232081208603	5.97996427696		*Stachys_betoniciflora*_0,02mg/ml_Starvation	104	1.43855008874	0.508882346812
	EAGPD_0,01mg/ml	108	-0.898359289453	0.455851069204		*Phlomis_sewertzovii*_0,1mg/ml	43	-0.322713757559	0.844952030047
	EAGPD_0,02mg/ml	108	-0.932171302329	0.313439522265		*Phlomis_salicifolia*_0,1mg/ml_Starvation	55	1.63113775218	1.05844632989
	EAGPD_0,05mg/ml	71	-0.846817526584	0.185566949604		*Phlomis_tadschikistanica*_0,1mg/ml	108	-0.620758086432	0.282371751034
	EAGPD_0,1mg/ml	97	-0.889833771958	0.374685474677		*Cousina_umbrosa*_0,01mg/ml	61	0.735484473829	1.65081661041
	EAGTM_0,01mg/ml	71	-1.08298247585	0.635593985338		*Cousina_umbrosa*_0,05mg/ml	56	0.71629358149	1.41646493944
	EAGTM_0,02mg/ml	105	-0.952263521303	0.676025083115		*Cousina_umbrosa*_0,01mg/ml_Starvation	91	1.96194305265	0.69348188766
	EAGTM_0,05mg/ml	94	-0.994997302797	0.453294982514		*Cousina_umbrosa*_0,02mg/ml_Starvation	111	1.88179614587	0.551190898786
	EAGTM_0,1mg/ml	62	-0.86498643468	0.293535477566		*Cousina_umbrosa*_0,05mg/ml_Starvation	77	1.71253912611	0.918900540306
	EAGC_0,01mg/ml	94	-1.07090082614	0.472179256705		Ctrl_Starvation	100	-0.165226692502	-0.371287249214
	EAGC_0,02mg/ml	96	-1.05268777828	0.579674471222		CtrlDMSO_Starvation	87	-0.0135777168692	-0.679626063698
	EAGC_0,05mg/ml	108	-1.16364502163	0.599973241155		EAGRC_0,05mg/ml	69	-0.275357491314	-0.312774938657
	EAGC_0,1mg/ml	91	-1.11477173544	0.565916581695		EAGRC_0,1mg/ml	88	-0.5558911338	-0.234797674283
	EFHA_0,01mg/ml	95	-0.963365827501	0.858762136361		EAGRC_0,02mg/ml_Starvation	106	0.120390032864	-0.939934183768
2	EFHA_0,02mg/ml	83	-1.01921240844	0.800695898504		EAGRC_0,05mg/ml_Starvation	94	0.379587912505	-0.69225111493
	EFHA_0,05mg/ml	83	-1.00629863083	0.571867444771		EAGRC_0,1mg/ml_Starvation	94	-0.0368437736605	-0.620041267277
	EFHA_0,1mg/ml	95	-1.04625457241	0.526102680248		ERAAlop_0,01mg/ml	88	-0.1201675203	0.00139901186479
	EAGSS_0,01mg/ml	113	-0.96415465925	0.32767771152		ERAAlop_0,02mg/ml	90	-0.233364995771	0.178485473511
	EAGSS_0,02mg/ml	87	-1.09508957287	0.399443804993		ERAAlop_0,05mg/ml	75	-0.220610542646	-0.512011330907
	EAGSS_0,05mg/ml	73	-0.949716549475	0.366469488336		ERAAlop_0,1mg/ml	65	0.0899019606709	-0.721103562743
	EAGSS_0,1mg/ml	109	-1.05221990876	0.575778340969		EAGPS_0,01mg/ml	98	-0.551846935979	0.153050798578
	EFIPS_0,01mg/ml	70	-1.10034506226	0.572050952445		EAGPS_0,02mg/ml	95	-0.625470450879	0.114711885815
	EFIPS_0,02mg/ml	80	-0.925988976508	0.572801971384		EAGPS_0,01mg/ml_Starvation	100	0.401235936176	-0.603513111783
	EFIPS_0,05mg/ml	75	-0.939524455081	0.285453327391		EAGPS_0,02mg/ml_Starvation	100	-0.110960328484	-0.120235413302
	EFIPS_0,1mg/ml	50	-1.00883620497	0.109174306998		EAGPS_0,05mg/ml_Starvation	55	0.0204122443841	0.275407806958
	EFIPS_0,1mg/ml_Starvation	59	-0.17982940221	0.719007946889		EAGAAsia_0,01mg/ml	83	-0.478221020828	-0.0444047363412
	EAGCB_0,01mg/ml	49	-0.799841416536	0.275544227182		EAGAAsia_0,02mg/ml	78	-0.150914009914	-0.379391683332
	EAGCB_0,02mg/ml	59	-0.813275294743	0.212154248241		EAGAAsia_0,05mg/ml	72	-0.426752584441	-0.43772649281
	EAGCB_0,05mg/ml	69	-0.799313317009	0.187587442315		EAGAAsia_0,1mg/ml	72	-0.299749595646	-0.25785792765
	EAGCB_0,1mg/ml	59	-0.957093496155	0.249383572022		EAGAAsia_0,01mg/ml_Starvation	61	0.715842761975	-0.512643584635
	EAGCB_0,05mg/ml_Starvation	68	0.0807563682876	0.934383100632		EAGAAsia_0,1mg/ml_Starvation	42	0.572572632976	-0.579250667069
	EAGCT_0,01mg/ml	104	-1.18304754379	1.4653039026		EAGAAlop_0,01mg/ml	101	-0.559833657737	0.0252942235056
	EAGCT_0,02mg/ml	82	-1.06168832006	0.742235613609		EAGAAlop_0,02mg/ml	77	-0.333449446251	-0.0956542110293
	EAGCT_0,05mg/ml	88	-1.19686487893	0.91487350932		EAGAAlop_0,05mg/ml	80	-0.276876493074	-0.241368267353
	EAGCT_0,1mg/ml	72	-1.06893055518	1.19578256763	3	EAGAAlop_0,1mg/ml	79	-0.305271812764	-0.0464816708846
	EAGOT_0,01mg/ml	98	-1.26063171203	1.05760656937		EAGAAlop_0,02mg/ml_Starvation	85	0.696697664508	-0.616475752524
	EAGOT_0,02mg/ml	98	-1.08360883353	0.698927795714		EAGAAlop_0,05mg/ml_Starvation	66	1.26145540033	-0.141264700735
	EAGOT_0,05mg/ml	88	-1.09636469093	0.983374565694		EAGAAlop_0,1mg/ml_Starvation	83	0.892830300118	0.155161112266
	EAGOT_0,1mg/ml	89	-1.05687235651	0.704047169045		EAGMA_0,01mg/ml	109	-0.40919622174	-0.0970817668217
	ERKL_0,01mg/ml	98	-1.17440602054	0.689584236967		EAGMA_0,02mg/ml	83	-0.124620012922	-0.697780403124
	ERKS_0,01mg/ml	96	-1.2137647911	0.674522577126		EAGMA_0,05mg/ml	74	-0.429450947532	-0.724800248085
	ERKS_0,02mg/ml	87	-1.13341433034	0.326968582117		EAGMA_0,1mg/ml	73	0.0468738134012	-0.640971898884
	ERKS_0,05mg/ml	77	-1.0395865722	0.570010972255		EAGMD_0,01mg/ml	96	-0.505991512849	-0.113452315959
	ERKS_0,1mg/ml	81	-0.943495040514	0.558853693882		EAGMD_0,02mg/ml	84	-0.399832887603	-0.214831104797
	ESHA_0,01mg/ml	98	-1.15480946763	1.16023091937		EAGMD_0,05mg/ml	81	-0.209898262171	-0.648704974465
	ESHA_0,02mg/ml	83	-0.990067927955	0.804160107399		EAGMD_0,1mg/ml	83	-0.429100045394	-0.727722345774
	ESHA_0,05mg/ml	92	-1.04899015872	0.7074418251		EAGKL_0,01mg/ml	76	-0.233314241729	-0.601539215756
	ESHA_0,1mg/ml	78	-1.07271204054	0.856800400629		EAGKL_0,02mg/ml	79	-0.0370867767514	-0.479175208866
	U-2C_0,01mg/ml	78	-0.231390812552	2.49394264849		EAGKL_0,05mg/ml	92	-0.172979101179	-0.699559726521
	U-2C_0,02mg/ml	87	-0.668031367434	1.29053684034		EAGKL_0,1mg/ml	63	-0.00760842400915	-0.66111201292
	U-2C_0,05mg/ml	90	-1.02403134433	0.81565155244		EAGAS_0,01mg/ml	99	-0.461803970823	0.0511446932001
	U-2C_0,1mg/ml	85	-1.03467952632	0.853586850015		EAGAS_0,02mg/ml	79	-0.121876190039	0.0336380559058
	C-3B_0,01mg/ml	63	-0.908729998453	1.36548094133		EAGAS_0,05mg/ml	76	-0.437799976345	-0.377730942404
	C-3B_0,02mg/ml	65	-0.630721811883	1.37109044999		EAGAS_0,1mg/ml	83	-0.246427645555	-0.267797310077
	C-3B_0,05mg/ml	78	-0.988409972393	1.03575811377		EAGAS_0,05mg/ml_Starvation	79	0.763776267882	-0.748197309032
	C-3B_0,1mg/ml	72	-1.11751277453	2.00351006176		EAGAS_0,1mg/ml_Starvation	73	0.907586553829	0.188281220398
	C-3B_0,01mg/ml_Starvation	57	0.904396360569	1.34628680076		EAGAT_0,02mg/ml_Starvation	73	0.531926309597	-0.322511871364
	C-3B_0,02mg/ml_Starvation	82	0.515308009539	0.984502724946		EAGAT_0,05mg/ml_Starvation	62	0.396377182889	-0.54785830215
	C-3B_0,1mg/ml_Starvation	71	1.0515819269	1.80803358382		EAGAT_0,1mg/ml_Starvation	69	0.659816126694	-0.107300765666

2 Hours Treatments - Clusters and Toxicity									
#C.	Treatments	%l.c.	PC1	PC2	#C.	Treatments	%l.c.	PC1	PC2
	EAGAU_0,02mg/ml	81	0.629190843568	-0.615891444509		*Verbascum_blattaria*_0,01mg/ml_Starvation	109	0.700622857611	-0.707134700519
	EAGAU_0,01mg/ml_Starvation	93	0.335367653229	-0.168304110597		*Stachys_hissarica*_0,01mg/ml_Starvation	106	0.590047438662	-0.693070330768
	EAGAU_0,02mg/ml_Starvation	73	0.209901134975	-0.203231882916		*Stachys_hissarica*_0,02mg/ml_Starvation	124	0.378896616434	-0.540236691748
	EAGAU_0,05mg/ml_Starvation	78	0.406518417709	0.168674312446		*Stachys_betoniciflora*_0,01mg/ml_Starvation	142	0.553656901092	-0.496208074804
	EAGAU_0,1mg/ml_Starvation	82	0.260671706291	-0.180405904784		*Stachys_betoniciflora*_0,1mg/ml_Starvation	107	0.549340029167	-0.183605188635
	ERPS_0,01mg/ml	72	-0.232066124701	-0.372039875966		*Phlomis_sewertzovii*_0,01mg/ml	71	-0.500476198331	-0.000637510486784
	ERPS_0,02mg/ml	69	-0.0418562311044	-0.439316880901		*Phlomis_sewertzovii*_0,02mg/ml	63	-0.412976493957	0.00260921018499
	ERPS_0,05mg/ml	60	-0.301184663146	-0.35499984291		*Phlomis_sewertzovii*_0,05mg/ml	76	-0.671439254662	0.0972671900029
	ERPS_0,1mg/ml	48	-0.0192632788473	-0.717889897262		*Phlomis_salicifolia*_0,01mg/ml	78	-0.346994942878	-0.390365039463
	EFIAAlop_0,01mg/ml_Starvation	95	-0.484247167193	-0.569429439025		*Phlomis_salicifolia*_0,02mg/ml	70	-0.462299210114	0.0619719513041
	EFIAAlop_0,02mg/ml_Starvation	97	-0.572373920324	-0.132103649225		*Phlomis_salicifolia*_0,05mg/ml	83	-0.576698399997	-0.194225823834
	EFIAAlop_0,05mg/ml_Starvation	87	-0.424546850807	-0.333666374828		*Phlomis_salicifolia*_0,1mg/ml	55	-0.274877229796	-0.23753347613
	EFIAAlop_0,1mg/ml_Starvation	123	-0.663143141344	-0.252665795672		*Phlomis_salicifolia*_0,02mg/ml_Starvation	139	0.94484336835	-0.0884393893274
	ERHA_0,01mg/ml_Starvation	144	-0.380178907758	-0.137386087811		*Silene_oreina*_0,01mg/ml	54	-0.17610470348	-0.317243201448
	ERHA_0,02mg/ml_Starvation	47	0.312086686769	-0.209599810383		*Silene_oreina*_0,02mg/ml	74	-0.193473670873	-0.391690457796
	ERHA_0,05mg/ml_Starvation	138	-0.593667931792	-0.365935234267		*Silene_oreina*_0,05mg/ml	56	-0.0950188053986	-0.530732811274
	ERHA_0,1mg/ml_Starvation	72	-0.340464310727	-0.379398637154		*Silene_oreina*_0,1mg/ml	88	-0.267039626554	-0.222590571624
	EAGAF_0,01mg/ml_Starvation	95	-0.00359823534878	0.33562792195		*Phlomis_tadschikistanica*_0,01mg/ml	90	-0.423384001861	-0.131913988791
	EAGAF_0,02mg/ml_Starvation	65	-0.0331630580306	-0.13302634136		*Phlomis_tadschikistanica*_0,02mg/ml	97	-0.306271191134	0.23841385236
	EAGAF_0,05mg/ml_Starvation	108	-0.560736749619	-0.208216843445	3	*Phlomis_tadschikistanica*_0,05mg/ml	63	-0.201669259518	-0.333463534816
	EAGAF_0,1mg/ml_Starvation	60	-0.524455419066	-0.658820140197		*Cousina_umbrosa*_0,02mg/ml	53	-0.325590622846	-0.260952642142
	EAGAA_0,01mg/ml_Starvation	77	-0.439632742068	-0.481080308148		*Cousina_umbrosa*_0,1mg/ml	62	-0.481540744078	0.116082165173
	EAGAA_0,02mg/ml_Starvation	101	-0.402751684902	-0.337252216569		*Nepeta_olgae*_0,01mg/ml	51	-0.0524915525723	0.00812924069275
	EAGAA_0,05mg/ml_Starvation	36	-0.480541773756	-0.568267675473		*Nepeta_olgae*_0,02mg/ml	65	-0.229765405964	-0.190767623296
	EAGAA_0,1mg/ml_Starvation	83	-0.601028794265	-0.474258991188		*Nepeta_olgae*_0,05mg/ml	115	-0.331220403428	0.0280888764733
	EAGFO_0,01mg/ml_Starvation	54	-0.777281465549	-0.647131398692		*Nepeta_olgae*_0,1mg/ml	74	-0.48026634817	0.249311751571
	EAGFO_0,02mg/ml_Starvation	86	-0.661689664417	-0.112618242095		*Scutellaria_scharistanica*_0,01mg/ml	57	-0.343773466332	0.131964987405
	EAGPD_0,01mg/ml_Starvation	135	-0.32360974184	0.146788774694		*Scutellaria_scharistanica*_0,02mg/ml	53	-0.0836798808423	-0.474122330324
	EAGPD_0,02mg/ml_Starvation	84	0.0299969497648	-0.456934216606		*Scutellaria_scharistanica*_0,05mg/ml	48	-0.26010647444	-0.622785748503
	EAGPD_0,05mg/ml_Starvation	111	-0.221261902605	0.302690799582		*Scutellaria_scharistanica*_0,1mg/ml	50	0.00138617392601	0.00767011611448
	EAGPD_0,1mg/ml_Starvation	100	-0.370087248549	-0.435400081841		*Schrophullaria_sp*_0,01mg/ml	63	-0.165894780652	-0.350492731023
	EAGTM_0,01mg/ml_Starvation	130	-0.547392486577	-0.607883377856		*Schrophullaria_sp*_0,02mg/ml	57	-0.0816039592562	-0.414104626138
	EAGTM_0,02mg/ml_Starvation	62	-0.323670800163	-0.658144133682		*Schrophullaria_sp*_0,05mg/ml	73	-0.0946650748407	-0.24463432161
	EAGTM_0,05mg/ml_Starvation	137	-0.261070774652	-0.329576203403		*Schrophullaria_sp*_0,1mg/ml	92	-0.319006358893	-0.324152147436
	EAGTM_0,1mg/ml_Starvation	110	-0.580142646517	-0.683384655245		*Leonurus_panzeroides*_0,01mg/ml	59	-0.0576913257336	-0.169394469015
	EAGC_0,01mg/ml_Starvation	95	-0.383905728519	-0.174657756582		*Leonurus_panzeroides*_0,02mg/ml	87	-0.338683645657	-0.338878002495
	EAGC_0,02mg/ml_Starvation	52	0.334451816632	0.439989282088		*Leonurus_panzeroides*_0,05mg/ml	49	0.0230735282534	-0.405354895906
	EAGC_0,05mg/ml_Starvation	69	-0.451872124843	-0.250677664754		*Leonurus_panzeroides*_0,1mg/ml	85	-0.285943895564	-0.23145117024
	EAGC_0,1mg/ml_Starvation	79	-0.396525199218	-0.188459887398		*Leonurus_panzeroides*_0,1mg/ml_Starvation	93	0.791381013894	-0.641789091516
	EFHA_0,01mg/ml_Starvation	35	0.0624591393973	-0.377979459272		EAGRC_0,01mg/ml_Starvation	99	0.638209516558	-0.962456173198
	EFHA_0,02mg/ml_Starvation	100	-0.268898738565	0.0687950958306		ERAAlop_0,01mg/ml_Starvation	60	1.00482657986	-0.771242791623
	EFHA_0,05mg/ml_Starvation	68	-0.496557737385	-0.407105323345		ERAAlop_0,02mg/ml_Starvation	67	0.962026997137	-0.705033193795
	EFHA_0,1mg/ml_Starvation	100	-0.802595451465	-0.0809019268546		ERAAlop_0,05mg/ml_Starvation	67	1.50266294383	-0.62241017582
	EAGSS_0,01mg/ml_Starvation	76	-0.202613791963	-0.432619861664		ERAAlop_0,1mg/ml_Starvation	51	1.02785262265	-0.638352648678
3	EAGSS_0,02mg/ml_Starvation	98	-0.0547594879242	-0.519258581238		EAGAAsia_0,02mg/ml_Starvation	64	0.861389838351	-0.991250870056
	EAGSS_0,05mg/ml_Starvation	99	-0.317556477649	-0.334786465776		EAGAAsia_0,05mg/ml_Starvation	45	1.04375673658	-0.586523336297
	EAGSS_0,1mg/ml_Starvation	107	-0.389952850942	-0.456015220218		EAGAAlop_0,01mg/ml_Starvation	83	0.945241162987	-0.473587299241
	EFIPS_0,01mg/ml_Starvation	119	-0.497982369461	-0.129645375494		EAGMA_0,01mg/ml_Starvation	36	1.40291902948	-1.03100028641
	EFIPS_0,02mg/ml_Starvation	40	0.138848314436	-0.0353984184583		EAGMA_0,02mg/ml_Starvation	68	1.53258733585	-1.26984083784
	EFIPS_0,05mg/ml_Starvation	88	-0.270441939116	-0.522568455888		EAGMA_0,05mg/ml_Starvation	57	1.46666600921	-1.33494498957
	EAGCB_0,01mg/ml_Starvation	84	-0.488795607937	-0.0115365391861		EAGMA_0,1mg/ml_Starvation	58	1.05597415051	-1.19840844665
	EAGCB_0,02mg/ml_Starvation	41	0.0836229210081	0.12530344605		EAGMD_0,01mg/ml_Starvation	94	0.847639474148	-1.21806900883
	EAGCB_0,1mg/ml_Starvation	56	-0.139748609602	-0.204086692826		EAGMD_0,02mg/ml_Starvation	109	0.711307305331	-1.07095615945
	EAGCT_0,01mg/ml_Starvation	91	-0.263557231399	-0.419155164561		EAGMD_0,05mg/ml_Starvation	77	0.803939843596	-0.964472464318
	EAGCT_0,02mg/ml_Starvation	92	-0.415856546334	-0.28574221692		EAGMD_0,1mg/ml_Starvation	74	0.917591536626	-0.987759089639
	EAGCT_0,05mg/ml_Starvation	72	-0.22214563042	-0.635154670615		EAGKL_0,01mg/ml_Starvation	51	0.928590062632	-0.978158757229
	EAGCT_0,1mg/ml_Starvation	64	-0.41780619811	-0.522144228693		EAGKL_0,02mg/ml_Starvation	79	1.11051447454	-0.937535181955
	EAGOT_0,01mg/ml_Starvation	102	-0.253291964445	-0.695573241788		EAGKL_0,05mg/ml_Starvation	83	1.13874598801	-0.82786584432
	EAGOT_0,02mg/ml_Starvation	103	-0.364146364816	-0.631013287087		EAGKL_0,1mg/ml_Starvation	74	0.993871408081	-0.887615056483
	EAGOT_0,05mg/ml_Starvation	97	-0.158218362507	-0.543936845635		EAGAS_0,01mg/ml_Starvation	113	1.19602331736	-0.81056181082
	EAGOT_0,1mg/ml_Starvation	100	-0.26442610487	-0.392878969942		EAGAS_0,02mg/ml_Starvation	77	1.06059496091	-0.372632054353
	ERKL_0,02mg/ml	88	-0.885204570184	-0.271048625314		EAGAT_0,01mg/ml	91	0.81776753495	-0.964291813965
	ERKL_0,05mg/ml	87	-0.980418943378	-0.395476843612		EAGAT_0,02mg/ml	74	1.15222560893	-1.08131622373
	ERKL_0,1mg/ml	82	-0.784100753804	-0.581764200173		EAGAT_0,05mg/ml	60	0.778377560642	-1.61983340834
	ERKL_0,01mg/ml_Starvation	100	0.026465045235	-0.478317605717	4	EAGAT_0,1mg/ml	78	0.800969583593	-0.987307439655
	ERKL_0,02mg/ml_Starvation	98	-0.00343736043343	-0.9243025298		EAGAT_0,01mg/ml_Starvation	32	0.794587273339	-0.376703755874
	ERKS_0,01mg/ml_Starvation	78	0.156127566478	-0.751031690074		EAGAU_0,01mg/ml	68	0.851957436835	-0.868858857675
	ERKS_0,02mg/ml_Starvation	84	-0.11521766897	-0.535518104803		EAGAU_0,05mg/ml	57	0.782722900701	-1.05504195187
	ERKS_0,05mg/ml_Starvation	75	0.418171834377	-0.746993942724		EAGAU_0,1mg/ml	58	0.484973226348	-1.19287056459
	ERKS_0,1mg/ml_Starvation	79	0.208860210618	-0.691144554747		ERPS_0,01mg/ml_Starvation	43	1.14299750967	-1.0903882502
	ESHA_0,01mg/ml_Starvation	91	0.0335542288703	-0.494657932736		ERPS_0,02mg/ml_Starvation	53	1.29294818431	-0.933380251808
	ESHA_0,02mg/ml_Starvation	84	-0.12458131942	-0.723251811561		ERPS_0,05mg/ml_Starvation	30	2.39144871392	-1.00145849005
	ESHA_0,05mg/ml_Starvation	85	0.294996150297	-0.660839411531		ERKL_0,05mg/ml_Starvation	96	0.370275550086	-1.08860438712
	ESHA_0,1mg/ml_Starvation	93	0.0992725436817	-0.576310890996		ERKL_0,1mg/ml_Starvation	47	1.49792507912	-1.15776574635
	U-2C_0,01mg/ml_Starvation	108	-0.133063893287	-0.351452359297		*Verbascum_blattaria*_0,02mg/ml_Starvation	102	0.66787702124	-0.836613729303
	U-2C_0,02mg/ml_Starvation	94	-0.0480130532035	-0.2267336689181		*Verbascum_blattaria*_0,05mg/ml_Starvation	65	0.96078335626	-0.594988496005
	U-2C_0,05mg/ml_Starvation	97	0.282220514838	-0.0484774079022		*Verbascum_blattaria*_0,1mg/ml_Starvation	43	1.34501182434	-0.586026268895
	U-2C_0,1mg/ml_Starvation	94	0.15035781381	-0.396550907406		*Stachys_hissarica*_0,05mg/ml_Starvation	82	1.02581517433	-0.854708704384
	C-3B_0,05mg/ml_Starvation	76	0.368903292157	0.379411211559		*Stachys_hissarica*_0,1mg/ml_Starvation	24	1.3879131213	-0.56790418247
	C-4B_0,01mg/ml_Starvation	71	-0.191693648683	0.0966506864717		*Verbascum_songoricum*_0,01mg/ml_Starvation	107	0.854532698658	-0.933316682407
	T-1B_0,01mg/ml_Starvation	70	0.195087802963	-0.410912454228		*Verbascum_songoricum*_0,02mg/ml_Starvation	103	0.695713761463	-0.866786799307
	T-1B_0,02mg/ml_Starvation	65	0.504833125885	0.54281556171		*Verbascum_songoricum*_0,05mg/ml_Starvation	59	1.26069609205	-0.735064767725
	T-1B_0,05mg/ml_Starvation	59	0.260770122885	-0.287359926278		*Verbascum_songoricum*_0,1mg/ml_Starvation	42	1.13205481492	-0.682454470762
	T-1B_0,1mg/ml_Starvation	26	0.777642803394	0.529666011924		*Stachys_betoniciflora*_0,05mg/ml_Starvation	46	0.984924282062	-0.584878044458
	T-1C_0,02mg/ml	63	-0.717433428926	-0.0280981271351		*Phlomis_sewertzovii*_0,01mg/ml_Starvation	132	0.825026227059	-0.976845832589
	T-1C_0,01mg/ml_Starvation	85	0.187195643589	-0.392083873116		*Phlomis_sewertzovii*_0,02mg/ml_Starvation	120	0.806580293268	-1.20721785042
	T-1C_0,02mg/ml_Starvation	82	0.399537004271	0.221190373916		*Phlomis_sewertzovii*_0,05mg/ml_Starvation	91	1.50780780164	-0.887394178342
	T-1C_0,05mg/ml_Starvation	86	0.324229477936	-0.379486312394		*Silene_oreina*_0,1mg/ml_Starvation	84	1.70878084469	-1.37836186815
	T-1C_0,1mg/ml_Starvation	91	0.612357044774	-0.18220989197		*Phlomis_tadschikistanica*_0,01mg/ml_Starvation	128	1.64254151974	-1.16129019225

2 Hours Treatments - Clusters and Toxicity									
#C.	Treatments	%l.c.	PC1	PC2	#C.	Treatments	%l.c.	PC1	PC2
	*Phlomis_tadschikistanica*_0,02mg/ml_Starvation	103	1.52405473397	-0.981287984115		*Scutellaria_scharistanica*_0,1mg/ml_Starvation	64	1.26793273643	-0.912176579825
	*Phlomis_tadschikistanica*_0,05mg/ml_Starvation	120	0.908393369693	-0.793563084088		*Schrophullaria_sp*_0,01mg/ml_Starvation	97	4.53384205014	-1.77383700054
	*Phlomis_tadschikistanica*_0,1mg/ml_Starvation	85	0.816933364887	-0.860102591812		*Schrophullaria_sp*_0,02mg/ml_Starvation	115	4.25871846674	-2.33304789834
	*Cousina_umbrosa*_0,1mg/ml_Starvation	120	1.57099466617	-0.552548899034		*Scutellaria_scharistanica*_0,01mg/ml_Starvation	91	1.15987228009	-0.848774596425
	*Nepeta_olgae*_0,01mg/ml_Starvation	141	0.900403972534	-0.96846815766		*Scutellaria_scharistanica*_0,02mg/ml_Starvation	102	1.15596411454	-0.756874668799
	*Nepeta_olgae*_0,02mg/ml_Starvation	130	1.22669378292	-0.738498290905		*Schrophullaria_sp*_0,05mg/ml_Starvation	24	4.86746752041	-2.46276780918
	*Nepeta_olgae*_0,05mg/ml_Starvation	94	1.4996633385	-0.808123624958		*Leonurus_panzeroides*_0,01mg/ml_Starvation	128	1.04143413397	-1.18432777984
	*Nepeta_olgae*_0,1mg/ml_Starvation	95	1.50121452248	-0.832294769001		*Leonurus_panzeroides*_0,02mg/ml_Starvation	112	0.990225896586	-0.754230264937
	*Scutellaria_scharistanica*_0,05mg/ml_Starvation	105	1.33549782557	-0.668180974199		*Leonurus_panzeroides*_0,05mg/ml_Starvation	152	0.962838225529	-0.808606443225

Table A5. Ordered list of all the treatments administrated for 2 hours is reported divided per cluster, which number is reported in the first column by the cluster number and identified in all the columns by the background stained with the same colour originally used to define the cluster after hierarchical cluster analysis. The factor scores and the coloured background can also help the individuation of each sample in the scatter plot reporting the planar distribution showed in figure 27. All the treatments producing a mortality rate greater then 50% are reported in red background.

20 Hours Treatments - Clusters and Toxicity									
#C.	Treatments x Cluster	%l.c.	PC1	PC2	#C.	Treatments x Cluster	%l.c.	PC1	PC2
1	ERPS_0,1mg/ml	5	6.37248382023	-1.69176118848		U-2C_0,02mg/ml	82	0.00404920232512	0.945507662858
	ERPS_0,01mg/ml_Starvation	58	3.90096847579	-0.581511286171		U-2C_0,05mg/ml	77	-0.0084278186583	1.05520979622
	ERPS_0,02mg/ml_Starvation	18	7.03959639626	-3.01448175483		U-2C_0,1mg/ml	87	0.304350863065	1.27405021153
	ERPS_0,05mg/ml_Starvation	16	6.75729216179	-1.15243872047		C-3B_0,01mg/ml	82	0.209268013543	1.08039210078
	ERPS_0,1mg/ml_Starvation	13	7.70016729145	-2.34283410911		C-3B_0,02mg/ml	79	0.342262455813	1.14279792551
	EAGFO_0,1mg/ml	3	8.00003801111	4.40716550835		C-3B_0,05mg/ml	66	0.453521046823	1.47393336569
	EAGFO_0,05mg/ml_Starvation	4	5.14449807382	1.95680323127		C-3B_0,1mg/ml	66	1.16929885062	1.57211374936
	EAGFO_0,1mg/ml_Starvation	5	5.16008708686	2.64446147407		C-3B_0,02mg/ml_Starvation	70	-0.110843737059	0.633147126576
	EAGPD_0,1mg/ml_Starvation	6	4.98822831872	-1.02036834311		C-3B_0,05mg/ml_Starvation	57	0.0387551763615	0.763101711785
	EFHA_0,1mg/ml_Starvation	7	4.05530772238	0.756210061023		C-3B_0,1mg/ml_Starvation	34	-0.141707339711	0.357027412728
	EFIPS_0,1mg/ml_Starvation	6	6.37374866205	0.394161048167		C-4B_0,01mg/ml	88	-0.327793741059	0.672474982034
	ERKL_0,1mg/ml	2	6.43347535454	-0.298080946185		C-4B_0,02mg/ml	80	-0.2398710424	0.740387808669
	ERKL_0,05mg/ml_Starvation	3	5.27819065766	-0.228074972664		C-4B_0,05mg/ml	49	-0.273928116119	0.893480972928
	ERKL_0,1mg/ml_Starvation	2	8.34075502867	-1.44288105465		C-4B_0,1mg/ml	14	0.767347057195	1.38113623916
	C-4B_0,1mg/ml_Starvation	1	8.47025845979	1.76329843956		C-4B_0,05mg/ml_Starvation	15	-0.121824349982	0.787524755466
	T-1B_0,05mg/ml	2	6.90897638525	-0.455877095651		T-1B_0,01mg/ml	77	-0.20080306149	0.906316138669
	T-1B_0,1mg/ml	3	6.25628962805	-0.516187450438		T-1B_0,02mg/ml	82	-0.609077175288	0.83318128439
	T-1B_0,02mg/ml_Starvation	6	3.86282029548	-1.44290376398		T-1C_0,01mg/ml	70	-0.0558634840033	0.805792157435
	T-1B_0,05mg/ml_Starvation	3	6.27891843337	0.359084450894		T-1C_0,02mg/ml	94	0.933852961693	0.168114028625
	T-1B_0,1mg/ml_Starvation	2	8.9349903708	0.721803898349		T-1C_0,05mg/ml	94	-0.254719216351	0.697332259966
	*Verbascum_blattaria*_0,1mg/ml_Starvation	23	4.52723056704	0.591451572975		*Verbascum_blattaria*_0,01mg/ml	81	-0.278109025786	0.766549291351
	*Schrophullaria_sp*_0,05mg/ml_Starvation	3	8.23683867818	-1.74968166954		*Verbascum_blattaria*_0,02mg/ml	56	-0.428848572871	0.685887905967
	*Schrophullaria_sp*_0,1mg/ml_Starvation	3	7.91119187648	-1.73121837181		*Verbascum_blattaria*_0,05mg/ml	112	-0.565976217921	0.739011491606
2	Ctrl	100	-0.462332915708	0.410375379506		*Verbascum_blattaria*_0,1mg/ml	82	-0.168956526032	1.04627705105
	CtrlDMSO	93	-0.443799259264	0.420230130368		*Stachys_hissarica*_0,01mg/ml	73	0.651996073746	1.35123586493
	EFIAAlop_0,01mg/ml	91	-0.50855957856	0.709917532922		*Stachys_hissarica*_0,02mg/ml	77	0.520947998551	1.38676066949
	EFIAAlop_0,02mg/ml	76	-0.14642014422	1.13018774203		*Stachys_hissarica*_0,05mg/ml	82	0.594825682795	1.52339003484
	EFIAAlop_0,05mg/ml	93	-0.42892271518	0.711724289432		*Stachys_hissarica*_0,1mg/ml	45	0.449745687275	1.41108177442
	EFIAAlop_0,1mg/ml	60	-0.381963953947	0.425478917631		*Verbascum_songoricum*_0,01mg/ml	91	0.341714496184	1.2532853483
	ERHA_0,01mg/ml	75	-0.517192943813	0.898397644868		*Verbascum_songoricum*_0,02mg/ml	88	0.300185927487	1.25208576939
	ERHA_0,02mg/ml	53	-0.238294784784	1.02394916405		*Verbascum_songoricum*_0,05mg/ml	84	0.2440580744	1.27546692487
	ERHA_0,05mg/ml	48	-0.730231542408	0.735526475519		*Verbascum_songoricum*_0,1mg/ml	39	0.132670103455	1.31122400177
	ERHA_0,1mg/ml	53	-0.752764559088	0.522946917309		*Stachys_betoniciflora*_0,01mg/ml	93	-0.0527407529089	0.796871125239
	EAGAF_0,01mg/ml	122	-0.516270067733	0.751230293529	2	*Stachys_betoniciflora*_0,02mg/ml	104	0.0376827671012	0.911754393476
	EAGAF_0,02mg/ml	92	-0.36178267468	0.887021524172		*Stachys_betoniciflora*_0,05mg/ml	90	-0.0109529048594	0.859930855811
	EAGAF_0,05mg/ml	82	-0.545232735349	0.808662723007		*Stachys_betoniciflora*_0,1mg/ml	111	0.329780735189	1.07645302538
	EAGAF_0,1mg/ml	113	-0.270701396047	0.750782588789		*Stachys_betoniciflora*_0,1mg/ml_Starvation	56	0.42421095314	0.360033984514
	EAGAA_0,01mg/ml	66	-0.464706814047	0.88606178469		*Phlomis_sewertzovii*_0,01mg/ml	113	-0.0422112154099	0.918771432987
	EAGAA_0,02mg/ml	48	-0.150989965565	1.11668126976		*Phlomis_sewertzovii*_0,02mg/ml	102	0.166595037278	0.913966631443
	EAGAA_0,05mg/ml	57	-0.41791476131	1.09871484817		*Phlomis_sewertzovii*_0,05mg/ml	95	0.0477595054349	1.12954837512
	EAGAA_0,1mg/ml	76	-0.228547417373	1.10217751354		*Phlomis_sewertzovii*_0,1mg/ml	87	0.290092205367	1.13976987958
	EAGAA_0,1mg/ml_Starvation	29	-0.605251223983	0.348241177953		*Phlomis_salicifolia*_0,01mg/ml	94	-0.0787636067143	0.790181809824
	EAGFO_0,01mg/ml	88	-0.610680922875	0.488594080056		*Phlomis_salicifolia*_0,02mg/ml	99	-0.159501672569	0.760573336001
	EAGFO_0,02mg/ml	50	-0.121999000882	1.35279744349		*Phlomis_salicifolia*_0,05mg/ml	96	0.055176579333	0.869947663514
	EAGFO_0,05mg/ml	40	-0.154596689873	1.39602245587		*Phlomis_salicifolia*_0,1mg/ml	67	0.679870980219	1.31516555391
	EAGFO_0,02mg/ml_Starvation	14	-0.453227785953	0.418538382175		*Phlomis_tadschikistanica*_0,01mg/ml	90	0.00559297720829	0.904422918666
	EAGPD_0,02mg/ml	70	-0.362779094147	0.478417063439		*Phlomis_tadschikistanica*_0,02mg/ml	76	-0.626193629685	0.371673758165
	EAGPD_0,05mg/ml	40	-0.129940121279	0.221327520625		*Phlomis_tadschikistanica*_0,05mg/ml	70	-0.386930049233	0.664513616069
	EAGPD_0,1mg/ml	22	-0.307203616901	0.301887087095		*Phlomis_tadschikistanica*_0,1mg/ml	57	-0.207493443346	0.868866536582
	EAGTM_0,01mg/ml	84	-0.373288791806	0.748920750118		*Cousina_umbrosa*_0,01mg/ml	62	-0.615004301706	0.43330848612
	EAGTM_0,02mg/ml	67	0.188269948318	0.940718362422		*Cousina_umbrosa*_0,02mg/ml	50	-0.520990574024	0.400931561473
	EAGTM_0,05mg/ml	60	-0.100187726814	0.297714335031		*Cousina_umbrosa*_0,05mg/ml	36	-0.111747023977	0.824283798375
	EAGTM_0,1mg/ml	58	0.232915641856	1.08594830204		*Cousina_umbrosa*_0,1mg/ml	51	-0.185625855368	0.83383683175
	EAGC_0,01mg/ml	43	-0.575426654117	0.377298680386		*Nepeta_olgae*_0,01mg/ml	84	-0.0896453865038	0.815272787135
	EAGC_0,02mg/ml	26	-0.336074143544	0.7994870657		*Nepeta_olgae*_0,02mg/ml	78	-0.193297841354	0.829482947959
	EAGC_0,05mg/ml	33	-0.512152809239	0.359868092003		*Nepeta_olgae*_0,05mg/ml	64	-0.10811919805	0.667006648335
	EFHA_0,01mg/ml	75	-0.601691119595	0.447198629619		*Nepeta_olgae*_0,1mg/ml	82	-0.424333393893	0.679876056428
	EFHA_0,02mg/ml	61	-0.504538003195	0.605274031706		*Scutellaria_scharistanica*_0,01mg/ml	59	-0.353346450174	0.5007477938
	EFHA_0,05mg/ml	95	-0.583593100405	0.376970266301		*Scutellaria_scharistanica*_0,02mg/ml	41	-0.0820374943374	0.861714650254
	EFHA_0,1mg/ml	65	-0.319498332592	0.773985143033		*Scutellaria_scharistanica*_0,05mg/ml	59	-0.397315360539	0.506533717022
	EAGSS_0,01mg/ml	68	-0.571408752455	0.395987742329		*Scutellaria_scharistanica*_0,1mg/ml	29	-0.278718084445	0.534935443574
	EAGSS_0,02mg/ml	39	-0.63758476644	0.292602803084		*Schrophullaria_sp*_0,01mg/ml	54	-0.369081422313	0.728585256012
	EAGSS_0,05mg/ml	59	-0.612437982029	0.450812245351		*Schrophullaria_sp*_0,02mg/ml	49	-0.233090457633	0.753737309317
	EFIPS_0,01mg/ml	87	-0.580144152548	0.371628500391		*Schrophullaria_sp*_0,05mg/ml	64	-0.256408754358	0.694391398782
	EFIPS_0,02mg/ml	47	-0.677181291086	0.780424033422		*Schrophullaria_sp*_0,1mg/ml	46	-0.35437505844	0.731676429884
	EFIPS_0,05mg/ml	70	-0.339405898009	0.995282355848		*Leonurus_panzeroides*_0,01mg/ml	69	0.0528278970335	0.721376123892
	EFIPS_0,1mg/ml	27	-0.693896235334	0.688979634761		*Leonurus_panzeroides*_0,02mg/ml	61	0.375217908809	0.948300874318
	EAGCB_0,01mg/ml	80	-0.157573878152	0.332044031894		*Leonurus_panzeroides*_0,05mg/ml	52	0.371352481042	1.0240106476
	EAGCB_0,02mg/ml	45	-0.0915009170388	1.09323561287		*Leonurus_panzeroides*_0,1mg/ml	72	0.290286748857	0.957365459156
	EAGCB_0,1mg/ml	65	0.163331149728	0.616843600657	3	EAGRC_0,1mg/ml_Starvation	28	1.04806967279	-1.96782369138
	EAGCT_0,01mg/ml	90	0.0586368408872	1.15432967885		EAGPS_0,1mg/ml	88	0.12943258018	-1.74370396264
	EAGCT_0,02mg/ml	95	0.492132222482	1.35729860798		EAGPS_0,01mg/ml_Starvation	180	0.316720694877	-2.12510934799
	EAGCT_0,05mg/ml	84	0.245711735413	1.30309068688		EAGPS_0,02mg/ml_Starvation	122	0.757789953469	-2.31609718775
	EAGCT_0,1mg/ml	83	0.598017834427	1.52779081334		EAGPS_0,05mg/ml_Starvation	127	0.886050571463	-2.60280853359
	EAGCT_0,02mg/ml_Starvation	82	-0.652952306093	0.29968295861		EAGPS_0,1mg/ml_Starvation	72	1.42692373862	-2.69559401665
	EAGCT_0,05mg/ml_Starvation	55	-0.308117174495	0.636285241408		EAGAAsia_0,02mg/ml_Starvation	49	0.388512699419	-1.41832890854
	EAGCT_0,1mg/ml_Starvation	49	0.128768666938	1.17321848359		EAGAAsia_0,05mg/ml_Starvation	23	0.628438765238	-1.72663061987
	EAGOT_0,01mg/ml	92	0.53686773345	1.12025370086		EAGAAsia_0,1mg/ml_Starvation	40	0.511821871865	-1.49108300083
	EAGOT_0,02mg/ml	99	1.19186448071	1.46691417479		EAGAAlop_0,1mg/ml_Starvation	51	1.53818337126	-2.71185673708
	EAGOT_0,05mg/ml	101	0.97309553673	1.52344481927		EAGKL_0,05mg/ml_Starvation	74	0.61516706707	-2.12058925857
	EAGOT_0,1mg/ml	89	0.825758206606	1.42114988207		EAGKL_0,1mg/ml_Starvation	31	0.584368607191	-2.00804464376
	ERKS_0,01mg/ml	90	0.149238116363	0.979026680246		EAGPD_0,01mg/ml_Starvation	68	0.669679827352	-2.20016193097
	ERKS_0,02mg/ml	91	0.00321316806138	0.853463896262		ERKS_0,02mg/ml_Starvation	103	0.304251293773	-1.43756268836
	ERKS_0,05mg/ml	86	-0.414109197573	0.644853694697		*Verbascum_songoricum*_0,1mg/ml_Starvation	15	1.52486226417	-2.07003260185
	ESHA_0,01mg/ml	96	-0.143736379703	1.08516439968		*Silene_oreina*_0,1mg/ml_Starvation	49	-0.178458313795	-1.79123411413
	ESHA_0,02mg/ml	88	0.161773080723	1.09124339207	4	Ctrl_Starvation	100	-0.810463832901	-0.319616223058
	ESHA_0,05mg/ml	94	0.0620445831785	1.13878360806		CtrlDMSO_Starvation	97	-0.827515568979	-0.306822191736
	ESHA_0,1mg/ml	89	0.258632430651	1.14620030173		EAGRC_0,01mg/ml	104	-0.867802947551	-0.156699534156
	U-2C_0,01mg/ml	97	0.0459052941315	0.999536456268		EAGRC_0,02mg/ml	98	-0.848761939931	-0.0878975252566

20 Hours Treatments - Clusters and Toxicity									
#C.	Treatments x Cluster	%l.c.	PC1	PC2	#C.	Treatments x Cluster	%l.c.	PC1	PC2
	EAGRC_0,05mg/ml	106	-0.849704558553	-0.0436337442209		EAGAA_0,01mg/ml_Starvation	80	-0.686728786832	-0.626692626118
	EAGRC_0,1mg/ml	96	-0.928187373233	-0.0811237117396		EAGAA_0,02mg/ml_Starvation	58	-0.856780146145	-0.669227586315
	EAGRC_0,01mg/ml_Starvation	134	-0.799480592361	-0.134337884664		EAGAA_0,05mg/ml_Starvation	59	-0.848129581985	-0.14625436544
	EAGRC_0,02mg/ml_Starvation	123	-0.701568062172	-0.153725636843		EAGFO_0,01mg/ml_Starvation	58	-1.00300532513	-0.681121020767
	EAGRC_0,05mg/ml_Starvation	119	-0.553876265916	-0.369854819128		EAGPD_0,01mg/ml	70	-0.501450921343	0.160463683218
	ERAAlop_0,01mg/ml	85	-0.757075783636	-0.297389822575		EAGPD_0,02mg/ml_Starvation	62	-0.604163667909	-0.900031020842
	ERAAlop_0,02mg/ml	73	-0.689506788122	-0.208636836742		EAGPD_0,05mg/ml_Starvation	39	-0.63053253342	-0.747465340803
	ERAAlop_0,05mg/ml	62	-0.574087040866	-0.310892470616		EAGTM_0,01mg/ml_Starvation	47	-0.916836452235	-0.872942647128
	ERAAlop_0,1mg/ml	47	-0.914244288017	0.102382851295		EAGTM_0,02mg/ml_Starvation	105	-0.786007507518	-0.964998199047
	ERAAlop_0,01mg/ml_Starvation	83	-0.617596258155	-0.370176370167		EAGTM_0,05mg/ml_Starvation	70	-0.750260722621	-0.585204293546
	ERAAlop_0,02mg/ml_Starvation	100	-0.618973912587	-0.218316284286		EAGTM_0,1mg/ml_Starvation	44	-0.594433197011	-0.618573787566
	ERAAlop_0,05mg/ml_Starvation	62	-0.286703275902	-0.48111706499		EAGC_0,1mg/ml	24	-0.862493765225	-0.0969667072852
	ERAAlop_0,1mg/ml_Starvation	68	-0.833799055733	0.096000307892		EAGC_0,01mg/ml_Starvation	48	-0.850252504404	-0.69472889633
	EAGPS_0,01mg/ml	104	-0.789549767346	-0.403541654985		EAGC_0,02mg/ml_Starvation	39	-0.906056242134	-1.15575025576
	EAGPS_0,02mg/ml	91	-0.921950149432	-0.780062263907		EAGC_0,05mg/ml_Starvation	23	-0.791227827775	-1.17344252256
	EAGPS_0,05mg/ml	84	-0.941146185427	-0.796110236404		EAGC_0,1mg/ml_Starvation	13	0.125612171229	-0.511503363286
	EAGAAsia_0,01mg/ml	75	-0.777510606885	-0.0845320384589		EFHA_0,01mg/ml_Starvation	90	-0.668530384525	-0.840813657907
	EAGAAsia_0,02mg/ml	93	-0.681941540103	-0.049247941123		EFHA_0,02mg/ml_Starvation	106	-0.977688890873	-0.738793146364
	EAGAAsia_0,05mg/ml	69	-0.364490077562	-0.266099155727		EFHA_0,05mg/ml_Starvation	103	-0.55549113169	-0.925773075672
	EAGAAsia_0,1mg/ml	58	-0.909088926465	0.246904176388		EAGSS_0,1mg/ml	39	-0.742406961794	0.11327275724
	EAGAAsia_0,01mg/ml_Starvation	59	-0.508520167579	-0.733324074352		EAGSS_0,01mg/ml_Starvation	82	-1.14978414526	-0.966357157189
	EAGAAlop_0,01mg/ml	105	-0.783719010703	-0.119538906314		EAGSS_0,02mg/ml_Starvation	47	-1.11005202934	-1.00887014707
	EAGAAlop_0,02mg/ml	88	-0.715005522091	-0.15781076992		EAGSS_0,05mg/ml_Starvation	66	-0.753936605106	-0.835388368535
	EAGAAlop_0,05mg/ml	98	-0.739965821899	-0.0688232050534		EAGSS_0,1mg/ml_Starvation	61	-0.854776711355	-1.00253009746
	EAGAAlop_0,1mg/ml	109	-0.805497561563	-0.0895235734856		EFIPS_0,01mg/ml_Starvation	79	-0.666725589907	-1.03337157885
	EAGAAlop_0,01mg/ml_Starvation	133	-0.710923754809	-0.368841629972		EFIPS_0,02mg/ml_Starvation	93	-0.569605177236	-1.06705352231
	EAGAAlop_0,02mg/ml_Starvation	222	-0.794706820739	-0.06556868374		EFIPS_0,05mg/ml_Starvation	30	-0.469009636593	-0.980662557472
	EAGAAlop_0,05mg/ml_Starvation	88	-0.600355958084	-0.560960148919		EAGCB_0,05mg/ml	50	-0.553902606238	0.244721337307
	EAGMA_0,01mg/ml	62	-0.956801786283	0.0100225160541		EAGCB_0,01mg/ml_Starvation	41	-0.920306943004	-0.823472703458
	EAGMA_0,02mg/ml	68	-0.967798776566	-0.0981199356164		EAGCB_0,02mg/ml_Starvation	57	-0.950471100979	-1.22451748531
	EAGMA_0,05mg/ml	84	-1.00707671449	0.0310816769927		EAGCB_0,05mg/ml_Starvation	48	-0.859701651227	-0.745293636915
	EAGMA_0,1mg/ml	81	-1.17852498263	0.00222631306232		EAGCB_0,1mg/ml_Starvation	52	-0.549854876136	-0.904375194774
	EAGMA_0,01mg/ml_Starvation	88	-1.12389039762	-0.12998383913		EAGCT_0,01mg/ml_Starvation	74	-0.851456050999	0.0668128846085
	EAGMA_0,02mg/ml_Starvation	85	-0.884375295896	-0.209067032148		EAGOT_0,01mg/ml_Starvation	106	-0.611625668495	-0.215166773659
	EAGMA_0,05mg/ml_Starvation	74	-0.836359176247	-0.211293432901		EAGOT_0,02mg/ml_Starvation	111	-0.706749256831	-0.0901118728133
	EAGMA_0,1mg/ml_Starvation	84	-1.21839285313	0.0288196337562		EAGOT_0,05mg/ml_Starvation	93	-0.647228629994	-0.0774056916685
	EAGMD_0,01mg/ml	84	-0.967046856225	-0.0965128215608		EAGOT_0,1mg/ml_Starvation	103	-0.668167856914	0.104810564339
	EAGMD_0,02mg/ml	81	-0.928363801043	-0.0974182599989		ERKL_0,01mg/ml	95	-0.777195427828	0.251598184689
	EAGMD_0,05mg/ml	77	-0.896543628433	-0.0376976537139		ERKL_0,02mg/ml	94	-0.603461183263	0.235738065618
	EAGMD_0,1mg/ml	67	-0.914094513913	-0.00295816780436		ERKL_0,05mg/ml	53	-0.73157733332	0.25221653525
	EAGMD_0,01mg/ml_Starvation	95	-0.954511759344	0.0380990641109		ERKL_0,01mg/ml_Starvation	84	-0.867502026421	-0.0829973670335
	EAGMD_0,02mg/ml_Starvation	107	-0.851835997404	-0.254417729066		ERKL_0,02mg/ml_Starvation	79	-0.746271796029	-0.139120034519
	EAGMD_0,05mg/ml_Starvation	75	-0.61362145332	-0.501032262556		ERKS_0,1mg/ml	84	-0.487049673006	-0.0345304840971
	EAGMD_0,1mg/ml_Starvation	44	-0.73907417841	-0.687691750786		ERKS_0,01mg/ml_Starvation	108	-0.787639600883	-0.365199044708
	EAGKL_0,01mg/ml	86	-0.705587571493	-0.226382454625	4	ERKS_0,05mg/ml_Starvation	95	-0.799555058807	-0.685211934467
	EAGKL_0,02mg/ml	80	-0.690981248195	-0.129650978092		ERKS_0,1mg/ml_Starvation	85	-0.788302094656	-0.839938013885
	EAGKL_0,05mg/ml	74	-0.664874446065	-0.163006067767		ESHA_0,01mg/ml_Starvation	99	-0.938598331093	-0.139116890234
	EAGKL_0,1mg/ml	78	-0.74991000206	-0.0613191175331		ESHA_0,02mg/ml_Starvation	95	-0.847613666097	-0.098005542074
	EAGKL_0,01mg/ml_Starvation	60	-0.788595380941	-0.196463663191		ESHA_0,05mg/ml_Starvation	87	-0.860519033219	-0.161265782339
	EAGKL_0,02mg/ml_Starvation	103	-0.861081430635	-0.300972675788		ESHA_0,1mg/ml_Starvation	80	-0.863930046832	-0.203331485394
	EAGAS_0,01mg/ml	92	-0.848211727621	-0.128493043699		U-2C_0,01mg/ml_Starvation	90	-0.797641433533	-0.107503598623
	EAGAS_0,02mg/ml	85	-0.687260463133	-0.192328555261		U-2C_0,02mg/ml_Starvation	80	-0.575974685769	0.0667502765839
	EAGAS_0,05mg/ml	88	-0.735985742018	-0.239292212761		U-2C_0,05mg/ml_Starvation	90	-0.6209803835	0.0408427282643
	EAGAS_0,1mg/ml	72	-0.832448640928	-0.281889811273		U-2C_0,1mg/ml_Starvation	70	-0.557562802015	-0.108682707411
	EAGAS_0,01mg/ml_Starvation	110	-0.65652125884	-0.167421306958		C-3B_0,01mg/ml_Starvation	82	-0.494603281004	0.0205757717671
	EAGAS_0,02mg/ml_Starvation	121	-0.76037634383	-0.29372553265		C-4B_0,01mg/ml_Starvation	71	-0.77904861315	-0.010639793618
	EAGAS_0,05mg/ml_Starvation	113	-0.658870763552	-0.427630362582		C-4B_0,02mg/ml_Starvation	64	-0.64699529929	0.139784283465
	EAGAS_0,1mg/ml_Starvation	212	-0.780496020148	-0.580150014494		T-1B_0,01mg/ml_Starvation	39	-0.781477381484	-0.0604119682815
	EAGAT_0,01mg/ml	81	-0.80833599167	0.038291099758		T-1C_0,1mg/ml	10	-0.207201298544	-0.341508946666
	EAGAT_0,02mg/ml	87	-0.700183546004	-0.0671859940258		T-1C_0,01mg/ml_Starvation	98	-0.767267449117	-0.301188579014
	EAGAT_0,05mg/ml	80	-0.665376674067	-0.135953972146		T-1C_0,02mg/ml_Starvation	81	-0.57669754772	-0.285113513183
	EAGAT_0,1mg/ml	91	-0.775863515976	-0.0553148302643		T-1C_0,05mg/ml_Starvation	84	-0.85849822351	-0.0887413453173
	EAGAT_0,01mg/ml_Starvation	81	-0.381522813339	-0.422695834431		T-1C_0,1mg/ml_Starvation	27	-0.658133733936	-0.119961677814
	EAGAT_0,02mg/ml_Starvation	107	-0.871931983121	-0.0332752850156		*Verbascum_blattaria*_0,01mg/ml_Starvation	112	-0.756101479977	-0.508724384767
	EAGAT_0,05mg/ml_Starvation	81	-0.680489136918	-0.48863333422		*Verbascum_blattaria*_0,02mg/ml_Starvation	103	-0.721852184384	-0.809938788506
	EAGAT_0,1mg/ml_Starvation	113	-0.854969973941	0.0603897601422		*Verbascum_blattaria*_0,05mg/ml_Starvation	82	-0.469559713419	-0.537117179287
	EAGAU_0,01mg/ml	88	-0.82935961242	-0.139420669232		*Stachys_hissarica*_0,01mg/ml_Starvation	106	-0.679998716634	-0.0991128440301
	EAGAU_0,02mg/ml	83	-0.779241352006	-0.107088965144		*Stachys_hissarica*_0,02mg/ml_Starvation	84	-0.420225763672	-0.153065765277
	EAGAU_0,05mg/ml	118	-0.661197359672	0.00344432087207		*Stachys_hissarica*_0,05mg/ml_Starvation	91	-0.389594929133	-0.677751952976
	EAGAU_0,1mg/ml	68	-0.829957573762	-0.00601785402816		*Stachys_hissarica*_0,1mg/ml_Starvation	60	-0.434227038376	-0.506195044637
	EAGAU_0,01mg/ml_Starvation	88	-0.698294628581	-0.365024915982		*Verbascum_songoricum*_0,01mg/ml_Starvation	154	-0.544254721661	0.222960400993
	EAGAU_0,02mg/ml_Starvation	72	-0.684042822787	-0.139694494935		*Verbascum_songoricum*_0,02mg/ml_Starvation	103	-0.480368524936	-0.168516435468
	EAGAU_0,05mg/ml_Starvation	104	-0.414505582016	-0.447342879783		*Verbascum_songoricum*_0,05mg/ml_Starvation	58	0.0282478307916	-0.549096356825
	EAGAU_0,1mg/ml_Starvation	72	-0.785812649432	-0.121331147028		*Stachys_betoniciflora*_0,01mg/ml_Starvation	110	-0.553068295926	-0.40325804889
	ERPS_0,01mg/ml	91	-0.644038254629	-0.267147103177		*Stachys_betoniciflora*_0,02mg/ml_Starvation	133	-0.52450317818	-0.353827181409
	ERPS_0,02mg/ml	70	-0.875047662193	-0.0874285569761		*Stachys_betoniciflora*_0,05mg/ml_Starvation	165	-0.30962419442	-0.160598421547
	ERPS_0,05mg/ml	78	-0.765007201659	-0.265606432277		*Phlomis_sewertzovii*_0,01mg/ml_Starvation	92	-0.572306086538	-0.501485777546
	EFIAAlop_0,01mg/ml_Starvation	86	-0.87979139902	-0.929172224152		*Phlomis_sewertzovii*_0,02mg/ml_Starvation	126	-0.584110504067	-0.515699447377
	EFIAAlop_0,02mg/ml_Starvation	75	-0.936790882425	-0.839282401021		*Phlomis_sewertzovii*_0,05mg/ml_Starvation	117	-0.560902547398	-0.576151591523
	EFIAAlop_0,05mg/ml_Starvation	69	-1.00702325059	-0.5752287781		*Phlomis_sewertzovii*_0,1mg/ml_Starvation	50	-0.265702463014	-0.870141457972
	EFIAAlop_0,1mg/ml_Starvation	117	-0.813693572417	-0.855950308939		*Phlomis_salicifolia*_0,01mg/ml_Starvation	111	-0.518638091711	-0.49873990395
	ERHA_0,01mg/ml_Starvation	57	-0.510853513076	-0.622376326794		*Phlomis_salicifolia*_0,02mg/ml_Starvation	115	-0.681848189292	-0.679092424013
	ERHA_0,02mg/ml_Starvation	59	-1.10387638014	-0.556464490289		*Phlomis_salicifolia*_0,05mg/ml_Starvation	122	-0.469207734294	-0.875543510836
	ERHA_0,05mg/ml_Starvation	44	-0.99201793733	-0.436527928881		*Phlomis_salicifolia*_0,1mg/ml_Starvation	95	-0.399832216634	-0.686129849662
	ERHA_0,1mg/ml_Starvation	39	-1.00807351523	-0.449887540308		*Silene_oreina*_0,01mg/ml	72	-0.66686686247	0.0493585507741
	EAGAF_0,01mg/ml_Starvation	83	-0.89401499158	-0.325502508593		*Silene_oreina*_0,02mg/ml	79	-0.593420032067	0.166946256849
	EAGAF_0,02mg/ml_Starvation	111	-0.753176276917	-0.51278735039		*Silene_oreina*_0,05mg/ml	76	-0.664773825695	0.0930176346626
	EAGAF_0,05mg/ml_Starvation	42	-0.979342740937	-0.715930229317		*Silene_oreina*_0,1mg/ml	61	-0.81030154915	-0.0327132765317
	EAGAF_0,1mg/ml_Starvation	29	-0.595635273766	-0.0371300047209		*Silene_oreina*_0,01mg/ml_Starvation	101	-0.923576412153	-0.883509685589

20 Hours Treatments - Clusters and Toxicity									
#C.	Treatments x Cluster	%l.c.	PC1	PC2	#C.	Treatments x Cluster	%l.c.	PC1	PC2
	*Silene_oreina*_0,02mg/ml_Starvation	118	-0.849070461127	-0.972652009503		*Nepeta_olgae*_0,05mg/ml_Starvation	97	-0.762481276729	-0.669435363239
	*Silene_oreina*_0,05mg/ml_Starvation	78	-0.538965045308	-1.2506448158		*Nepeta_olgae*_0,1mg/ml_Starvation	81	-0.731364538943	-0.641965731634
	*Phlomis_tadschikistanica*_0,01mg/ml_Starvation	100	-0.652623131702	-0.75348578121		*Scutellaria_scharistanica*_0,01mg/ml_Starvation	107	-0.594092781074	-0.546204929067
	*Phlomis_tadschikistanica*_0,02mg/ml_Starvation	116	-0.834872889197	-0.838843902823		*Scutellaria_scharistanica*_0,02mg/ml_Starvation	82	-0.409642151036	-0.638526153891
	*Phlomis_tadschikistanica*_0,05mg/ml_Starvation	109	-0.698127228594	-1.04894017211		*Scutellaria_scharistanica*_0,05mg/ml_Starvation	63	-0.332039747082	-1.09461802458
	*Phlomis_tadschikistanica*_0,1mg/ml_Starvation	76	-0.638937121065	-0.944413097664		*Scutellaria_scharistanica*_0,1mg/ml_Starvation	98	-0.20820860479	-0.817680280555
	*Cousina_umbrosa*_0,01mg/ml_Starvation	48	-0.731467398115	-0.362404169694		*Schrophullaria_sp*_0,01mg/ml_Starvation	103	-0.580626626509	-0.564711712725
	*Cousina_umbrosa*_0,02mg/ml_Starvation	62	-0.650011755989	-0.741362040559		*Schrophullaria_sp*_0,02mg/ml_Starvation	70	-0.647608299593	-0.493361523587
	*Cousina_umbrosa*_0,05mg/ml_Starvation	58	-0.48649957441	-0.918851777787		*Leonurus_panzeroides*_0,01mg/ml_Starvation	90	-0.480535446204	-0.460229647089
	*Cousina_umbrosa*_0,1mg/ml_Starvation	32	0.0106515487209	-0.89873136847		*Leonurus_panzeroides*_0,02mg/ml_Starvation	85	-0.325229045867	-0.569881792836
	*Nepeta_olgae*_0,01mg/ml_Starvation	103	-0.433541495296	-0.956201364712		*Leonurus_panzeroides*_0,05mg/ml_Starvation	61	-0.32130931758	-0.462044350614
	*Nepeta_olgae*_0,02mg/ml_Starvation	110	-0.564176176438	-0.740303846879		*Leonurus_panzeroides*_0,1mg/ml_Starvation	34	0.349453115729	-0.487177291812

Table A6. Ordered list of all the treatments administrated for 20 hours is reported divided per cluster, which number is reported in the first column by the cluster number and identified in all the columns by the background stained with the same colour originally used to define the cluster after hierarchical cluster analysis. The factor scores and the coloured background can also help the individuation of each sample in the scatter plot reporting the planar distribution showed in figure 29. All the treatments producing a mortality rate greater then 50% are reported in red background.

Toxic Treatments after both 2 and 20 hours							
#	**Treatment**	**% living cells after 2h**	**% living cells after 20h**	**#**	**Treatment**	**% living cells after 2h**	**% living cells after 20h**
1	EAGAAsia_0,05mg/ml_Starvation	45	23	**9**	C-4B_0,1mg/ml	45	14
2	EAGAAsia_0,1mg/ml_Starvation	42	40	**10**	C-4B_0,1mg/ml_Starvation	37	1
3	ERPS_0,1mg/ml	48	5	**11**	T-1B_0,1mg/ml_Starvation	26	2
4	ERPS_0,05mg/ml_Starvation	30	16	**12**	*Verbascum_blattaria*_0,1mg/ml_Starvation	43	23
5	ERPS_0,1mg/ml_Starvation	5	13	**13**	*Verbascum_songoricum*_0,1mg/ml_Starvation	42	15
6	EAGFO_0,05mg/ml_Starvation	24	4	**14**	*Schrophullaria_sp*_0,05mg/ml_Starvation	24	3
7	EAGFO_0,1mg/ml_Starvation	3	5	**15**	*Schrophullaria_sp*_0,1mg/ml_Starvation	2	3
8	ERKL_0,1mg/ml_Starvation	47	2				

Table A7. List of treatments resulting to produce a toxicity rate greater then 50% after both 2 and 20 hours, and associated percentages of living cells detected after both treatments time durations.

Treatments Not Toxic after 2 hours and Toxic after 20 hours							
#	Treatment	% living cells after 2h	% living cells after 20h	#	Treatment	% living cells after 2h	% living cells after 20h
1	EAGRC_0,1mg/ml_Starvation	94	28	**34**	EAGSS_0,02mg/ml_Starvation	98	47
2	ERAAlop_0,1mg/ml	65	47	**35**	EFIPS_0,02mg/ml	80	47
3	EAGAAsia_0,02mg/ml_Starvation	64	49	**36**	EFIPS_0,1mg/ml	50	27
4	EAGMD_0,1mg/ml_Starvation	74	44	**37**	EFIPS_0,05mg/ml_Starvation	88	30
5	EAGKL_0,1mg/ml_Starvation	74	31	**38**	EFIPS_0,1mg/ml_Starvation	59	6
6	ERPS_0,02mg/ml_Starvation	53	18	**39**	EAGCB_0,02mg/ml	59	45
7	ERHA_0,05mg/ml	80	48	**40**	EAGCB_0,01mg/ml_Starvation	84	41
8	ERHA_0,05mg/ml_Starvation	138	44	**41**	EAGCB_0,05mg/ml_Starvation	68	48
9	ERHA_0,1mg/ml_Starvation	72	39	**42**	EAGCT_0,1mg/ml_Starvation	64	49
10	EAGAF_0,05mg/ml_Starvation	108	42	**43**	ERKL_0,1mg/ml	82	2
11	EAGAF_0,1mg/ml_Starvation	60	29	**44**	ERKL_0,05mg/ml_Starvation	96	3
12	EAGAA_0,02mg/ml	96	48	**45**	C-3B_0,1mg/ml_Starvation	71	34
13	EAGAA_0,1mg/ml_Starvation	83	29	**46**	C-4B_0,05mg/ml	73	49
14	EAGFO_0,05mg/ml	67	40	**47**	C-4B_0,05mg/ml_Starvation	71	15
15	EAGFO_0,1mg/ml	88	3	**48**	T-1B_0,02mg/ml	67	26
16	EAGFO_0,02mg/ml_Starvation	86	14	**49**	T-1B_0,05mg/ml	79	2
17	EAGPD_0,05mg/ml	71	40	**50**	T-1B_0,1mg/ml	51	3
18	EAGPD_0,1mg/ml	97	22	**51**	T-1B_0,01mg/ml_Starvation	70	39
19	EAGPD_0,05mg/ml_Starvation	111	39	**52**	T-1B_0,02mg/ml_Starvation	65	6
20	EAGPD_0,1mg/ml_Starvation	100	6	**53**	T-1B_0,05mg/ml_Starvation	59	3
21	EAGTM_0,01mg/ml_Starvation	130	47	**54**	T-1C_0,1mg/ml	64	10
22	EAGTM_0,1mg/ml_Starvation	110	44	**55**	T-1C_0,1mg/ml_Starvation	91	27
23	EAGC_0,01mg/ml	94	43	**56**	*Stachys_hissarica*_0,1mg/ml	91	45
24	EAGC_0,02mg/ml	96	26	**57**	*Verbascum_songoricum*_0,1mg/ml	85	39
25	EAGC_0,05mg/ml	108	33	**58**	*Silene_oreina*_0,1mg/ml_Starvation	84	49
26	EAGC_0,1mg/ml	91	24	**59**	*Cousina_umbrosa*_0,05mg/ml	56	36
27	EAGC_0,01mg/ml_Starvation	95	48	**60**	*Cousina_umbrosa*_0,01mg/ml_Starvation	91	48
28	EAGC_0,02mg/ml_Starvation	52	39	**61**	*Cousina_umbrosa*_0,1mg/ml_Starvation	120	32
29	EAGC_0,05mg/ml_Starvation	69	23	**62**	*Scutellaria_scharistanica*_0,02mg/ml	53	41
30	EAGC_0,1mg/ml_Starvation	79	13	**63**	*Scutellaria_scharistanica*_0,1mg/ml	50	29
31	EFHA_0,1mg/ml_Starvation	100	7	**64**	*Schrophullaria_sp*_0,02mg/ml	57	49
32	EAGSS_0,02mg/ml	87	39	**65**	*Schrophullaria_sp*_0,1mg/ml	92	46
33	EAGSS_0,1mg/ml	109	39	**66**	*Leonurus_panzeroides*_0,1mg/ml_Starvation	93	34

Table A8. List of the treatments producing a mortality rate lower than 50% after 2 hours but greater than the threshold value after 20 hours since treatments administration.

Treatments Toxic after 2 hours and Not Toxic after 20 hours							
#	**Treatment**	**% living cells after 2h**	**% living cells after 20h**	**#**	**Treatment**	**% living cells after 2h**	**% living cells after 20h**
1	EAGMA_0,01mg/ml_Starvation	36	88	**8**	EAGCB_0,01mg/ml	49	80
2	EAGAT_0,01mg/ml_Starvation	32	81	**9**	EAGCB_0,02mg/ml_Starvation	41	57
3	ERPS_0,01mg/ml_Starvation	43	58	**10**	*Stachys_hissarica*_0,1mg/ml_Starvation	24	60
4	ERHA_0,02mg/ml_Starvation	47	59	**11**	*Stachys_betoniciflora*_0,05mg/ml_Starvation	46	165
5	EAGAA_0,05mg/ml_Starvation	36	59	**12**	*Phlomis_sewertzovii*_0,1mg/ml	43	87
6	EFHA_0,01mg/ml_Starvation	35	90	**13**	*Scutellaria_scharistanica*_0,05mg/ml	48	59
7	EFIPS_0,02mg/ml_Starvation	40	93	**14**	*Leonurus_panzeroides*_0,05mg/ml	49	52

Table A9. List of the treatments producing a mortality rate greater than 50% after 2 hours but lower than the threshold value after 20 hours since treatments administration, and associated percentages of living cells detected after both treatments time durations.

Not Toxic Treatments after both 2 and 20 hours							
#	Treatment	% living cells after 2h	% living cells after 20h	#	Treatment	% living cells after 2h	% living cells after 20h
1	Ctrl	100	100	**66**	EAGAS_0,02mg/ml_Starvation	77	121
2	CtrlDMSO	107	93	**67**	EAGAS_0,05mg/ml_Starvation	79	113
3	Ctrl_Starvation	100	100	**68**	EAGAS_0,1mg/ml_Starvation	73	212
4	CtrlDMSO_Starvation	87	97	**69**	EAGAT_0,01mg/ml	91	81
5	EAGRC_0,01mg/ml	102	104	**70**	EAGAT_0,02mg/ml	74	87
6	EAGRC_0,02mg/ml	90	98	**71**	EAGAT_0,05mg/ml	60	80
7	EAGRC_0,05mg/ml	69	106	**72**	EAGAT_0,1mg/ml	78	91
8	EAGRC_0,1mg/ml	88	96	**73**	EAGAT_0,02mg/ml_Starvation	73	107
9	EAGRC_0,01mg/ml_Starvation	99	134	**74**	EAGAT_0,05mg/ml_Starvation	62	81
10	EAGRC_0,02mg/ml_Starvation	106	123	**75**	EAGAT_0,1mg/ml_Starvation	69	113
11	EAGRC_0,05mg/ml_Starvation	94	119	**76**	EAGAU_0,01mg/ml	85	88
12	ERAAlop_0,01mg/ml	88	85	**77**	EAGAU_0,02mg/ml	81	83
13	ERAAlop_0,02mg/ml	90	73	**78**	EAGAU_0,05mg/ml	77	118
14	ERAAlop_0,05mg/ml	75	62	**79**	EAGAU_0,1mg/ml	70	68
15	ERAAlop_0,01mg/ml_Starvation	60	83	**80**	EAGAU_0,01mg/ml_Starvation	93	88
16	ERAAlop_0,02mg/ml_Starvation	67	100	**81**	EAGAU_0,02mg/ml_Starvation	73	72
17	ERAAlop_0,05mg/ml_Starvation	67	62	**82**	EAGAU_0,05mg/ml_Starvation	78	104
18	ERAAlop_0,1mg/ml_Starvation	51	68	**83**	EAGAU_0,1mg/ml_Starvation	82	72
19	EAGPS_0,01mg/ml	98	104	**84**	ERPS_0,01mg/ml	72	91
20	EAGPS_0,02mg/ml	95	91	**85**	ERPS_0,02mg/ml	69	70
21	EAGPS_0,05mg/ml	90	84	**86**	ERPS_0,05mg/ml	60	78
22	EAGPS_0,1mg/ml	84	88	**87**	EFIAAlop_0,01mg/ml	102	91
23	EAGPS_0,01mg/ml_Starvation	100	180	**88**	EFIAAlop_0,02mg/ml	86	76
24	EAGPS_0,02mg/ml_Starvation	100	122	**89**	EFIAAlop_0,05mg/ml	85	93
25	EAGPS_0,05mg/ml_Starvation	55	127	**90**	EFIAAlop_0,1mg/ml	79	60
26	EAGPS_0,1mg/ml_Starvation	66	72	**91**	EFIAAlop_0,01mg/ml_Starvation	95	86
27	EAGAAsia_0,01mg/ml	83	75	**92**	EFIAAlop_0,02mg/ml_Starvation	97	75
28	EAGAAsia_0,02mg/ml	78	93	**93**	EFIAAlop_0,05mg/ml_Starvation	87	69
29	EAGAAsia_0,05mg/ml	72	69	**94**	EFIAAlop_0,1mg/ml_Starvation	123	117
30	EAGAAsia_0,1mg/ml	72	58	**95**	ERHA_0,01mg/ml	113	75
31	EAGAAsia_0,01mg/ml_Starvation	61	59	**96**	ERHA_0,02mg/ml	115	53
32	EAGAAlop_0,01mg/ml	101	105	**97**	ERHA_0,1mg/ml	83	53
33	EAGAAlop_0,02mg/ml	77	88	**98**	ERHA_0,01mg/ml_Starvation	144	57
34	EAGAAlop_0,05mg/ml	80	98	**99**	EAGAF_0,01mg/ml	61	122
35	EAGAAlop_0,1mg/ml	79	109	**100**	EAGAF_0,02mg/ml	114	92
36	EAGAAlop_0,01mg/ml_Starvation	83	133	**101**	EAGAF_0,05mg/ml	86	82
37	EAGAAlop_0,02mg/ml_Starvation	85	222	**102**	EAGAF_0,1mg/ml	67	113
38	EAGAAlop_0,05mg/ml_Starvation	66	88	**103**	EAGAF_0,01mg/ml_Starvation	95	83
39	EAGAAlop_0,1mg/ml_Starvation	83	51	**104**	EAGAF_0,02mg/ml_Starvation	65	111
40	EAGMA_0,01mg/ml	109	62	**105**	EAGAA_0,01mg/ml	105	66
41	EAGMA_0,02mg/ml	83	68	**106**	EAGAA_0,05mg/ml	76	57
42	EAGMA_0,05mg/ml	74	84	**107**	EAGAA_0,1mg/ml	85	76
43	EAGMA_0,1mg/ml	73	81	**108**	EAGAA_0,01mg/ml_Starvation	77	80
44	EAGMA_0,02mg/ml_Starvation	68	85	**109**	EAGAA_0,02mg/ml_Starvation	101	58
45	EAGMA_0,05mg/ml_Starvation	57	74	**110**	EAGFO_0,01mg/ml	102	88
46	EAGMA_0,1mg/ml_Starvation	58	84	**111**	EAGFO_0,02mg/ml	71	50
47	EAGMD_0,01mg/ml	96	84	**112**	EAGFO_0,01mg/ml_Starvation	54	58
48	EAGMD_0,02mg/ml	84	81	**113**	EAGPD_0,01mg/ml	108	70
49	EAGMD_0,05mg/ml	81	77	**114**	EAGPD_0,02mg/ml	108	70
50	EAGMD_0,1mg/ml	83	67	**115**	EAGPD_0,01mg/ml_Starvation	135	68
51	EAGMD_0,01mg/ml_Starvation	94	95	**116**	EAGPD_0,02mg/ml_Starvation	84	62
52	EAGMD_0,02mg/ml_Starvation	109	107	**117**	EAGTM_0,01mg/ml	71	84
53	EAGMD_0,05mg/ml_Starvation	77	75	**118**	EAGTM_0,02mg/ml	105	67
54	EAGKL_0,01mg/ml	76	86	**119**	EAGTM_0,05mg/ml	94	60
55	EAGKL_0,02mg/ml	79	80	**120**	EAGTM_0,1mg/ml	62	58
56	EAGKL_0,05mg/ml	92	74	**121**	EAGTM_0,02mg/ml_Starvation	62	105
57	EAGKL_0,1mg/ml	63	78	**122**	EAGTM_0,05mg/ml_Starvation	137	70
58	EAGKL_0,01mg/ml_Starvation	51	60	**123**	EFHA_0,01mg/ml	95	75
59	EAGKL_0,02mg/ml_Starvation	79	103	**124**	EFHA_0,02mg/ml	83	61
60	EAGKL_0,05mg/ml_Starvation	83	74	**125**	EFHA_0,05mg/ml	83	95
61	EAGAS_0,01mg/ml	99	92	**126**	EFHA_0,1mg/ml	95	65
62	EAGAS_0,02mg/ml	79	85	**127**	EFHA_0,02mg/ml_Starvation	100	106
63	EAGAS_0,05mg/ml	76	88	**128**	EFHA_0,05mg/ml_Starvation	68	103
64	EAGAS_0,1mg/ml	83	72	**129**	EAGSS_0,01mg/ml	113	68
65	EAGAS_0,01mg/ml_Starvation	113	110	**130**	EAGSS_0,05mg/ml	73	59

Not Toxic Treatments after both 2 and 20 hours							
#	Treatment	% living cells after 2h	% living cells after 20h	#	Treatment	% living cells after 2h	% living cells after 20h
131	EAGSS_0,01mg/ml_Starvation	76	82	**196**	T-1C_0,01mg/ml	69	77
132	EAGSS_0,05mg/ml_Starvation	99	66	**197**	T-1C_0,02mg/ml	63	82
133	EAGSS_0,1mg/ml_Starvation	107	61	**198**	T-1C_0,05mg/ml	88	70
134	EFIPS_0,01mg/ml	70	87	**199**	T-1C_0,01mg/ml_Starvation	85	98
135	EFIPS_0,05mg/ml	75	70	**200**	T-1C_0,02mg/ml_Starvation	82	81
136	EFIPS_0,01mg/ml_Starvation	119	79	**201**	T-1C_0,05mg/ml_Starvation	86	84
137	EAGCB_0,05mg/ml	69	50	**202**	*Verbascum_blattaria*_0,01mg/ml	81	94
138	EAGCB_0,1mg/ml	59	65	**203**	*Verbascum_blattaria*_0,02mg/ml	97	94
139	EAGCB_0,1mg/ml_Starvation	56	52	**204**	*Verbascum_blattaria*_0,05mg/ml	96	81
140	EAGCT_0,01mg/ml	104	90	**205**	*Verbascum_blattaria*_0,1mg/ml	70	56
141	EAGCT_0,02mg/ml	82	95	**206**	*Verbascum_blattaria*_0,01mg/ml_Starvation	109	112
142	EAGCT_0,05mg/ml	88	84	**207**	*Verbascum_blattaria*_0,02mg/ml_Starvation	102	103
143	EAGCT_0,1mg/ml	72	83	**208**	*Verbascum_blattaria*_0,05mg/ml_Starvation	65	82
144	EAGCT_0,01mg/ml_Starvation	91	74	**209**	*Stachys_hissarica*_0,01mg/ml	91	112
145	EAGCT_0,02mg/ml_Starvation	92	82	**210**	*Stachys_hissarica*_0,02mg/ml	85	82
146	EAGCT_0,05mg/ml_Starvation	72	55	**211**	*Stachys_hissarica*_0,05mg/ml	51	73
147	EAGOT_0,01mg/ml	98	92	**212**	*Stachys_hissarica*_0,01mg/ml_Starvation	106	106
148	EAGOT_0,02mg/ml	98	99	**213**	*Stachys_hissarica*_0,02mg/ml_Starvation	124	84
149	EAGOT_0,05mg/ml	88	101	**214**	*Stachys_hissarica*_0,05mg/ml_Starvation	82	91
150	EAGOT_0,1mg/ml	89	89	**215**	*Verbascum_songoricum*_0,01mg/ml	84	91
151	EAGOT_0,01mg/ml_Starvation	102	106	**216**	*Verbascum_songoricum*_0,02mg/ml	105	88
152	EAGOT_0,02mg/ml_Starvation	103	111	**217**	*Verbascum_songoricum*_0,05mg/ml	60	84
153	EAGOT_0,05mg/ml_Starvation	97	93	**218**	*Verbascum_songoricum*_0,01mg/ml_Starvation	107	154
154	EAGOT_0,1mg/ml_Starvation	100	103	**219**	*Verbascum_songoricum*_0,02mg/ml_Starvation	103	103
155	ERKL_0,01mg/ml	98	95	**220**	*Verbascum_songoricum*_0,05mg/ml_Starvation	59	58
156	ERKL_0,02mg/ml	88	94	**221**	*Stachys_betoniciflora*_0,01mg/ml	82	93
157	ERKL_0,05mg/ml	87	53	**222**	*Stachys_betoniciflora*_0,02mg/ml	88	104
158	ERKL_0,01mg/ml_Starvation	100	84	**223**	*Stachys_betoniciflora*_0,05mg/ml	78	90
159	ERKL_0,02mg/ml_Starvation	98	79	**224**	*Stachys_betoniciflora*_0,1mg/ml	79	111
160	ERKS_0,01mg/ml	96	90	**225**	*Stachys_betoniciflora*_0,01mg/ml_Starvation	142	110
161	ERKS_0,02mg/ml	87	91	**226**	*Stachys_betoniciflora*_0,02mg/ml_Starvation	104	133
162	ERKS_0,05mg/ml	77	86	**227**	*Stachys_betoniciflora*_0,1mg/ml_Starvation	107	56
163	ERKS_0,1mg/ml	81	84	**228**	*Phlomis_sewertzovii*_0,01mg/ml	71	113
164	ERKS_0,01mg/ml_Starvation	78	108	**229**	*Phlomis_sewertzovii*_0,02mg/ml	63	102
165	ERKS_0,02mg/ml_Starvation	84	103	**230**	*Phlomis_sewertzovii*_0,05mg/ml	76	95
166	ERKS_0,05mg/ml_Starvation	75	95	**231**	*Phlomis_sewertzovii*_0,01mg/ml_Starvation	132	92
167	ERKS_0,1mg/ml_Starvation	79	85	**232**	*Phlomis_sewertzovii*_0,02mg/ml_Starvation	120	126
168	ESHA_0,01mg/ml	98	96	**233**	*Phlomis_sewertzovii*_0,05mg/ml_Starvation	91	117
169	ESHA_0,02mg/ml	83	88	**234**	*Phlomis_sewertzovii*_0,1mg/ml_Starvation	75	50
170	ESHA_0,05mg/ml	92	94	**235**	*Phlomis_salicifolia*_0,01mg/ml	78	94
171	ESHA_0,1mg/ml	78	89	**236**	*Phlomis_salicifolia*_0,02mg/ml	70	99
172	ESHA_0,01mg/ml_Starvation	91	99	**237**	*Phlomis_salicifolia*_0,05mg/ml	83	96
173	ESHA_0,02mg/ml_Starvation	84	95	**238**	*Phlomis_salicifolia*_0,1mg/ml	55	67
174	ESHA_0,05mg/ml_Starvation	85	87	**239**	*Phlomis_salicifolia*_0,01mg/ml_Starvation	114	111
175	ESHA_0,1mg/ml_Starvation	93	80	**240**	*Phlomis_salicifolia*_0,02mg/ml_Starvation	139	115
176	U-2C_0,01mg/ml	78	97	**241**	*Phlomis_salicifolia*_0,05mg/ml_Starvation	59	122
177	U-2C_0,02mg/ml	87	82	**242**	*Phlomis_salicifolia*_0,1mg/ml_Starvation	55	95
178	U-2C_0,05mg/ml	90	77	**243**	*Silene_oreina*_0,01mg/ml	54	72
179	U-2C_0,1mg/ml	85	87	**244**	*Silene_oreina*_0,02mg/ml	74	79
180	U-2C_0,01mg/ml_Starvation	108	90	**245**	*Silene_oreina*_0,05mg/ml	56	76
181	U-2C_0,02mg/ml_Starvation	94	80	**246**	*Silene_oreina*_0,1mg/ml	88	61
182	U-2C_0,05mg/ml_Starvation	97	90	**247**	*Silene_oreina*_0,01mg/ml_Starvation	123	101
183	U-2C_0,1mg/ml_Starvation	94	70	**248**	*Silene_oreina*_0,02mg/ml_Starvation	113	118
184	C-3B_0,01mg/ml	63	82	**249**	*Silene_oreina*_0,05mg/ml_Starvation	95	78
185	C-3B_0,02mg/ml	65	79	**250**	*Phlomis_tadschikistanica*_0,01mg/ml	90	90
186	C-3B_0,05mg/ml	78	66	**251**	*Phlomis_tadschikistanica*_0,02mg/ml	97	76
187	C-3B_0,1mg/ml	72	66	**252**	*Phlomis_tadschikistanica*_0,05mg/ml	63	70
188	C-3B_0,01mg/ml_Starvation	57	82	**253**	*Phlomis_tadschikistanica*_0,1mg/ml	108	57
189	C-3B_0,02mg/ml_Starvation	82	70	**254**	*Phlomis_tadschikistanica*_0,01mg/ml_Starvation	128	100
190	C-3B_0,05mg/ml_Starvation	76	57	**255**	*Phlomis_tadschikistanica*_0,02mg/ml_Starvation	103	116
191	C-4B_0,01mg/ml	59	88	**256**	*Phlomis_tadschikistanica*_0,05mg/ml_Starvation	120	109
192	C-4B_0,02mg/ml	63	80	**257**	*Phlomis_tadschikistanica*_0,1mg/ml_Starvation	85	76
193	C-4B_0,01mg/ml_Starvation	71	71	**258**	*Cousina_umbrosa*_0,01mg/ml	61	62
194	C-4B_0,02mg/ml_Starvation	68	64	**259**	*Cousina_umbrosa*_0,02mg/ml	53	50
195	T-1B_0,01mg/ml	67	58	**260**	*Cousina_umbrosa*_0,1mg/ml	62	51

Not Toxic Treatments after both 2 and 20 hours							
#	Treatment	% living cells after 2h	% living cells after 20h	#	Treatment	% living cells after 2h	% living cells after 20h
261	*Cousina_umbrosa*_0,02mg/ml_Starvation	111	62	**274**	*Scutellaria_scharistanica*_0,02mg/ml_Starvation	102	82
262	*Cousina_umbrosa*_0,05mg/ml_Starvation	77	58	**275**	*Scutellaria_scharistanica*_0,05mg/ml_Starvation	105	63
263	*Nepeta_olgae*_0,01mg/ml	51	84	**276**	*Scutellaria_scharistanica*_0,1mg/ml_Starvation	64	98
264	*Nepeta_olgae*_0,02mg/ml	65	78	**277**	*Schrophullaria_sp*_0,01mg/ml	63	54
265	*Nepeta_olgae*_0,05mg/ml	115	64	**278**	*Schrophullaria_sp*_0,05mg/ml	73	64
266	*Nepeta_olgae*_0,1mg/ml	74	82	**279**	*Schrophullaria_sp*_0,01mg/ml_Starvation	97	103
267	*Nepeta_olgae*_0,01mg/ml_Starvation	141	103	**280**	*Schrophullaria_sp*_0,02mg/ml_Starvation	115	70
268	*Nepeta_olgae*_0,02mg/ml_Starvation	130	110	**281**	*Leonurus_panzeroides*_0,01mg/ml	59	69
269	*Nepeta_olgae*_0,05mg/ml_Starvation	94	97	**282**	*Leonurus_panzeroides*_0,02mg/ml	87	61
270	*Nepeta_olgae*_0,1mg/ml_Starvation	95	81	**283**	*Leonurus_panzeroides*_0,1mg/ml	85	72
271	*Phlomis_tadschikistanica*_0,1mg/ml_Starvation	85	76	**284**	*Leonurus_panzeroides*_0,01mg/ml_Starvation	128	90
272	*Scutellaria_scharistanica*_0,01mg/ml	57	59	**285**	*Leonurus_panzeroides*_0,02mg/ml_Starvation	112	85
273	*Scutellaria_scharistanica*_0,01mg/ml_Starvation	91	107				

Table A10. List of treatments resulting to produce a toxicity rate lower then 50% after both 2 and 20 hours, and associated percentages of living cells detected after both treatments time durations.

Treatments Administrated in Normal Conditions Producing Effects Similar to Starvation Controls After Both 2 and 20 Hours							
#	Treatments	2 hours			20 hours		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	Ctrl_Starvation	100	-0.165226692502	-0.371287249214	100	-0.810463832901	-0.319616223058
2	CtrlDMSO_Starvation	87	-0.0135777168692	-0.679626063698	97	-0.827515568979	-0.306822191736
3	EAGRC_0,05mg/ml	69	-0.275357491314	-0.312774938657	106	-0.849704558553	-0.0436337442209
4	EAGRC_0,1mg/ml	88	-0.5558911338	-0.234797674283	96	-0.928187373233	-0.0811237117396
5	ERAAlop_0,01mg/ml	88	-0.1201675203	0.00139901186479	85	-0.757075783636	-0.297389822575
6	ERAAlop_0,02mg/ml	90	-0.233364995771	0.178485473511	73	-0.689506788122	-0.208636836742
7	ERAAlop_0,05mg/ml	75	-0.220610542646	-0.512011330907	62	-0.574087040866	-0.310892470616
8	EAGPS_0,01mg/ml	98	-0.551846935979	0.153050798578	104	-0.789549767346	-0.403541654985
9	EAGPS_0,02mg/ml	95	-0.625470450879	0.114711885815	91	-0.921950149432	-0.780062263907
10	EAGAAsia_0,01mg/ml	83	-0.478221020828	-0.0444047363412	75	-0.777510606885	-0.0845320384589
11	EAGAAsia_0,02mg/ml	78	-0.150914009914	-0.379391683332	93	-0.681941540103	-0.049247941123
12	EAGAAsia_0,05mg/ml	72	-0.426752584441	-0.43772649281	69	-0.364490077562	-0.266099155727
13	EAGAAsia_0,1mg/ml	72	-0.299749595646	-0.25785792765	58	-0.909088926465	0.246904176388
14	EAGAAlop_0,01mg/ml	101	-0.559833657737	0.0252942235056	105	-0.783719010703	-0.119538906314
15	EAGAAlop_0,02mg/ml	77	-0.333449446251	-0.0956542110293	88	-0.715005522091	-0.15781076992
16	EAGAAlop_0,05mg/ml	80	-0.276876493074	-0.241368267353	98	-0.739965821899	-0.0688232050534
17	EAGAAlop_0,1mg/ml	79	-0.305271812764	-0.0464816708846	109	-0.805497561563	-0.0895235734856
18	EAGMA_0,01mg/ml	109	-0.40919622174	-0.0970817668217	62	-0.956801786283	0.0100225160541
19	EAGMA_0,02mg/ml	83	-0.124620012922	-0.697780403124	68	-0.967798776566	-0.0981199356164
20	EAGMA_0,05mg/ml	74	-0.429450947532	-0.724800248085	84	-1.00707671449	0.0310816769927
21	EAGMA_0,1mg/ml	73	0.0468738134012	-0.640971898884	81	-1.17852498263	0.00222631306232
22	EAGMD_0,01mg/ml	96	-0.505991512849	-0.113452315959	84	-0.967046856225	-0.0965128215608
23	EAGMD_0,02mg/ml	84	-0.399832887603	-0.214831104797	81	-0.928363801043	-0.0974182599989
24	EAGMD_0,05mg/ml	81	-0.209898262171	-0.648704974465	77	-0.896543628433	-0.0376976537139
25	EAGMD_0,1mg/ml	83	-0.429100045394	-0.727722345774	67	-0.914094513913	-0.00295816780436
26	EAGKL_0,01mg/ml	76	-0.233314241729	-0.601539215756	86	-0.705587571493	-0.226382454625
27	EAGKL_0,02mg/ml	79	-0.0370867767514	-0.479175208866	80	-0.690981248195	-0.129650978092
28	EAGKL_0,05mg/ml	92	-0.172979101179	-0.699559726521	74	-0.664874446065	-0.163006067767
29	EAGKL_0,1mg/ml	63	-0.00760842400915	-0.66111201292	78	-0.74991000206	-0.0613191175331
30	EAGAS_0,01mg/ml	99	-0.461803970823	0.0511446932001	92	-0.848211727621	-0.128493043699
31	EAGAS_0,02mg/ml	79	-0.121876190039	0.0336380559058	85	-0.687260463133	-0.192328555261
32	EAGAS_0,05mg/ml	76	-0.437799976345	-0.377730942404	88	-0.735985742018	-0.239292212761
33	EAGAS_0,1mg/ml	83	-0.246427645555	-0.267797310077	72	-0.832448640928	-0.281889811273
34	EAGAU_0,02mg/ml	81	0.629190843568	-0.615891444509	83	-0.779241352006	-0.107088965144
35	ERPS_0,01mg/ml	72	-0.232066124701	-0.372039875966	91	-0.644038254629	-0.267147103177
36	ERPS_0,02mg/ml	69	-0.0418562311044	-0.439316880901	70	-0.875047662193	-0.0874285569761
37	ERPS_0,05mg/ml	60	-0.301184663146	-0.35499984291	78	-0.765007201659	-0.265606432277
38	ERKL_0,02mg/ml	88	-0.885204570184	-0.271048625314	94	-0.603461183263	0.235738065618
39	ERKL_0,05mg/ml	87	-0.980418943378	-0.395476843612	53	-0.73157733332	0.25221653525
40	*Silene_oreina*_0,01mg/ml	54	-0.17610470348	-0.317243201448	72	-0.66686686247	0.0493585507741
41	*Silene_oreina*_0,02mg/ml	74	-0.193473670873	-0.391690457796	79	-0.593420032067	0.166946256849
42	*Silene_oreina*_0,05mg/ml	56	-0.0950188053986	-0.530732811274	76	-0.664773825695	0.0930176346626
43	*Silene_oreina*_0,1mg/ml	88	-0.267039626554	-0.222590571624	61	-0.81030154915	-0.0327132765317

Table A11. List of treatments administrated in normal culturing conditions and producing an effects comparable to starvation controls after both 2 and 20 hours, as described in figure 36.

Treatments Administrated in Starvation Conditions Producing Effects Similar to Normal Controls After Both 2 and 20 Hours							
#	Treatments	2 hours			20 hours		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	Ctrl	100	-1.04335413742	0.839364068725	100	-0.462332915708	0.410375379506
2	CtrlDMSO	107	-1.10850318842	0.897022520147	93	-0.443799259264	0.420230130368
3	C-3B_0,02mg/ml_Starvation	82	0.515308009539	0.984502724946	70	-0.110843737059	0.633147126576

Table A12. List of treatments administrated in starvation culturing conditions and producing an effects comparable to normal controls after both 2 and 20 hours, as described in figure 37.

Treatments Producing Effects Different from Both Control Samples After Both 2 and 20 Hours Independently from The Culturing Conditions							
#	Treatments	2 hours			20 hours		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	EAGKL_0,05mg/ml_Starvation	83	1.13874598801	-0.82786584432	74	0.61516706707	-2.12058925857

Table A13. List of treatments producing an effects different from either normal and starvation controls after both 2 and 20 hours, as described in figure 38.

Treatments Producing Not Interesting Effects Independently from the culturing conditions After Both 2 and 20 Hours							
#	Treatments	2 hours			20 hours		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	Ctrl	100	-1.04335413742	0.839364068725	100	-0.462332915708	0.410375379506
2	CtrlDMSO	107	-1.10850318842	0.897022520147	93	-0.443799259264	0.420230130368
3	EFIAAlop_0,01mg/ml	102	-0.904611208563	1.03003246002	91	-0.50855957856	0.709917532922
4	EFIAAlop_0,02mg/ml	86	-1.0854714113	1.01604724431	76	-0.14642014422	1.13018774203
5	EFIAAlop_0,05mg/ml	85	-1.15507687995	1.20390296261	93	-0.42892271518	0.711724289432
6	EFIAAlop_0,1mg/ml	79	-1.11334018484	0.470134208102	60	-0.381963953947	0.425478917631
7	ERHA_0,01mg/ml	113	-1.16369699196	0.674144645741	75	-0.517192943813	0.898397644868
8	ERHA_0,02mg/ml	115	-1.16635980951	0.673895514039	53	-0.238294784784	1.02394916405
9	ERHA_0,1mg/ml	83	-1.12524649543	1.04641885678	53	-0.752764559088	0.522946917309
10	EAGAF_0,01mg/ml	61	-1.12273068481	0.691238164154	122	-0.516270067733	0.751230293529
11	EAGAF_0,02mg/ml	114	-1.19492178781	1.30841039732	92	-0.36178267468	0.887021524172
12	EAGAF_0,05mg/ml	86	-0.969712248422	1.3651337605	82	-0.545232735349	0.808662723007
13	EAGAF_0,1mg/ml	67	-1.19922929229	0.488671329649	113	-0.270701396047	0.750782588789
14	EAGAA_0,01mg/ml	105	-1.0093522106	0.680466369341	66	-0.464706814047	0.88606178469
15	EAGAA_0,05mg/ml	76	-1.14819160758	0.711229712077	57	-0.41791476131	1.09871484817
16	EAGAA_0,1mg/ml	85	-1.028264054	0.884736051197	76	-0.228547417373	1.10217751354
17	EAGFO_0,01mg/ml	102	-1.29703016701	0.867969401831	88	-0.610680922875	0.488594080056
18	EAGFO_0,02mg/ml	71	-1.14272207535	0.980791958657	50	-0.121999000882	1.35279744349
19	EAGPD_0,02mg/ml	108	-0.932171302329	0.313439522265	70	-0.362779094147	0.478417063439
20	EAGTM_0,01mg/ml	71	-1.08298247585	0.635593985338	84	-0.373288791806	0.748920750118
21	EAGTM_0,02mg/ml	105	-0.952263521303	0.676025083115	67	0.188269948318	0.940718362422
22	EAGTM_0,05mg/ml	94	-0.994997302797	0.453294982514	60	-0.100187726814	0.297714335031
23	EAGTM_0,1mg/ml	62	-0.86498643468	0.293535477566	58	0.232915641856	1.08594830204
24	EFHA_0,01mg/ml	95	-0.963365827501	0.858762136361	75	-0.601691119595	0.447198629619
25	EFHA_0,02mg/ml	83	-1.01921240844	0.800695898504	61	-0.504538003195	0.605274031706
26	EFHA_0,05mg/ml	83	-1.00629863083	0.571867444771	95	-0.583593100405	0.376970266301
27	EFHA_0,1mg/ml	95	-1.04625457241	0.526102680248	65	-0.319498332592	0.773985143033
28	EAGSS_0,01mg/ml	113	-0.96415465925	0.32767771152	68	-0.571408752455	0.395987742329
29	EAGSS_0,05mg/ml	73	-0.949716549475	0.366469488336	59	-0.612437982029	0.450812245351
30	EFIPS_0,01mg/ml	70	-1.10034506226	0.572050952445	87	-0.580144152548	0.371628500391
31	EFIPS_0,05mg/ml	75	-0.939524455081	0.285453327391	70	-0.339405898009	0.995282355848
32	EAGCB_0,1mg/ml	59	-0.957093496155	0.249383572022	65	0.163331149728	0.616843600657
33	EAGCT_0,01mg/ml	104	-1.18304754379	1.4653039026	90	0.0586368408872	1.15432967885
34	EAGCT_0,02mg/ml	82	-1.06168832006	0.742235613609	95	0.492132222482	1.35729860798
35	EAGCT_0,05mg/ml	88	-1.19686487893	0.91487350932	84	0.245711735413	1.30309068688
36	EAGCT_0,1mg/ml	72	-1.06893055518	1.19578256763	83	0.598017834427	1.52779081334
37	EAGOT_0,01mg/ml	98	-1.26063171203	1.05760656937	92	0.53686773345	1.12025370086
38	EAGOT_0,02mg/ml	98	-1.08360883353	0.698927795714	99	1.19186448071	1.46691417479
39	EAGOT_0,05mg/ml	88	-1.09636469093	0.983374565694	101	0.97309553673	1.52344481927
40	EAGOT_0,1mg/ml	89	-1.05687235651	0.704047169045	89	0.825758206606	1.42114988207
41	ERKS_0,01mg/ml	96	-1.2137647911	0.674522577126	90	0.149238116363	0.979026680246
42	ERKS_0,02mg/ml	87	-1.13341433034	0.326968582117	91	0.00321316806138	0.853463896262
43	ERKS_0,05mg/ml	77	-1.0395865722	0.570010972255	86	-0.414109197573	0.644853694697
44	ESHA_0,01mg/ml	98	-1.15480946763	1.16023091937	96	-0.143736379703	1.08516439968
45	ESHA_0,02mg/ml	83	-0.990067927955	0.804160107399	88	0.161773080723	1.09124339207
46	ESHA_0,05mg/ml	92	-1.04899015872	0.7074418251	94	0.0620445831785	1.13878360806
47	ESHA_0,1mg/ml	78	-1.07271204054	0.856800400629	89	0.258632430651	1.14620030173
48	U-2C_0,01mg/ml	78	-0.231390812552	2.49394264849	97	0.0459052941315	0.999536456268
49	U-2C_0,02mg/ml	87	-0.668031367434	1.29053684034	82	0.00404920232512	0.945507662858
50	U-2C_0,05mg/ml	90	-1.02403134433	0.81565155244	77	-0.0084278186583	1.05520979622
51	U-2C_0,1mg/ml	85	-1.03467952632	0.853586850015	87	0.304350863065	1.27405021153
52	C-3B_0,01mg/ml	63	-0.908729998453	1.36548094133	82	0.209268013543	1.08039210078
53	C-3B_0,02mg/ml	65	-0.630721811883	1.37109044999	79	0.342262455813	1.14279792551
54	C-3B_0,05mg/ml	78	-0.988409972393	1.03575811377	66	0.453521046823	1.47393336569
55	C-3B_0,1mg/ml	72	-1.11751277453	2.00351006176	66	1.16929885062	1.57211374936
56	C-4B_0,01mg/ml	59	-0.363201578074	1.83197725291	88	-0.327793741059	0.672474982034
57	C-4B_0,02mg/ml	63	-0.888619417594	0.852697591061	80	-0.2398710424	0.740387808669
58	T-1B_0,01mg/ml	67	-0.697180295332	0.405341072522	58	-0.20080306149	0.906316138669
59	T-1C_0,01mg/ml	69	-0.669804074408	0.70263124393	77	-0.0558634840033	0.805792157435
60	T-1C_0,05mg/ml	88	-0.893216597821	0.418465825513	70	-0.254719216351	0.697332259966
61	*Verbascum_blattaria*_0,01mg/ml	81	-0.961462720502	1.25753963484	94	-0.278109025786	0.766549291351
62	*Verbascum_blattaria*_0,02mg/ml	97	-1.09490689276	1.0138926622	94	-0.428848572871	0.685887905967
63	*Verbascum_blattaria*_0,05mg/ml	96	-1.08578516672	1.48037445376	81	-0.565976217921	0.739011491606
64	*Verbascum_blattaria*_0,1mg/ml	70	-1.17465170675	1.16928839583	56	-0.168956526032	1.04627705105
65	*Stachys_hissarica*_0,01mg/ml	91	-0.94782947574	0.650902944467	112	0.651996073746	1.35123586493

Treatments Producing Not Interesting Effects Independently from the culturing conditions After Both 2 and 20 Hours							
#	Treatments	2 hours			20 hours		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
66	*Stachys_hissarica*_0,02mg/ml	85	-0.870092120736	1.22222322442	82	0.520947998551	1.38676066949
67	*Stachys_hissarica*_0,05mg/ml	51	-0.874508978023	1.14705881337	73	0.594825682795	1.52339003484
68	*Verbascum_songoricum*_0,01mg/ml	84	-0.956294330606	0.51436448722	91	0.341714496184	1.2532853483
69	*Verbascum_songoricum*_0,02mg/ml	105	-0.786660928316	0.52038065249	88	0.300185927487	1.25208576939
70	*Verbascum_songoricum*_0,05mg/ml	60	-0.824826319278	0.749256377789	84	0.2440580744	1.27546692487
71	*Stachys_betoniciflora*_0,01mg/ml	82	-0.605281545302	0.437427782048	93	-0.0527407529089	0.796871125239
72	*Stachys_betoniciflora*_0,02mg/ml	88	-0.489206299629	0.458320251422	104	0.0376827671012	0.911754393476
73	*Stachys_betoniciflora*_0,05mg/ml	78	-0.480594015472	0.705362767779	90	-0.0109529048594	0.859930855811
74	*Stachys_betoniciflora*_0,1mg/ml	79	-0.705722651803	0.694444810975	111	0.329780735189	1.07645302538
75	*Phlomis_tadschikistanica*_0,1mg/ml	108	-0.620758086432	0.282371751034	57	-0.207493443346	0.868866536582
76	*Cousina_umbrosa*_0,01mg/ml	61	0.735484473829	1.65081661041	62	-0.615004301706	0.43330848612
77	Ctrl_Starvation	100	-0.165226692502	-0.371287249214	100	-0.810463832901	-0.319616223058
78	CtrlDMSO_Starvation	87	-0.0135777168692	-0.679626063698	97	-0.827515568979	-0.306822191736
79	EAGRC_0,02mg/ml_Starvation	106	0.120390032864	-0.939934183768	123	-0.701568062172	-0.153725636843
80	EAGRC_0,05mg/ml_Starvation	94	0.379587912505	-0.69225111493	119	-0.553876265916	-0.369854819128
81	EAGAAsia_0,01mg/ml_Starvation	61	0.715842761975	-0.512643584635	59	-0.508520167579	-0.733324074352
82	EAGAAlop_0,02mg/ml_Starvation	85	0.696697664508	-0.616475752524	222	-0.794706820739	-0.06556868374
83	EAGAAlop_0,05mg/ml_Starvation	66	1.26145540033	-0.141264700735	88	-0.600355958084	-0.560960148919
84	EAGAS_0,05mg/ml_Starvation	79	0.763776267882	-0.748197309032	113	-0.658870763552	-0.427630362582
85	EAGAS_0,1mg/ml_Starvation	73	0.907586553829	0.188281220398	212	-0.780496020148	-0.580150014494
86	EAGAT_0,02mg/ml_Starvation	73	0.531926309597	-0.322511871364	107	-0.871931983121	-0.0332752850156
87	EAGAT_0,05mg/ml_Starvation	62	0.396377182889	-0.54785830215	81	-0.680489136918	-0.48863333422
88	EAGAT_0,1mg/ml_Starvation	69	0.659816126694	-0.107300765666	113	-0.854969973941	0.0603897601422
89	EAGAU_0,01mg/ml_Starvation	93	0.335367653229	-0.168304110597	88	-0.698294628581	-0.365024915982
90	EAGAU_0,02mg/ml_Starvation	73	0.209901134975	-0.203231882916	72	-0.684042822787	-0.139694494935
91	EAGAU_0,05mg/ml_Starvation	78	0.406518417709	0.168674312446	104	-0.414505582016	-0.447342879783
92	EAGAU_0,1mg/ml_Starvation	82	0.260671706291	-0.180405904784	72	-0.785812649432	-0.121331147028
93	EFIAAlop_0,01mg/ml_Starvation	95	-0.484247167193	-0.569429439025	86	-0.87979139902	-0.929172224152
94	EFIAAlop_0,02mg/ml_Starvation	97	-0.572373920324	-0.132103649225	75	-0.936790882425	-0.839282401021
95	EFIAAlop_0,05mg/ml_Starvation	87	-0.424546850807	-0.333666374828	69	-1.00702325059	-0.5752287781
96	EFIAAlop_0,1mg/ml_Starvation	123	-0.663143141344	-0.252665795672	117	-0.813693572417	-0.855950308939
97	ERHA_0,01mg/ml_Starvation	144	-0.380178907758	-0.137386087811	57	-0.510853513076	-0.622376326794
98	EAGAF_0,01mg/ml_Starvation	95	-0.00359823534878	0.33562792195	83	-0.89401499158	-0.325502508593
99	EAGAF_0,02mg/ml_Starvation	65	-0.0331630580306	-0.13302634136	111	-0.753176276917	-0.51278735039
100	EAGAA_0,01mg/ml_Starvation	77	-0.439632742068	-0.481080308148	80	-0.686728786832	-0.626692626118
101	EAGAA_0,02mg/ml_Starvation	101	-0.402751684902	-0.337252216569	58	-0.856780146145	-0.669227586315
102	EAGFO_0,01mg/ml_Starvation	54	-0.777281465549	-0.647131398692	58	-1.00300532513	-0.681121020767
103	EAGPD_0,02mg/ml_Starvation	84	0.0299969497648	-0.456934216606	62	-0.604163667909	-0.900031020842
104	EAGTM_0,02mg/ml_Starvation	62	-0.323670800163	-0.658144133682	105	-0.786007507518	-0.964998199047
105	EAGTM_0,05mg/ml_Starvation	137	-0.261070774652	-0.329576203403	70	-0.750260722621	-0.585204293546
106	EFHA_0,02mg/ml_Starvation	100	-0.268898738565	0.0687950958306	106	-0.977688890873	-0.738793146364
107	EFHA_0,05mg/ml_Starvation	68	-0.496557737385	-0.407105323345	103	-0.55549113169	-0.925773075672
108	EAGSS_0,01mg/ml_Starvation	76	-0.202613791963	-0.432619861664	82	-1.14978414526	-0.966357157189
109	EAGSS_0,05mg/ml_Starvation	99	-0.317556477649	-0.334786465776	66	-0.753936605106	-0.835388368535
110	EAGSS_0,1mg/ml_Starvation	107	-0.389952850942	-0.456015220218	61	-0.854776711355	-1.00253009746
111	EFIPS_0,01mg/ml_Starvation	119	-0.497982369461	-0.129645375494	79	-0.666725589907	-1.03337157885
112	EAGCB_0,1mg/ml_Starvation	56	-0.139748609602	-0.204086692826	52	-0.549854876136	-0.904375194774
113	EAGCT_0,01mg/ml_Starvation	91	-0.263557231399	-0.419155164561	74	-0.851456050999	0.0668128846085
114	EAGOT_0,01mg/ml_Starvation	102	-0.253291964445	-0.695573241788	106	-0.611625668495	-0.215166773659
115	EAGOT_0,02mg/ml_Starvation	103	-0.364146364816	-0.631013287087	111	-0.706749256831	-0.0901118728133
116	EAGOT_0,05mg/ml_Starvation	97	-0.158218362507	-0.543936845635	93	-0.647228629994	-0.0774056916685
117	EAGOT_0,1mg/ml_Starvation	100	-0.26442610487	-0.392878969942	103	-0.668167856914	0.104810564339
118	ERKL_0,01mg/ml_Starvation	100	0.026465045235	-0.478317605717	84	-0.867502026421	-0.0829973670335
119	ERKL_0,02mg/ml_Starvation	98	-0.00343736043343	-0.9243025298	79	-0.746271796029	-0.139120034519
120	ERKS_0,01mg/ml_Starvation	78	0.156127566478	-0.751031690074	108	-0.787639600883	-0.365199044708
121	ERKS_0,05mg/ml_Starvation	75	0.418171834377	-0.746993942724	95	-0.799555058807	-0.685211934467
122	ERKS_0,1mg/ml_Starvation	79	0.208860210618	-0.691144554747	85	-0.788302094656	-0.839938013885
123	ESHA_0,01mg/ml_Starvation	91	0.0335542288703	-0.494657932736	99	-0.938598331093	-0.139116890234
124	ESHA_0,02mg/ml_Starvation	84	-0.12458131942	-0.723251811561	95	-0.847613666097	-0.098005542074
125	ESHA_0,05mg/ml_Starvation	85	0.294996150297	-0.660839411531	87	-0.860519033219	-0.161265782339
126	ESHA_0,1mg/ml_Starvation	93	0.0992725436817	-0.576310890996	80	-0.863930046832	-0.203331485394
127	U-2C_0,01mg/ml_Starvation	108	-0.133063893287	-0.351452359297	90	-0.797641433533	-0.107503598623
128	U-2C_0,02mg/ml_Starvation	94	-0.0480130532035	-0.226733689181	80	-0.575974685769	0.0667502765839
129	U-2C_0,05mg/ml_Starvation	97	0.282220514838	-0.0484774079022	90	-0.6209803835	0.0408427282643
130	U-2C_0,1mg/ml_Starvation	94	0.15035781381	-0.396550907406	70	-0.557562802015	-0.108682707411

Treatments Producing Not Interesting Effects Independently from the culturing conditions After Both 2 and 20 Hours							
#	Treatments	2 hours			20 hours		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
131	C-4B_0,01mg/ml_Starvation	71	-0.191693648683	0.0966506864717	71	-0.77904861315	-0.010639793618
132	T-1C_0,01mg/ml_Starvation	85	0.187195643589	-0.392083873116	98	-0.767267449117	-0.301188579014
133	T-1C_0,02mg/ml_Starvation	82	0.399537004271	0.221190373916	81	-0.57669754772	-0.285113513183
134	T-1C_0,05mg/ml_Starvation	86	0.324229477936	-0.379486312394	84	-0.85849822351	-0.0887413453173
135	*Verbascum_blattaria*_0,01mg/ml_Starvation	109	0.700622857611	-0.707134700519	112	-0.756101479977	-0.508724384767
136	*Stachys_hissarica*_0,01mg/ml_Starvation	106	0.590047438662	-0.693070330768	106	-0.679998716634	-0.0991128440301
137	*Stachys_hissarica*_0,02mg/ml_Starvation	124	0.378896616434	-0.540236691748	84	-0.420225763672	-0.153065765277
138	*Stachys_betoniciflora*_0,01mg/ml_Starvation	142	0.553656901092	-0.496208074804	110	-0.553068295926	-0.40325804889
139	*Phlomis_salicifolia*_0,02mg/ml_Starvation	139	0.94484336835	-0.0884393893274	115	-0.681848189292	-0.679092424013

Table A14. List of treatments producing not interesting effects after both 2 and 20 hours since their administration, independently from the culturing conditions, as described in figure 39.

Treatments Administrated in Normal Conditions Producing Effects Considered as Not Interesting After 2 and Interesting After 20 Hours							
#	Treatments	2 hours			20 hours - Ctrl Starvation Like Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	EAGRC_0,01mg/ml	102	-0.751420786794	0.278918480514	104	-0.867802947551	-0.156699534156
2	EAGRC_0,02mg/ml	90	-0.612062306209	0.317656244559	98	-0.848761939931	-0.0878975252566
3	EAGPS_0,05mg/ml	90	-0.646494242198	0.348646655413	84	-0.941146185427	-0.796110236404
4	EAGPD_0,01mg/ml	108	-0.898359289453	0.455851069204	70	-0.501450921343	0.160463683218
5	EAGCB_0,05mg/ml	69	-0.799313317009	0.187587442315	50	-0.553902606238	0.244721337307
6	ERKL_0,01mg/ml	98	-1.17440602054	0.689584236967	95	-0.777195427828	0.251598184689
7	ERKS_0,1mg/ml	81	-0.943495040514	0.558853693882	84	-0.487049673006	-0.0345304840971
#	Treatments	2 hours			20 hours - Other Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
8	EAGPS_0,1mg/ml	84	-0.815011566829	0.710707029355	88	0.12943258018	-1.74370396264

Table A15. List of treatments administrated in normal conditions producing effects considered as not interesting after 2 hours and becoming interesting after 20 hours, because similar to starvation control samples (Ctrl Starvation Like Treatments) or because different from both control samples (Other Treatments).

Treatments Administrated in Starvation Conditions Producing Effects Considered as Not Interesting After 2 and Interesting After 20 Hours							
#	Treatments	2 hours			20 hours - Ctrl Like Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	EAGCT_0,02mg/ml_Starvation	92	-0.415856546334	-0.28574221692	82	-0.652952306093	0.29968295861
2	EAGCT_0,05mg/ml_Starvation	72	-0.22214563042	-0.635154670615	55	-0.308117174495	0.636285241408
3	C-3B_0,05mg/ml_Starvation	76	0.368903292157	0.379411211559	57	0.0387551763615	0.763101711785
4	*Stachys_betoniciflora*_0,1mg/ml_Starvation	107	0.549340029167	-0.183605188635	56	0.42421095314	0.360033984514
#	Treatments	2 hours			20 hours - Other Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
5	EAGPS_0,01mg/ml_Starvation	100	0.401235936176	-0.603513111783	180	0.316720694877	-2.12510934799
6	EAGPS_0,02mg/ml_Starvation	100	-0.110960328484	-0.120235413302	122	0.757789953469	-2.31609718775
7	EAGPS_0,05mg/ml_Starvation	55	0.0204122443841	0.275407806958	127	0.886050571463	-2.60280853359
8	EAGAAlop_0,1mg/ml_Starvation	83	0.892830300118	0.155161112266	51	1.53818337126	-2.71185673708
9	EAGPD_0,01mg/ml_Starvation	135	-0.32360974184	0.146788774694	68	0.669679827352	-2.20016193097
10	ERKS_0,02mg/ml_Starvation	84	-0.11521766897	-0.535518104803	103	0.304251293773	-1.43756268836

Table A16. List of treatments administrated in starvation conditions producing effects considered as not interesting after 2 hours and becoming interesting after 20 hours, because similar to normal control samples (Ctrl Starvation Like Treatments) or because different from both control samples (Other Treatments).

Treatments Administrated in Starvation Conditions Producing Effects Considered as Interesting After 2 and Producing Effects Different Form Both Control Samples Taken as References or Toxic After 20 Hours							
#	Treatments	2 hours			20 hours - Other Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	EAGPS_0,1mg/ml_Starvation	66	0.0331223168076	0.835518866459	72	1.42692373862	-2.69559401665
#	Treatments	2 hours			20 hours - Toxic Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
2	EFIPS_0,1mg/ml_Starvation	59	-0.17982940221	0.719007946889	6	6.37374866205	0.394161048167
3	EAGCB_0,05mg/ml_Starvation	68	0.0807563682876	0.934383100632	48	-0.859701651227	-0.745293636915
4	C-3B_0,1mg/ml_Starvation	71	1.0515819269	1.80803358382	34	-0.141707339711	0.357027412728
5	C-4B_0,05mg/ml_Starvation	71	0.301213903815	1.1418797022	15	-0.121824349982	0.787524755466
6	*Cousina umbrosa*_0,01mg/ml_Starvation	91	1.96194305265	0.69348188766	48	-0.731467398115	-0.362404169694

Table A17. List of treatments administrated in starvation conditions producing effects considered as interesting after 2 hours and becoming not interesting, because similar to normal control samples (Ctrl Starvation Like Treatments), or different from both control samples (Other Treatments) after 20 hours.

Treatments Administrated in Physiological Conditions Producing Effects Considered as Interesting After 2 and Producing Effects Different Form Both Control Samples Taken as References or Toxic After 20 Hours							
#	Treatments	2 hours			20 hours - Other Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
/	/	/	/	/	/	/	/
#	Treatments	2 hours			20 hours - Toxic Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	ERAAlop_0,1mg/ml	65	0.0899019606709	-0.721103562743	47	-0.914244288017	0.102382851295
2	ERKL_0,1mg/ml	82	-0.784100753804	-0.581764200173	2	6.43347535454	-0.298080946185
3	*Scutellaria_scharistanica*_0,02mg/ml	53	-0.0836798808423	-0.474122330324	41	-0.0820374943374	0.861714650254
4	*Scutellaria_scharistanica*_0,1mg/ml	50	0.00138617392601	0.00767011611448	29	-0.278718084445	0.534935443574
5	*Schrophullaria_sp*_0,02mg/ml	57	-0.0816039592562	-0.414104626138	49	-0.233090457633	0.753737309317
6	*Schrophullaria_sp*_0,1mg/ml	92	-0.319006358893	-0.324152147436	46	-0.35437505844	0.731676429884

Table A18. List of treatments administrated in normal conditions producing effects considered as not interesting after 2 hours and becoming interesting, because similar to normal control samples (Ctrl Starvation Like Treatments), or different from both control samples (Other Treatments) after 20 hours.

Treatments Producing Effects Different From Both Control Samples Taken As Reference After 2 Hours But Similar To One Of Them And Considered As Interesting or Toxic After 20 Hours							
#	Treatments	2 hours			20 hours - Ctrl Like Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
/	/	/	/	/	/	/	/
#	Treatments	2 hours			20 hours - Ctrl Starvation Like Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	EAGAT_0,01mg/ml	91	0.81776753495	-0.964291813965	81	-0.80833599167	0.038291099758
2	EAGAT_0,02mg/ml	74	1.15222560893	-1.08131622373	87	-0.700183546004	-0.0671859940258
3	EAGAT_0,05mg/ml	60	0.778377560642	-1.61983340834	80	-0.665376674067	-0.135953972146
4	EAGAT_0,1mg/ml	78	0.800969583593	-0.987307439655	91	-0.775863515976	-0.0553148302643
5	EAGAU_0,01mg/ml	85	0.851957436835	-0.868858857675	88	-0.82935961242	-0.139420669232
6	EAGAU_0,05mg/ml	77	0.782722900701	-1.05504195187	118	-0.661197359672	0.00344432087207
7	EAGAU_0,1mg/ml	70	0.484973226348	-1.19287056459	68	-0.829957573762	-0.00601785402816
#	Treatments	2 hours			20 hours - Toxic Treatments		
		% l.c.	PC1	PC2	% l.c.	PC1	PC2
1	EAGAAsia_0,02mg/ml_Starvation	64	0.861389838351	-0.991250870056	49	0.388512699419	-1.41832890854
2	EAGMD_0,1mg/ml_Starvation	74	0.917591536626	-0.987759089639	44	-0.73907417841	-0.687691750786
3	EAGKL_0,1mg/ml_Starvation	74	0.993871408081	-0.887615056483	31	0.584368607191	-2.00804464376
4	ERPS_0,02mg/ml_Starvation	53	1.29294818431	-0.933380251808	18	7.03959639626	-3.01448175483
5	ERKL_0,05mg/ml_Starvation	96	0.370275550086	-1.08860438712	3	5.27819065766	-0.228074972664
6	*Silene_oreina*_0,1mg/ml_Starvation	84	1.70878084469	-1.37836186815	49	-0.178458313795	-1.79123411413
7	*Cousina_umbrosa*_0,1mg/ml_Starvation	120	1.57099466617	-0.552548899034	32	0.0106515487209	-0.89873136847

Table A19. List of treatments considered as interesting because producing effects different from both control sample types after 2 hours but not interesting after 20 hours since their administration.

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS SINCE TREATMENT ADMINISTRATION ON SH-SY5Y																		
		2 HOUR TREATMENTS								20 HOUR TREATMENTS								
				LYSOSOMES			LC3B II PROTEINS					LYSOSOMES			LC3B II PROTEINS		
		% Living Cells	% Living Cells (per sample)	%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area	% Living Cells	% Living Cells (per sample)	%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
Controls	Ctrl	100	100	79	2	38	100	27	26	100	100	95	16	66	100	84	24
	Ctrl DMSO	107	94	100	7	42	100	53	25	93	100	100	31	66	100	115	23
	Ctrl Starvation	100	100	76	2	38	100	52	25	100	100	93	17	67	100	98	24
	Ctrl DMSO Starvation	87	93	100	8	42	100	81	24	97	100	100	32	68	100	121	23
EAGRC	0,01mg/ml	102	100	88	3	41	100	54	25	104	100	100	20	50	100	85	24
	0,02mg/ml	90	97	87	3	42	100	65	25	98	100	100	19	51	100	91	24
	0,05mg/ml	69	100	96	6	43	100	79	26	106	100	100	18	50	100	93	24
	0,1mg/ml	88	100	94	5	40	100	87	25	96	100	100	18	47	100	105	24
	0,01mg/ml Starvation	99	94	100	13	44	100	88	25	134	95	100	16	47	100	158	23
	0,02mg/ml Starvation	106	97	100	10	42	100	111	24	123	94	100	17	50	100	107	24
	0,05mg/ml Starvation	94	94	100	11	42	100	59	26	119	91	100	18	50	100	119	24
	0,1mg/ml Starvation	94	100	100	9	42	100	56	25	28	100	100	22	56	100	66	24
ERAAlop	0,01mg/ml	88	96	95	6	43	100	29	27	85	100	100	21	50	100	24	24
	0,02mg/ml	90	97	94	6	40	93	18	27	73	100	100	20	52	100	13	24
	0,05mg/ml	75	97	100	6	43	100	77	25	62	100	100	19	51	100	19	24
	0,1mg/ml	65	95	100	7	46	100	120	25	47	100	100	17	52	100	143	24
	0,01mg/ml Starvation	60	81	100	13	44	100	58	26	83	94	100	21	51	100	88	25
	0,02mg/ml Starvation	67	86	100	13	46	100	41	26	100	91	100	17	52	100	152	23
	0,05mg/ml Starvation	67	79	100	15	48	100	22	26	62	83	100	17	52	100	131	25
	0,1mg/ml Starvation	51	78	100	11	47	100	60	26	68	100	100	17	54	100	117	24
EAGPS	0,01mg/ml	98	100	93	4	41	100	31	27	104	100	100	25	53	100	36	24
	0,02mg/ml	95	100	92	4	40	100	50	26	91	100	100	33	51	100	47	25
	0,05mg/ml	90	100	88	4	40	100	29	26	84	100	100	35	53	100	61	25
	0,1mg/ml	84	96	80	2	39	100	62	26	88	100	100	32	52	100	58	25
	0,01mg/ml Starvation	100	91	100	11	41	100	36	26	180	93	100	34	55	100	217	22
	0,02mg/ml Starvation	100	90	96	7	39	100	19	26	122	88	100	31	56	100	131	23
	0,05mg/ml Starvation	55	78	90	6	38	100	25	27	127	79	100	34	55	100	156	24
	0,1mg/ml Starvation	66	70	88	3	37	87	10	27	72	76	100	30	57	0	0	25
EAGAAsia	0,01mg/ml	83	100	92	5	42	100	60	25	75	100	100	18	51	100	61	24
	0,02mg/ml	78	97	100	6	44	100	56	26	93	100	100	17	51	100	39	25
	0,05mg/ml	72	96	100	5	41	100	70	25	69	100	100	15	52	100	41	24
	0,1mg/ml	72	98	96	6	42	100	60	25	58	100	100	15	54	100	148	22
	0,01mg/ml Starvation	61	87	100	11	46	100	26	26	59	84	100	23	50	100	165	23
	0,02mg/ml Starvation	64	87	100	14	45	100	80	24	49	100	100	20	51	100	147	22
	0,05mg/ml Starvation	45	83	100	13	45	97	35	26	23	100	100	21	50	100	2	22
	0,1mg/ml Starvation	42	77	100	9	43	100	71	26	40	100	100	21	54	100	108	23
EAGAAlop	0,01mg/ml	101	97	92	4	41	100	60	25	105	100	100	19	50	100	67	25
	0,02mg/ml	77	100	97	6	41	100	22	26	88	100	100	19	51	100	36	25
	0,05mg/ml	80	100	100	6	42	100	29	26	98	100	100	18	51	100	59	25
	0,1mg/ml	79	100	97	6	41	94	26	26	109	100	100	19	51	100	82	25
	0,01mg/ml Starvation	83	89	100	13	47	97	21	26	133	89	100	20	49	100	153	23
	0,02mg/ml Starvation	85	87	100	12	43	100	29	26	222	96	100	17	49	100	124	25
	0,05mg/ml Starvation	66	80	100	13	46	86	10	26	88	90	100	24	51	100	103	26
	0,1mg/ml Starvation	83	75	100	8	44	87	7	28	51	75	100	27	57	100	75	25
EAGMA	0,01mg/ml	109	100	93	5	42	100	72	26	62	100	100	18	49	100	140	24
	0,02mg/ml	83	100	100	7	44	100	109	25	68	100	100	20	50	100	125	23
	0,05mg/ml	74	100	100	6	41	100	114	24	84	100	100	18	50	100	155	23
	0,1mg/ml	73	100	100	8	45	100	87	26	81	100	100	19	48	100	196	21
	0,01mg/ml Starvation	36	67	100	13	46	100	115	25	88	94	100	19	47	100	293	21
	0,02mg/ml Starvation	68	92	100	18	50	100	96	25	85	96	100	18	48	100	233	23
	0,05mg/ml Starvation	57	90	100	18	48	100	115	25	74	93	100	18	51	100	261	22
	0,1mg/ml Starvation	58	86	100	15	46	100	119	24	84	100	100	19	46	100	221	22
EAGMD	0,01mg/ml	96	100	94	5	40	100	65	26	84	100	100	21	50	100	135	24
	0,02mg/ml	84	100	96	5	42	100	76	26	81	100	100	20	50	100	119	24
	0,05mg/ml	81	97	100	7	41	100	93	25	77	100	100	19	51	100	117	24
	0,1mg/ml	83	100	100	6	41	100	115	24	67	100	100	19	51	100	128	24
	0,01mg/ml Starvation	94	96	100	15	47	100	121	24	95	93	100	16	48	100	247	23
	0,02mg/ml Starvation	109	97	100	14	46	100	109	25	107	97	100	19	47	100	143	23
	0,05mg/ml Starvation	77	93	100	13	47	100	93	25	75	83	100	22	54	100	201	23
	0,1mg/ml Starvation	74	92	100	13	49	100	102	25	44	94	100	28	54	100	164	23
EAGKL	0,01mg/ml	76	100	100	7	43	100	91	25	86	99	100	20	51	100	66	25
	0,02mg/ml	79	100	100	8	43	100	49	26	80	100	100	18	49	100	34	26
	0,05mg/ml	92	100	100	7	43	100	108	25	74	100	100	20	51	100	35	27
	0,1mg/ml	63	100	100	8	45	100	85	25	78	100	100	18	51	100	64	25
	0,01mg/ml Starvation	51	86	100	13	46	100	93	25	60	97	100	19	50	100	159	24
	0,02mg/ml Starvation	79	96	100	16	48	100	57	26	103	97	100	23	52	100	129	23
	0,05mg/ml Starvation	83	94	100	15	49	100	43	26	74	98	100	30	52	100	94	24
	0,1mg/ml Starvation	74	91	100	15	45	100	53	26	31	100	100	27	50	0	0	23
EAGAS	0,01mg/ml	99	100	95	5	40	100	28	27	92	100	100	19	49	100	79	24
	0,02mg/ml	79	100	100	6	43	88	9	27	85	100	100	19	50	100	19	25
	0,05mg/ml	76	100	100	5	41	100	66	26	88	100	100	20	49	100	29	25
	0,1mg/ml	83	100	95	6	43	100	83	26	72	100	100	23	51	100	71	25
	0,01mg/ml Starvation	113	97	100	16	49	100	39	26	110	100	100	17	48	100	87	25
	0,02mg/ml Starvation	77	87	100	13	46	91	26	25	121	94	100	20	47	100	127	25

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS SINCE TREATMENT ADMINISTRATION ON SH-SY5Y																		
		2 HOUR TREATMENTS								20 HOUR TREATMENTS								
		% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS		
				%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area			%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
	0,05mg/ml Starvation	79	92	100	13	45	100	48	26	113	90	100	23	52	100	139	25
	0,1mg/ml Starvation	73	92	100	11	44	72	5	28	212	96	100	28	53	100	88	25
EAGAT	0,01mg/ml	91	97	100	15	44	100	69	26	81	100	100	16	50	100	86	24
	0,02mg/ml	74	100	100	18	46	100	55	26	87	100	100	16	48	100	32	25
	0,05mg/ml	60	100	100	16	45	100	184	23	80	100	100	18	50	100	27	26
	0,1mg/ml	78	100	100	16	44	100	66	26	91	100	100	18	51	100	74	25
	0,01mg/ml Starvation	32	80	100	7	51	100	80	27	81	100	100	17	51	100	107	24
	0,02mg/ml Starvation	73	89	100	8	50	100	35	26	107	100	100	17	48	100	177	24
	0,05mg/ml Starvation	62	92	100	8	50	100	75	25	81	93	100	22	49	100	136	24
	0,1mg/ml Starvation	69	85	100	7	50	100	12	27	113	100	100	17	51	100	122	25
EAGAU	0,01mg/ml	85	100	100	17	42	100	30	27	88	100	100	19	48	100	76	25
	0,02mg/ml	81	98	95	15	42	100	39	26	83	100	100	18	49	100	61	25
	0,05mg/ml	77	100	97	16	43	100	103	26	118	100	100	15	49	100	38	26
	0,1mg/ml	70	100	100	14	42	100	120	25	68	100	100	17	49	100	97	25
	0,01mg/ml Starvation	93	89	100	6	49	100	30	26	88	90	100	19	46	100	130	24
	0,02mg/ml Starvation	73	89	100	5	49	100	38	26	72	100	100	16	49	100	150	24
	0,05mg/ml Starvation	78	93	100	6	48	100	12	28	104	85	100	17	49	100	109	24
	0,1mg/ml Starvation	82	90	100	6	47	100	30	27	72	100	100	21	53	100	79	25
ERPS	0,01mg/ml	72	100	100	6	43	100	53	26	91	100	100	18	47	98	20	25
	0,02mg/ml	69	100	100	7	45	100	57	26	70	100	100	17	45	100	90	25
	0,05mg/ml	60	100	98	6	43	100	93	25	78	100	100	19	44	100	27	26
	0,1mg/ml	48	91	100	8	40	100	85	24	5	50	100	9	74	0	0	/
	0,01mg/ml Starvation	43	81	100	14	46	100	122	25	58	100	100	16	48	100	130	23
	0,02mg/ml Starvation	53	87	100	16	45	100	65	27	18	98	100	10	54	0	0	25
	0,05mg/ml Starvation	30	69	100	17	58	100	68	25	16	47	100	11	77	58	1	50
	0,1mg/ml Starvation	5	48	0	0	79	100	77	23	13	30	100	16	71	25	0	56
EFIAAlop	0,01mg/ml	102	100	71	2	41	100	67	25	91	100	95	13	73	100	172	22
	0,02mg/ml	86	100	74	2	36	100	49	26	76	100	88	8	79	100	177	21
	0,05mg/ml	85	100	73	1	37	100	26	26	93	100	94	14	76	100	162	22
	0,1mg/ml	79	100	79	2	37	100	100	24	60	100	100	16	76	100	65	25
	0,01mg/ml Starvation	95	94	96	5	39	100	137	24	86	96	100	45	74	100	145	23
	0,02mg/ml Starvation	97	96	92	5	38	100	76	25	75	100	100	44	73	100	114	24
	0,05mg/ml Starvation	87	93	94	5	39	100	114	25	69	100	100	40	73	100	170	23
	0,1mg/ml Starvation	123	95	93	5	37	100	95	24	117	100	100	43	71	100	69	25
ERHA	0,01mg/ml	113	100	76	2	35	100	92	25	75	100	96	12	79	100	206	22
	0,02mg/ml	115	100	78	2	35	100	77	25	53	100	91	11	83	100	185	22
	0,05mg/ml	80	100	84	3	37	100	93	25	48	100	100	15	78	100	225	22
	0,1mg/ml	83	100	76	1	36	100	46	27	53	100	100	19	79	100	202	21
	0,01mg/ml Starvation	144	93	92	5	40	100	82	25	57	100	100	34	72	100	47	24
	0,02mg/ml Starvation	47	64	94	5	39	100	113	25	59	100	100	40	71	100	205	23
	0,05mg/ml Starvation	138	100	96	5	39	100	92	25	44	100	100	38	76	100	182	22
	0,1mg/ml Starvation	72	95	95	6	41	100	110	24	39	100	100	38	74	100	178	22
EAGAF	0,01mg/ml	61	100	79	2	36	100	58	25	122	100	96	13	76	100	174	21
	0,02mg/ml	114	100	71	1	35	100	35	27	92	100	93	11	77	100	167	21
	0,05mg/ml	86	100	75	2	37	85	9	25	82	100	96	13	77	100	199	22
	0,1mg/ml	67	100	81	2	34	100	86	25	113	100	94	13	78	100	116	23
	0,01mg/ml Starvation	95	73	89	4	41	100	89	25	83	100	100	35	75	100	152	22
	0,02mg/ml Starvation	65	79	92	6	39	100	86	25	111	99	100	36	74	100	121	24
	0,05mg/ml Starvation	108	92	95	5	37	100	92	25	42	100	100	44	77	100	147	22
	0,1mg/ml Starvation	60	98	100	5	40	100	112	24	29	100	100	30	81	100	108	24
EAGAA	0,01mg/ml	105	100	80	2	37	100	65	26	66	100	94	13	78	100	212	21
	0,02mg/ml	96	100	76	2	36	100	61	26	48	100	92	9	83	100	176	21
	0,05mg/ml	76	100	79	2	36	100	59	25	57	100	95	9	81	100	206	21
	0,1mg/ml	85	100	77	2	37	100	44	26	76	100	90	8	81	100	174	20
	0,01mg/ml Starvation	77	93	100	5	37	100	90	26	80	100	100	40	77	100	65	25
	0,02mg/ml Starvation	101	88	96	5	37	100	90	25	58	96	100	41	77	100	169	22
	0,05mg/ml Starvation	36	100	100	5	37	100	100	25	59	100	100	33	80	100	173	22
	0,1mg/ml Starvation	83	94	100	4	38	100	91	25	29	100	100	25	87	100	161	22
EAGFO	0,01mg/ml	102	100	75	1	34	100	79	26	88	100	96	18	73	100	180	22
	0,02mg/ml	71	100	72	1	38	100	84	25	50	100	86	6	82	100	229	21
	0,05mg/ml	67	100	79	2	36	100	83	25	40	100	82	7	81	100	274	20
	0,1mg/ml	88	100	88	3	39	100	84	25	3	100	0	0	242	50	1	80
	0,01mg/ml Starvation	54	100	100	4	35	100	125	25	58	100	100	42	75	100	151	22
	0,02mg/ml Starvation	86	97	92	4	38	100	105	25	14	100	100	20	85	100	94	22
	0,05mg/ml Starvation	24	100	50	1	41	100	51	26	4	100	0	0	89	67	1	63
	0,1mg/ml Starvation	3	100	0	0	52	100	17	20	5	100	0	0	113	78	2	62
EAGPD	0,01mg/ml	108	100	87	3	37	100	34	26	70	100	100	22	75	100	70	24
	0,02mg/ml	108	100	88	3	36	100	56	26	70	100	100	15	77	100	57	24
	0,05mg/ml	71	100	91	3	38	100	75	26	40	100	100	21	79	100	7	24
	0,1mg/ml	97	100	87	3	37	100	48	26	22	100	100	21	83	100	38	24
	0,01mg/ml Starvation	135	98	97	6	37	93	13	27	68	100	100	44	76	15	0	26
	0,02mg/ml Starvation	84	82	100	6	39	100	77	26	62	99	100	43	77	100	31	24
	0,05mg/ml Starvation	111	97	96	6	39	87	8	26	39	100	100	42	78	100	34	25
	0,1mg/ml Starvation	100	95	100	6	39	100	52	25	6	100	100	27	82	0	0	28

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS SINCE TREATMENT ADMINISTRATION ON SH-SY5Y																	
		2 HOUR TREATMENTS								20 HOUR TREATMENTS							
				LYSOSOMES			LC3B II PROTEINS					LYSOSOMES			LC3B II PROTEINS		
		% Living Cells	% Living Cells (per sample)	%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area	% Living Cells	% Living Cells (per sample)	%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
EAGTM	0,01mg/ml	71	100	79	2	36	100	81	26	84	100	96	12	76	100	131	23
	0,02mg/ml	105	100	84	2	37	100	31	27	67	100	88	8	79	100	46	24
	0,05mg/ml	94	100	86	2	37	100	51	26	60	100	90	22	78	100	64	25
	0,1mg/ml	62	100	84	3	40	100	109	25	58	100	86	9	85	100	82	23
	0,01mg/ml Starvation	130	96	100	5	38	100	106	25	47	100	100	46	76	100	108	23
	0,02mg/ml Starvation	62	100	100	7	40	100	93	25	105	100	100	44	72	100	38	24
	0,05mg/ml Starvation	137	95	96	6	40	100	84	26	70	100	100	38	75	100	81	24
	0,1mg/ml Starvation	110	100	100	6	38	100	99	24	44	100	100	39	78	100	33	25
EAGC	0,01mg/ml	94	100	83	2	36	100	81	26	43	100	100	19	75	100	119	23
	0,02mg/ml	96	100	83	2	36	100	51	26	26	100	100	14	81	100	160	23
	0,05mg/ml	108	100	81	2	35	100	61	25	33	100	100	20	79	100	104	23
	0,1mg/ml	91	100	83	2	36	100	50	25	24	100	100	31	77	100	174	22
	0,01mg/ml Starvation	95	90	94	5	39	100	74	25	48	100	100	42	76	100	113	24
	0,02mg/ml Starvation	52	64	83	5	38	100	74	27	39	100	100	50	75	100	127	24
	0,05mg/ml Starvation	69	94	97	5	38	100	70	26	23	100	100	50	78	100	113	24
	0,1mg/ml Starvation	79	94	94	5	39	100	89	26	13	100	100	26	80	100	69	26
EFHA	0,01mg/ml	95	100	79	2	37	100	44	27	75	100	100	17	74	100	132	23
	0,02mg/ml	83	100	78	2	37	100	60	26	61	100	100	14	76	100	118	23
	0,05mg/ml	83	100	85	2	37	100	37	26	95	100	100	18	74	100	116	23
	0,1mg/ml	95	100	83	2	36	100	65	26	65	100	97	13	81	100	129	23
	0,01mg/ml Starvation	35	67	100	4	36	100	90	26	90	96	100	41	74	100	82	25
	0,02mg/ml Starvation	100	80	98	4	37	100	25	26	106	100	100	43	73	100	138	23
	0,05mg/ml Starvation	68	92	100	5	35	100	69	26	103	95	100	43	77	100	60	25
	0,1mg/ml Starvation	100	100	96	4	36	100	59	26	7	100	100	17	99	100	43	22
EAGSS	0,01mg/ml	113	100	86	3	36	100	59	26	68	100	100	19	76	100	131	24
	0,02mg/ml	87	100	83	2	36	100	91	25	39	100	100	21	74	100	138	24
	0,05mg/ml	73	100	85	2	39	100	80	25	59	100	100	19	78	100	152	23
	0,1mg/ml	109	100	84	2	36	100	52	26	39	100	100	26	76	100	155	23
	0,01mg/ml Starvation	76	93	100	7	39	100	58	26	82	100	100	49	75	100	177	22
	0,02mg/ml Starvation	98	82	100	5	40	100	97	25	47	100	100	50	75	100	167	23
	0,05mg/ml Starvation	99	96	96	6	39	100	100	26	66	100	100	44	76	100	64	25
	0,1mg/ml Starvation	107	98	97	6	40	100	95	25	61	100	100	49	78	100	95	25
EFIPS	0,01mg/ml	70	100	83	2	35	100	52	26	87	100	97	20	74	100	153	23
	0,02mg/ml	80	98	82	3	37	100	79	26	47	100	100	12	76	100	191	21
	0,05mg/ml	75	100	85	3	37	100	85	25	70	100	95	12	85	100	185	22
	0,1mg/ml	50	100	89	2	36	100	101	26	27	100	100	16	81	100	204	21
	0,01mg/ml Starvation	119	97	95	5	38	100	58	26	79	92	100	45	77	100	111	23
	0,02mg/ml Starvation	40	91	100	5	45	100	27	29	93	88	100	43	78	100	136	22
	0,05mg/ml Starvation	88	89	100	5	40	100	91	25	30	87	100	42	82	100	143	22
	0,1mg/ml Starvation	59	100	95	5	39	100	71	27	6	100	75	4	125	0	0	31
EAGCB	0,01mg/ml	49	100	89	3	38	100	64	27	80	100	93	19	75	100	49	26
	0,02mg/ml	59	98	89	3	36	100	68	26	45	100	87	10	80	100	191	23
	0,05mg/ml	69	100	92	4	38	100	62	26	50	100	100	20	73	100	94	24
	0,1mg/ml	59	100	90	3	36	100	66	26	65	100	89	15	76	100	50	25
	0,01mg/ml Starvation	84	100	100	5	38	100	40	28	41	100	100	44	74	100	118	24
	0,02mg/ml Starvation	41	100	100	6	43	100	16	29	57	100	100	51	75	100	128	23
	0,05mg/ml Starvation	68	98	95	5	39	100	15	28	48	100	100	44	77	100	114	24
	0,1mg/ml Starvation	56	96	100	6	39	100	51	27	52	96	100	42	77	100	35	25
EAGCT	0,01mg/ml	104	98	68	1	37	99	17	25	90	100	80	5	72	100	147	20
	0,02mg/ml	82	97	76	2	37	100	76	24	95	100	72	3	75	100	116	21
	0,05mg/ml	88	98	73	1	37	100	77	24	84	100	78	4	77	100	144	21
	0,1mg/ml	72	96	69	1	38	100	66	25	83	98	70	2	82	100	163	20
	0,01mg/ml Starvation	91	97	98	7	40	100	60	25	74	100	100	24	71	100	147	20
	0,02mg/ml Starvation	92	96	96	6	40	100	45	24	82	99	100	20	76	100	144	21
	0,05mg/ml Starvation	72	94	97	7	41	100	102	24	55	97	94	15	80	100	153	21
	0,1mg/ml Starvation	64	94	97	6	40	100	90	23	49	98	83	7	82	100	166	21
EAGOT	0,01mg/ml	98	100	70	2	36	100	70	24	92	100	76	3	70	100	31	24
	0,02mg/ml	98	99	77	2	38	100	70	24	99	100	60	1	77	100	21	24
	0,05mg/ml	88	100	73	2	37	100	56	25	101	100	61	1	76	100	87	22
	0,1mg/ml	89	98	77	2	37	100	77	25	89	100	67	1	76	100	59	22
	0,01mg/ml Starvation	102	98	98	8	41	100	86	23	106	100	100	25	66	100	25	23
	0,02mg/ml Starvation	103	98	97	7	41	100	92	23	111	100	100	24	67	100	73	22
	0,05mg/ml Starvation	97	98	98	8	42	100	63	24	93	100	100	24	68	100	64	23
	0,1mg/ml Starvation	100	99	98	7	41	100	55	25	103	100	100	21	69	100	89	22
ERKL	0,01mg/ml	98	100	81	2	35	100	38	25	95	100	100	15	60	100	107	21
	0,02mg/ml	88	98	93	4	37	100	87	23	94	100	100	15	62	100	62	23
	0,05mg/ml	87	98	93	4	36	100	103	22	53	98	100	18	68	100	146	21
	0,1mg/ml	82	97	98	5	37	100	87	22	2	100	0	0	105	0	0	24
	0,01mg/ml Starvation	100	98	100	9	42	100	32	26	84	100	100	25	66	100	126	21
	0,02mg/ml Starvation	98	97	100	10	41	100	93	23	79	98	100	26	70	100	121	21
	0,05mg/ml Starvation	96	98	100	13	43	100	91	23	3	90	75	9	77	33	0	32
	0,1mg/ml Starvation	47	67	100	14	50	100	113	22	2	55	57	3	125	0	0	32
ERKS	0,01mg/ml	96	98	72	2	37	100	104	22	90	100	88	5	75	100	34	23
	0,02mg/ml	87	98	80	2	38	100	107	22	91	100	90	7	71	100	55	24

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS SINCE TREATMENT ADMINISTRATION ON SH-SY5Y																	
		2 HOUR TREATMENTS								20 HOUR TREATMENTS							
		% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS		
				%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area			%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
	0,05mg/ml	77	99	79	2	38	100	83	24	86	100	93	13	72	100	132	21
	0,1mg/ml	81	99	80	3	38	100	57	24	84	100	100	20	66	98	15	23
	0,01mg/ml Starvation	78	92	100	9	43	100	81	24	108	100	100	30	66	100	79	23
	0,02mg/ml Starvation	84	95	100	7	42	100	67	25	103	100	100	31	67	50	1	23
	0,05mg/ml Starvation	75	88	100	11	42	100	71	25	95	98	100	37	69	100	73	22
	0,1mg/ml Starvation	79	94	100	10	42	100	61	25	85	98	100	39	69	100	60	22
ESHA	0,01mg/ml	98	99	71	2	37	100	41	25	96	100	86	5	71	100	149	20
	0,02mg/ml	83	98	77	2	38	100	55	25	88	100	83	5	73	100	95	22
	0,05mg/ml	92	98	77	2	38	100	72	24	94	100	83	4	71	100	127	22
	0,1mg/ml	78	98	77	2	37	100	41	25	89	100	82	5	76	100	92	23
	0,01mg/ml Starvation	91	97	100	9	43	100	34	25	99	100	100	26	63	100	137	21
	0,02mg/ml Starvation	84	96	100	8	41	100	85	24	95	100	100	25	66	100	116	21
	0,05mg/ml Starvation	85	95	100	10	44	100	56	25	87	100	100	28	69	100	130	22
	0,1mg/ml Starvation	93	98	100	9	44	100	48	25	80	100	100	29	70	100	125	21
Ulva sp. (U-2C)	0,01mg/ml	78	98	79	2	36	9	0	25	97	100	87	5	71	100	79	23
	0,02mg/ml	87	100	83	3	37	83	5	26	82	100	89	6	72	100	74	23
	0,05mg/ml	90	100	79	2	37	100	39	26	77	100	87	6	74	100	107	22
	0,1mg/ml	85	100	80	2	37	98	27	26	87	100	78	4	76	100	124	22
	0,01mg/ml Starvation	108	100	100	8	41	99	20	26	90	100	100	26	68	100	112	22
	0,02mg/ml Starvation	94	98	98	8	41	100	16	27	80	100	100	22	70	100	70	24
	0,05mg/ml Starvation	97	98	100	9	42	87	4	28	90	100	100	23	71	100	79	23
	0,1mg/ml Starvation	94	99	100	9	43	100	26	27	70	98	100	27	75	100	77	23
Cryptomenia sp. (C-3B)	0,01mg/ml	63	100	80	2	36	86	6	27	82	100	85	4	74	100	57	23
	0,02mg/ml	65	98	82	3	37	82	4	26	79	100	83	4	76	100	48	24
	0,05mg/ml	78	100	79	2	37	95	9	26	66	100	75	2	80	100	135	21
	0,1mg/ml	72	100	55	2	37	100	27	26	66	100	61	1	81	100	51	24
	0,01mg/ml Starvation	57	97	100	8	41	22	0	28	82	100	100	22	70	100	36	25
	0,02mg/ml Starvation	82	97	96	8	41	37	0	26	70	100	92	12	73	100	55	24
	0,05mg/ml Starvation	76	98	97	9	42	65	4	26	57	100	88	11	75	100	64	24
	0,1mg/ml Starvation	71	99	90	9	43	1	0	28	34	95	93	20	82	100	126	23
Ciona intestinalis (C-4B)	0,01mg/ml	59	100	81	3	37	57	1	27	88	100	96	10	70	100	83	23
	0,02mg/ml	63	98	82	2	39	98	19	26	80	100	96	9	72	100	61	23
	0,05mg/ml	73	100	77	2	37	100	26	26	49	100	94	10	80	100	124	21
	0,1mg/ml	45	100	65	1	37	100	23	26	14	100	73	8	90	100	101	21
	0,01mg/ml Starvation	71	98	98	6	41	91	14	26	71	100	100	25	70	100	124	22
	0,02mg/ml Starvation	68	98	92	4	38	81	6	28	64	100	100	23	74	100	103	22
	0,05mg/ml Starvation	71	96	96	6	39	71	2	29	15	100	100	16	91	100	110	22
	0,1mg/ml Starvation	37	73	95	4	39	60	1	30	1	100	0	0	221	0	0	38
Heliotropium sp. (T-1B)	0,01mg/ml	67	99	90	3	40	100	22	26	58	100	95	9	81	100	96	22
	0,02mg/ml	67	98	92	4	39	100	32	26	26	100	100	14	84	100	200	21
	0,05mg/ml	79	97	90	3	39	100	32	26	2	50	0	0	110	0	0	28
	0,1mg/ml	51	89	85	3	43	100	47	25	3	100	40	0	91	0	0	/
	0,01mg/ml Starvation	70	96	100	9	44	100	26	26	39	98	100	30	78	100	180	22
	0,02mg/ml Starvation	65	91	98	7	43	87	4	28	6	53	95	9	93	77	25	24
	0,05mg/ml Starvation	59	88	100	7	44	100	35	27	3	100	25	0	110	0	0	33
	0,1mg/ml Starvation	26	71	94	6	47	96	14	26	2	100	0	0	141	0	0	89
Heliotropium sp. (T-1C)	0,01mg/ml	69	100	90	3	38	97	10	28	77	100	92	8	72	100	55	24
	0,02mg/ml	63	100	94	4	40	100	57	24	82	100	87	6	72	50	1	24
	0,05mg/ml	88	100	86	3	37	100	47	26	70	100	94	10	71	100	79	23
	0,1mg/ml	64	98	88	4	38	100	34	26	10	80	100	19	68	100	136	21
	0,01mg/ml Starvation	85	98	100	10	43	100	23	26	98	100	100	28	67	100	62	21
	0,02mg/ml Starvation	82	100	100	9	43	84	4	29	81	100	100	27	68	100	15	24
	0,05mg/ml Starvation	86	100	100	10	44	100	20	28	84	100	100	26	67	100	136	22
	0,1mg/ml Starvation	91	99	100	11	45	91	5	28	27	100	100	27	74	100	74	22
Verbascum blattaria	0,01mg/ml	81	98	68	1	40	100	78	26	94	100	94	11	73	100	123	24
	0,02mg/ml	97	98	75	2	36	100	44	26	94	100	98	11	70	100	113	24
	0,05mg/ml	96	97	66	1	37	100	44	26	81	100	98	11	71	100	166	23
	0,1mg/ml	70	97	71	1	35	100	52	26	56	100	93	8	80	100	140	23
	0,01mg/ml Starvation	109	69	100	10	40	100	82	25	112	96	100	35	73	100	134	23
	0,02mg/ml Starvation	102	75	100	11	40	100	97	25	103	100	100	41	71	100	47	26
	0,05mg/ml Starvation	65	60	100	9	41	100	78	26	82	93	100	33	75	100	76	24
	0,1mg/ml Starvation	43	44	100	10	38	100	65	26	23	100	100	12	97	100	82	23
Stachys hissarica	0,01mg/ml	91	100	79	2	39	100	80	26	112	100	71	2	74	100	83	24
	0,02mg/ml	85	100	71	2	40	100	41	27	82	100	75	3	78	100	114	24
	0,05mg/ml	51	93	71	1	40	100	109	25	73	100	75	2	83	100	119	24
	0,1mg/ml	91	100	68	1	36	100	47	27	45	100	76	4	79	100	150	24
	0,01mg/ml Starvation	106	77	100	9	43	100	86	25	106	100	100	28	74	100	106	24
	0,02mg/ml Starvation	124	86	100	9	42	100	55	26	84	97	100	27	76	100	71	25
	0,05mg/ml Starvation	82	69	100	11	42	100	101	25	91	94	100	35	73	100	35	27
	0,1mg/ml Starvation	24	54	100	10	42	100	69	27	60	94	100	33	77	100	88	25
Verbascum songoricum	0,01mg/ml	84	97	83	3	37	100	63	25	91	100	79	4	76	100	108	24
	0,02mg/ml	105	97	83	3	38	100	55	26	88	100	81	4	74	100	113	24
	0,05mg/ml	60	91	78	2	38	100	65	25	84	100	77	5	74	100	155	22
	0,1mg/ml	85	93	83	2	38	100	54	26	39	100	86	7	86	100	168	23

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS SINCE TREATMENT ADMINISTRATION ON SH-SY5Y																		
		2 HOUR TREATMENTS								20 HOUR TREATMENTS								
		% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS		
				%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area			%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
	0,01mg/ml Starvation	107	71	100	10	44	100	144	25	154	100	99	23	76	100	121	23
	0,02mg/ml Starvation	103	73	100	9	43	100	132	25	103	99	100	28	76	100	72	25
	0,05mg/ml Starvation	59	61	100	10	45	100	99	26	58	83	100	29	82	100	80	25
	0,1mg/ml Starvation	42	70	100	11	45	100	72	26	15	100	100	31	82	100	80	28
Stachys betoniciflora	0,01mg/ml	82	100	93	4	39	95	22	26	93	100	92	9	73	100	64	25
	0,02mg/ml	88	98	93	4	38	90	7	28	104	100	89	8	75	100	75	24
	0,05mg/ml	78	88	81	2	42	100	60	26	90	100	89	9	73	100	89	24
	0,1mg/ml	79	95	83	3	39	100	34	27	111	100	84	5	75	100	50	26
	0,01mg/ml Starvation	142	79	100	9	42	100	47	26	110	98	100	32	74	100	54	25
	0,02mg/ml Starvation	104	68	100	10	43	63	1	26	133	96	100	30	73	100	77	25
	0,05mg/ml Starvation	46	59	100	9	42	100	65	25	165	93	100	24	74	100	58	24
	0,1mg/ml Starvation	107	69	100	7	41	95	21	26	56	90	93	15	82	100	65	29
Phlomis sewertzovii	0,01mg/ml	71	95	93	4	40	100	62	26	113	100	89	9	74	100	115	25
	0,02mg/ml	63	94	93	4	41	100	69	26	102	100	86	8	73	100	58	25
	0,05mg/ml	76	98	89	4	39	100	73	25	95	100	87	5	74	100	121	24
	0,1mg/ml	43	97	90	3	41	100	80	26	87	100	82	6	74	100	103	24
	0,01mg/ml Starvation	132	85	100	12	45	100	113	25	92	93	100	34	76	100	130	24
	0,02mg/ml Starvation	120	86	100	12	45	100	171	25	126	98	100	34	73	100	56	25
	0,05mg/ml Starvation	91	67	100	13	47	100	102	26	117	95	100	35	75	100	74	24
	0,1mg/ml Starvation	75	73	100	10	45	100	131	25	50	86	100	37	79	100	85	25
Phlomis salicifolia	0,01mg/ml	78	94	97	5	41	100	94	25	94	100	91	10	74	100	79	24
	0,02mg/ml	70	96	92	5	40	100	50	26	99	100	93	10	72	100	88	25
	0,05mg/ml	83	94	93	4	40	100	95	24	96	100	85	9	72	100	93	24
	0,1mg/ml	55	90	93	4	43	100	112	24	67	100	74	3	77	100	62	25
	0,01mg/ml Starvation	114	85	100	10	44	100	142	24	111	98	100	34	74	100	42	25
	0,02mg/ml Starvation	139	82	100	11	44	100	5	26	115	97	100	37	71	100	82	25
	0,05mg/ml Starvation	59	70	100	12	45	100	81	24	122	94	100	40	74	100	37	26
	0,1mg/ml Starvation	55	80	100	10	44	100	50	26	95	91	100	35	76	100	78	25
Silene oreina	0,01mg/ml	54	95	96	6	42	100	96	26	72	100	100	24	71	100	109	24
	0,02mg/ml	74	96	100	6	42	100	58	26	79	100	100	21	70	100	100	25
	0,05mg/ml	56	90	100	6	42	100	83	25	76	100	100	22	69	100	104	24
	0,1mg/ml	88	97	96	6	41	100	59	26	61	100	100	26	69	100	143	23
	0,01mg/ml Starvation	123	80	100	13	46	100	142	25	101	97	100	43	71	100	137	23
	0,02mg/ml Starvation	113	83	100	16	47	100	108	26	118	96	100	44	71	100	121	24
	0,05mg/ml Starvation	95	69	100	16	48	100	141	25	78	87	100	47	77	100	125	24
	0,1mg/ml Starvation	84	71	100	17	46	100	155	25	49	78	100	51	76	100	74	26
Phlomis tadschikistanica	0,01mg/ml	90	99	97	5	41	100	42	26	90	100	91	9	78	100	78	25
	0,02mg/ml	97	98	97	5	40	88	4	27	76	100	100	18	72	100	134	24
	0,05mg/ml	63	95	100	6	42	100	42	26	70	100	97	13	74	100	113	23
	0,1mg/ml	108	97	88	3	41	100	64	26	57	100	92	9	73	100	121	24
	0,01mg/ml Starvation	128	83	100	17	50	100	112	26	100	96	100	39	73	100	101	25
	0,02mg/ml Starvation	103	81	100	16	48	100	77	27	116	100	100	42	70	100	76	25
	0,05mg/ml Starvation	120	90	100	12	48	100	77	26	109	94	100	44	72	100	91	25
	0,1mg/ml Starvation	85	83	100	12	44	100	85	25	76	95	100	43	75	100	82	25
Cousina umbrosa	0,01mg/ml	61	96	93	6	41	100	63	28	62	100	100	19	74	100	156	25
	0,02mg/ml	53	96	94	5	41	100	126	27	50	100	100	18	76	100	109	24
	0,05mg/ml	56	95	92	5	43	100	143	27	36	100	93	13	82	100	108	25
	0,1mg/ml	62	94	90	3	41	100	100	27	51	100	95	13	83	100	113	24
	0,01mg/ml Starvation	91	76	100	12	46	100	92	24	48	100	100	37	80	100	130	25
	0,02mg/ml Starvation	111	80	100	12	45	100	138	25	62	100	100	41	75	100	41	26
	0,05mg/ml Starvation	77	87	100	11	47	/	/	/	58	91	100	41	77	100	89	25
	0,1mg/ml Starvation	120	93	100	11	46	100	419	24	32	82	100	32	77	100	121	24
Nepeta olgae	0,01mg/ml	51	86	92	5	41	100	103	26	84	100	92	10	74	100	95	25
	0,02mg/ml	65	93	94	5	42	100	89	26	78	100	93	10	75	100	112	24
	0,05mg/ml	115	94	93	4	43	100	68	26	64	100	93	13	76	100	66	25
	0,1mg/ml	74	95	90	3	42	100	84	26	82	100	100	13	77	100	109	24
	0,01mg/ml Starvation	141	82	100	12	45	100	112	25	103	100	100	38	69	98	31	26
	0,02mg/ml Starvation	130	80	100	13	48	100	56	26	110	97	100	38	73	100	45	26
	0,05mg/ml Starvation	94	71	100	14	47	100	56	26	97	100	100	39	72	100	75	25
	0,1mg/ml Starvation	95	64	100	13	45	100	77	26	81	100	100	41	77	100	84	25
Scutellaria scharistanica	0,01mg/ml	57	95	93	4	43	100	44	27	59	100	99	16	76	100	93	24
	0,02mg/ml	53	94	100	6	41	100	92	27	41	100	94	9	75	100	95	26
	0,05mg/ml	48	94	100	6	40	100	123	26	59	100	100	14	72	100	79	26
	0,1mg/ml	50	91	96	6	43	100	22	27	29	100	98	17	75	100	116	25
	0,01mg/ml Starvation	91	81	100	12	49	100	100	26	107	97	100	35	72	100	68	26
	0,02mg/ml Starvation	102	81	100	14	47	100	41	25	82	100	100	35	75	100	48	26
	0,05mg/ml Starvation	105	66	100	12	45	100	55	26	63	86	100	41	74	100	83	26
	0,1mg/ml Starvation	64	67	100	12	44	100	115	26	98	85	100	35	78	100	74	25
Schrophullaria sp.	0,01mg/ml	63	96	96	6	43	100	94	26	54	100	100	10	74	100	91	25
	0,02mg/ml	57	97	100	7	42	100	54	27	49	100	100	9	76	100	54	26
	0,05mg/ml	73	96	100	7	40	100	27	28	64	100	100	10	75	100	54	26
	0,1mg/ml	92	98	100	6	39	100	48	27	46	100	100	13	80	100	109	25
	0,01mg/ml Starvation	97	85	100	43	48	88	19	27	103	97	100	35	74	100	73	25
	0,02mg/ml Starvation	115	78	100	42	45	100	31	27	70	98	100	36	76	100	98	25

FEATURES MEASUREMENTS AFTER 2 AND 20 HOURS SINCE TREATMENT ADMINISTRATION ON SH-SY5Y																	
		2 HOUR TREATMENTS								20 HOUR TREATMENTS							
		% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS			% Living Cells	% Living Cells (per sample)	LYSOSOMES			LC3B II PROTEINS		
				%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area			%Active Cells	# Per Cell	Area	%Active Cells	# Per Cell	Area
	0,05mg/ml Starvation	24	50	100	40	47	100	108	26	3	100	100	6	112	100	9	37
	0,1mg/ml Starvation	2	0	75	39	100	0	0	124	3	100	33	1	107	0	0	/
Leonurus panzeroides	0,01mg/ml	59	95	96	6	44	100	77	27	69	100	95	12	78	100	43	26
	0,02mg/ml	87	100	97	6	42	100	87	26	61	100	88	9	79	100	31	27
	0,05mg/ml	49	93	100	7	44	100	79	26	52	100	92	8	80	100	50	26
	0,1mg/ml	85	97	96	6	43	100	55	25	72	100	87	7	74	100	42	27
	0,01mg/ml Starvation	128	88	100	14	47	100	131	25	90	100	100	33	75	100	39	26
	0,02mg/ml Starvation	112	82	100	14	45	100	13	24	85	95	100	33	75	100	38	26
	0,05mg/ml Starvation	152	89	100	13	47	100	85	27	61	94	100	31	76	100	56	26
	0,1mg/ml Starvation	93	85	100	11	45	100	61	27	34	81	100	23	80	100	44	27

Table A20. Complete list of the treatments which show a cytotoxic activity or able to perturb the physiological conditions of the autophagic flux after 2 and 20 hours since their administration, reported along with the real values of the measurements of the features considered as descriptive of the activities and described through a colour system: the name of the most interesting treatments have been reported on an orange background; treatments increasing the autophagic activity have been reported on a green background; treatments decreasing the autophagic activity have been reported on a red background; treatments producing phenotypes different from both those chosen as reference have been reported on a blue background; treatments showing a cytotoxic effects have been reported on a grey background.

List of Publications

Bernardini, S, Tiezzi, A, Laghezza Masci, V & Ovidi, E 2017, 'Natural products for human health: an historical overview of the drug discovery approaches', *Natural Product Research*, pp. 1-25. doi: 10.1080/14786419.2017.1356838.

Bernardini, S, Donoso-Fierro, C, Tiezzi, A, Tassoni, M, Fava, E, Becerra, J, Silva, M & Ovidi, E 2017, 'Evaluation of the effects of *Ulva* sp., *Cryptomenia* sp., *Ciona intestinalis* and *Heliotropium* sp. crude extracts as possible interfering agents to the Autophagocytosis pathway: preliminary results', *The International Journal of Molecular Sciences*, in print.

Bernardini, S, Osorio, MS & Tiezzi, A (2018) Plants: an infinite source of molecules useful for pharmaceuticals, *Current Traditional Medicine*, in print.