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„Phenotypic screening of selected extracts extending the lifespan of *Caenorhabditis elegans*“

verfasst von / submitted by

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List of abbreviations

AMPK	5’ Adenosine Monophosphate Activated Protein Kinase
BCE	Before Common Era
C. elegans	*Caenorhabditis elegans*
DT50	Death Time; Time Point, when 50 % of all Nematodes of one Population Died
EtOAc	Ethyl Acetate
FUdR	5-Fluoro-2´-deoxyuridine
IGF-1	Insulin-like Growth Factor 1
HEMWat	Quaternary Mixture of *n*-Hexane, Ethyl Acetate, Methanol and Water that forms two immiscible Layers
HPCCC	High-Performance Counter-Current Chromatography
HPLC	High-Performance Liquid Chromatography
K_D	Partition Coefficient
LLE	Lead-like Enhanced
MeOH	Methanol
N2	Wild-type strain of *C. elegans*
NF-κB	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells
n-hex	*n*-Hexane
OP50	*Escherichia coli*, Strain OP50
PLE	Pressurized Liquid Extraction
Rt	Retention Time
TCM	Traditional Chinese Medicine
TLC	Thin Layer Chromatography
TOR	Target of Rapamycin
UHPLC	Ultra High-Performance Liquid Chromatography

1. Abstract

In this diploma thesis we phenotypically screened 41 selected plant and mushroom small-scale extracts for their potential lifespan prolonging effect in the model organism *Caenorhabditis elegans (C. elegans).* One of the active mushroom extracts, namely *Lignosus rhinocerus* (Cooke) Ryvarden (*L. rhinocerus*) was further mycochemically investigated. For the *C. elegans* screening 28 lead-like enhanced (LLE) extracts and one CO_2 extract of different herbal substances as well as 12 ethanolic extracts derived from different polypore mushrooms were tested in the *C. elegans* lifespan assay at two concentrations, 25 and 100 µg/ml, respectively. Out of the 41 screened extracts, eleven showed significant lifespan extending effects (given in %):

Cynanchum stauntonii (Decne.) Schltr. ex H.Lév (rhizome extract): 8,76% (100 µM, $p < 0.01$),
Cynanchum paniculatum (Bunge) Kitag. ex H.Hara (rhizome extract): 6,12% (25 µM, $p < 0.05$),
Eriobotrya japonica (Thunb.) Lindl. (leaf extract): 12.20 % (25 µM, $p < 0.05$) and 17.35 % (100 µM, $p < 0.05$),
Euphrasia officinalis L. (herb extract): 9.76 % (25 µM, $p < 0.01$) and 16.46 % (100 µM, $p < 0.01$),
Gardenia jasminoides J.Ellis (fruit extract): 9.68 % (25 µM, $p < 0.05$),
Gloeophyllum odoratum (Wulfen) Imazeki (fruiting body extract): 13.41 % (100 µM, $p < 0.01$),
Lignosus rhinocerus (Cooke) Ryvarden (sclerotia extract): 11.73 % (25 µM, $p < 0.01$),
Pimenta dioica (L.) Merr (fruit extract): 10.22 % (100 µM, $p < 0.05$),
Pterocarpus santalinus L.f (wood extract): 15.54 % (25 µM, $p < 0.01$),
Syzygium aromaticum (L.) Merr. & L.M.Perry (flower bud extract): 5.24 % (100 µM, $p < 0.05$),
Zanthoxylum armatum DC (pericarp extract): 8.93 % (25 µM, $p < 0.05$).

Of all active extracts we selected *L. rhinocerus* for a further mycochemical investigation as it showed on the one hand a promising lifespan prolonging effect on the nematodes and on the other hand its secondary metabolite profile is scarcely investigated until now. Thus, a large-scale ethanolic extract of *L. rhinocerus* sclerotia was generated, which was further fractionated by High-Performance Counter-Current Chromatography (HPCCC). The HPCCC fractionation resulted in 14 pooled fractions, namely LR01_01 to LR01_14. These fractions were further tested in the *C. elegans* lifespan assay and analyzed by LC-MS to narrow down

the search of putatively active constituents. In the *C. elegans* lifespan assay, at 25 µg/ml fractions LR01_03 and LR01_07 showed a statistically significant increase of lifespan of 5.41 % ($p < 0.05$) and 9.07 % ($p = 0.005$), respectively. In the subsequent dereplication step, some fatty acids and steroid-like compounds were identified and matched with findings of previous studies, while some remained unidentified, and will be worth to be studies in future. This diploma thesis offered insight into the lifespan increasing potential of extracts from selected medicinal plants and mushrooms. Thus, it will be the basis for further phyto- and mycochemical research to further isolate bioactive constituents from these promising natural materials.

Zusammenfassung

In dieser Diplomarbeit wurden 41 Pflanzen- und Pilzextrakte phenotypisch im *Caenorhabditis elegans* (*C. elegans*) Assay auf lebensspannenerweiternde Eigenschaften untersucht. Im Anschluss wurde einer der aktiven Pilzextrakte, *Lignosus rhinocerus* (Cooke) Ryvarden (*L. rhinocerus*) mykochemisch aufgearbeitet. Es wurden insgesamt 28 Extrakte, die durch das Lead-Like Enhanced-Verfahren gewonnen wurden, sowie ein CO_2 Extrakt aus ausgesuchten Medizinalpflanzen, sowie 12 ethanolische Extrakte von ausgewählten Porlingen im *C. elegans* lifespan Assay in zwei Konzentrationen, 25 und 100 µg/ml, untersucht. Von den 41 untersuchten Extrakten, zeigten elf einen statistisch signifikanten positiven Effekt auf die Lebensspanne von *C. elegans* (angegeben in %):

Cynanchum stauntonii (Decne.) Schltr. ex H.Lév (Rhizomextrakt): 8,76% (100 µM),

Cynanchum paniculatum (Bunge) Kitag. ex H.Hara (Rhizomextrakt): 6,12% (25 µM),

Eriobotrya japonica (Thunb.) Lindl. (Blattextrakt): 12.20 % (25 µM, $p < 0.05$) and 17.35 % (100 µM, $p < 0.05$),

Euphrasia officinalis L. (Krautextrakt): 9.76 % (25 µM, $p < 0.01$) and 16.46 % (100 µM, $p < 0.01$),

Gardenia jasminoides J.Ellis (Fruchtextrakt): 9.68 % (25 µM, $p < 0.05$)

Gloeophyllum odoratum (Wulfen) Imazeki (Fruchtkörperextrakt): 13.41 % (100 µM, $p < 0.01$)

Lignosus rhinocerus (Cooke) Ryvarden (Sklerotienextrakt): 11.73 % (25 µM, $p < 0.01$),

Pimenta dioica (L.) Merr (Fruchtextrakt): 10.22 % (100 µM, $p < 0.05$)

Pterocarpus santalinus L.f (Holzextrakt): 15.54 % (25 µM, $p < 0.01$)

Syzygium aromaticum (L.) Merr. & L.M.Perry (Blütenknospenextrakt): 5.24 % (100 µM, $p < 0.05$).

Zanthoxylum armatum DC (Perikarpextrakt): 8.93 % (25 µM, $p < 0.05$)

Aus den Extrakten, die einen positiven Effekt zeigten, wurde *L. rhinocerus* für eine weitere mykochemische Aufarbeitung und Fraktionierung ausgewählt, da dieser Extrakt einerseits eine vielversprechende lebensspannerweiternde Wirkung auf Nematoden zeigte, und andererseits noch unzureichend mykochemisch untersucht ist. Daher wurde ein Extrakt im Großmaßstab aus Sklerotien von *L. rhinocerus* gewonnen. Dieser wurde anschließend durch High-Performance Counter-Current Chromatography (HPCCC) fraktioniert. Die 14 daraus gewonnenen Fraktionen (LR01_01-14) wurden wiederum im *C. elegans* lifespan Assay auf deren biologische Aktivität getestet und mittels Flüssigchromatographie mit kombinierter

Massenspektrometrie analysiert, um die Suche nach aktiven Komponenten einzuengen. Im *C. elegans* lifespan Assay zeigten Fraktionen LR01_03 und LR01_07 bei einer Konzentration von 25 µg/ml einen statistisch signifikanten Anstieg der Lebenspanne, um 5.41 % bzw 9.07 %. In der anschließenden Dereplikation wurden verschiedene Fettsäuren sowie Komponenten mit Steroid-Grundgerüst identifiziert. Weitere bislang nicht identifizierbare Bestandteile werden Gegenstand weiterer Untersuchungen an Department sein. Diese Diplomarbeit ermöglichte Einsichten in die biologische Aktivität und das Potential von ausgewählten Medizinalpflanzen und Pilzen für weiterführende Forschung und einen möglichen medizinischen Einsatz.

2. Aim of the Work

2.1. Phenotypic Screening of Selected Plant/Mushroom Extracts Prolonging the Lifespan of *Caenorhabditis elegans*

In a vast multitude of various plants and fungi used as traditional remedies with distinct uses, the mechanism of action on the one hand and their bioactive constituents on the other hand is mostly unknown. The aim of this diploma thesis is to identify crude extracts with the ability to delay aging in the model organism *C. elegans*. We therefore investigated the lifespan prolonging effect on the nematodes in a screening of selected small-scale herbal and fungal extracts, in order to phytochemically and mycochemically analyze those with a significant lifespan prolonging effect. We used the protocol of Solis & Petraschek (2011) to measure lifespan of wild type *C. elegans* (N2) treated with different extracts in two concentrations (100 µg/ml and 25 µg/ml) by using the anti-hypertensive drug reserpine at 30 µM as positive control (Srivastava et al., 2008). The lifespan assay was carried out in 96-well microtiter plates in liquid media. All extracts were tested at a final concentration of 1 % DMSO which served as vehicle control.

2.2. Mycochemical Investigation of the Lifespan Prolonging Extract of *Lignosus rhinocerus*

After the completion of the phenotypic screening of 41 selected plant/mushroom extracts a literature research with focus on all plants/mushrooms that exhibited a positive effect on the lifespan of *C. elegans* was conducted. The aim was to find a promising target for a large-scale extraction with subsequent fractionation. The polypore mushroom *L. rhinocerus* was selected as attractive material for a mycochemical assessment, i.e. extraction and fractionation with HPCCC. The HPCCC fractions were further analyzed in the *C. elegans* phenotypic lifespan assay to determine their bioactivity and to pave the road for further fractionation and isolation of bioactive compounds.

3. Introduction

3.1. Phenotypic Screening of Selected Plant/Mushroom Extracts Prolonging the Lifespan of *Caenorhabditis elegans*

3.1.1. *Caenorhabditis elegans* as a Model Organism

Caenorhabditis elegans, a small, free living soil nematode has gained great importance by its extensive use in aging studies. *C. elegans* offers vast benefits for aging research because of a rather short and reproducible lifespan of two to three weeks and a completely elucidated genome. Its transparent body, ease of cultivation and morphological simplicity as well as suitability for genetic analysis are factors that contribute to its wide utilization as a model organism.

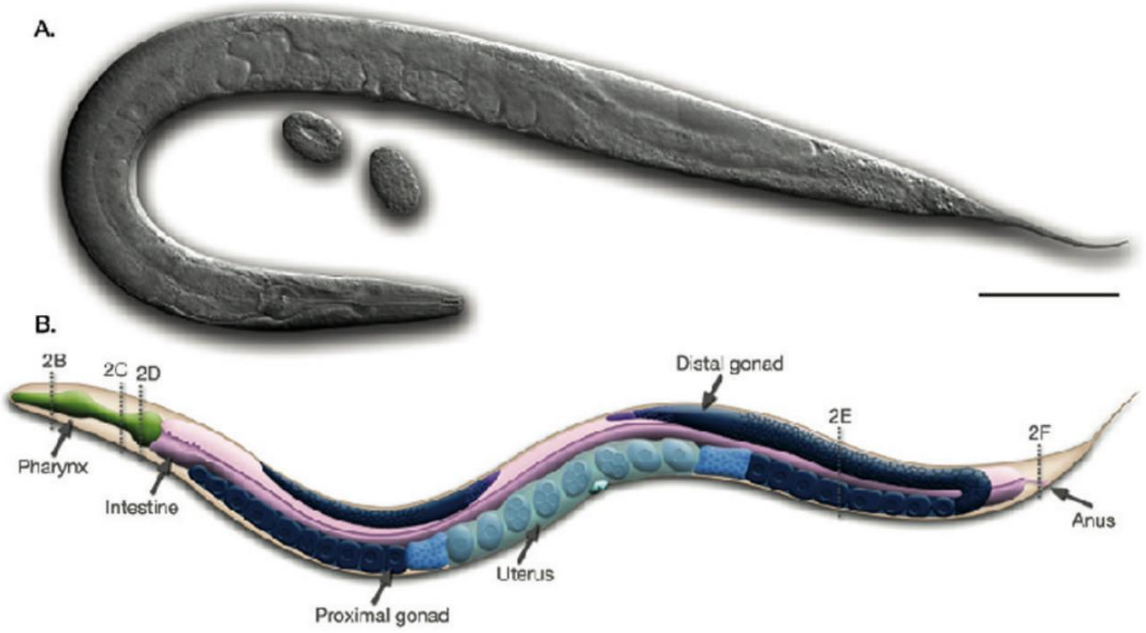


Figure 1 *C. elegans* hermaphrodite. (A) Microscopic picture of a *C. elegans* hermaphrodite. (B) Diagram of *C. elegans* hermaphrodite and its most important anatomical features. (Corsi, Markaki, Tavernarakis, Sawa, & Korswagen, 2013).

Medium to high-throughput screening investigations are available due to its small size and ability to grow in microtiter plates. A 40 % similarity of genes between *C. elegans* and humans is crucial to its application in a multitude of bioassays (Corsi et al., 2013; Solis & Petrascheck, 2011; Stiernagle, 2006).

Lifespan itself is a complex biological process that involves a plethora of different genetic pathways. To investigate the biology of aging, one can use two strategies:

(i) One is the investigation of animals and organisms that show mutations in age-regulatory pathways, should these perturb the function of one of these pathways and alter the lifespan of the whole organism, insights in the mechanism of said pathway may be gained.

(ii) The other strategy involves use of small molecules to influence pathways that regulate aging. Only a small number of molecules have been identified to date that show a positive effect on lifespan.

One approach for instance is to measure the lifespan of the nematodes in 96-well microtiter plates by counting the number of living/dead worms two to three times per week. With this robust and miniaturized assay a screening of a moderate-sized number of animals – tested under the same assay condition - is feasible and therefore allows an investigation of lifespan-extending properties of various plant/mushroom samples within the same assay plate, see Chapter 6.2.4 (Solis & Petrascheck, 2011).

3.1.2. Aging and Related Effects

The most significant risk factor associated with chronic diseases is aging. It leads to a plethora of biological changes, including aberrations in protein homeostasis, metabolic dysfunction and altered cellular signaling. Most common age-related diseases like cardiovascular diseases, cancers, neurodegenerative disorders, metabolic disorders such as diabetes, osteoporosis and age-related macular degeneration are rooted in these changes (Luu & Palczewski, 2018). But aging is still not fully understood throughout the scientific world, although many involved pathways have already been elucidated. In the past, aging was considered to be an exclusively passive, entropic process rather than being influenced by signaling pathways and transcription factors. But many different signals can affect and regulate the process of aging, e.g. dietary restriction, oxidative stress, heat, low ambient temperature as well as thermosensory and chemosensory signals have been reported to extend *C. elegans* lifespan. Signals from the reproductive system and reductions in the rate of respiration and translation have been reported to have a lifespan prolonging effect as well. Insulin/IGF-1 signaling, TOR (target of

rapamycin) signaling, AMPK (5' adenosine monophosphate activated protein kinase), sirtuins, inhibition of respiration, signals from the reproductive system and telomeres are some of the already elucidated pathways of aging. While one would expect that due to an increased lifespan, age-related diseases (e.g. cancer) would become more prevalent, a contrary trend has been observed (= healthspan). The involved pathways mentioned above can not only extend the lifespan but also protect the organism from age-related diseases (Kenyon, 2010).

3.1. Generated Plant and Mushroom Extracts Tested in the *Caenorhabditis elegans* Lifespan Assay

Andrographis paniculata

Kalmegh or "King of bitters", as *Andrographis paniculata* (Burm.f.) Nees (Acanthaceae) is most often referred to, is the most popular medicinal herb of the genus Andrographis. It is incorporated in traditional medicines such as Ayurveda, Unani and Siddha with extensive use as a home remedy in the Indian traditional system and even in tribal medicine. Its mechanism of action is mostly facilitated by enzyme induction. It has a wide scope of uses and activities, being used to treat gastrointestinal tract and upper respiratory infections, fever, herpes, hepatitis and other chronic and infectious diseases. Some activities that have been reported include anti-bacterial, anti-malarial, filaricidal, anti-diarrhoeal and cardiovascular activities as well as hepatoprotective and fertility effects (Niranjan, Tewari, & Lehri, 2010).

Antrodia cinnamomea

Antrodia cinnamomea T.T. Chang & W.N. Chou (Fomitopsidaceae) is a mushroom originating from Taiwan, where it has been used by local tribes for centuries as a treatment for food poisoning and to enhance liver functions. It gained importance in the last decades after being included in Asian folk medicine showing anti-inflammatory, anti-cancer, anti-hypertensive and anti-diabetic activities as well as being used to treat hepatitis. Due to this reputation it gained the status of a luxury dietary supplement (Lu et al., 2013).

Aquilegia vulgaris

Aquilegia vulgaris L. (Ranunculaceae), otherwise known as common columbine is known in traditional medicine to alleviate symptoms of fever, inflammation and pain. An anti-inflammatory activity has been indicated in recent studies (Malik et al., 2017).

Azaridachta indica

Neem, Indian Lilac or Margosa are common names for *Azadirachta indica* A.Juss. (Meliaceae). This common evergreen tree, that can be found in India, Africa and America has been in use in traditional Ayurvedic medicine since before 2000 BCE. The positive effects of the plant, its extracts and isolated compounds have been reported in a multitude of pharmacological studies, in which antidiabetic and antihyperlipaemic, anti-bacterial, anti-malarial and anti-fungal, anti-tumor as well as anti-ulcer and anti-inflammatory activities in conjunction with positive effects on wound-healing have been observed (Bijauliya, Alok, Chanchal, Sabharwal, & Yadav, 2008).

Calendula officinalis

Pot Marigold, or *Calendula officinalis* L. (Asteraceae), is a common herb found across Europe and Asia. It is extensively used etnomedicinally for various ailments and diseases e.g. various inflammations, ulcers, wounds, burns, sprains and conjunctivitis in both topical and oral formulations (Arora, Rani, & Sharma, 2013). In pharmacological studies *C. officinalis* exhibited anti-inflammatory, antibacterial and antifungal, anti-HIV and antiviral, anti-cancer, hepatoprotective, antioxidative, immunostimulating and wound healing, insecticidal activities as well as spasmolytic and spasmogenic, genotoxic and antigenotoxic dual activities (Vinod, Singh, Pradhan, Iyer, & Tripathi, 2019).

Cannabis sativa

Indian hemp, the common name *Cannabis sativa* L. (Cannabaceae) is known under, is an annual plant whose cultivation can be traced back to the dawn of agriculture. It has been valued for its wide range of applications, i.e. as a food, fibre and oil source as well as its medical and euphoric drug. Its medicinal application can be traced back to 3000 BCE, when it was incorporated in the first Chinese pharmacopeia. There its indications covered fatigue, rheumatism and malaria as well as eczema, psoriasis and inflammatory ailments. Around the Mediterranean Sea its applications were mostly centered around its analgesic and mood-stabilizing properties. In recent pharmacological studies, a vast multitude of effects have been reported, anti-convulsive, anti-spasmic, anxiolytic, antidiabetic, neuroprotective as well as analgesic, anti-nausea and anti-rheumatoid, to name at least a few. Positive effects on ailments like pain, colitis, anorexia, sleep disorders, spasticity, epilepsy, Alzheimer's disease and many more have been reported as well (Bonini et al., 2018).

Cetraria islandica

Iceland moss, *Cetraria islandica* (L.) Ach. (Parmeliaceae), is a commonly used medicinal lichen in Europe. It is traditionally used to alleviate symptoms of cold and lung diseases as well as stomach ailments and against inflammation and other minor ailments. Several biological activities have been reported for *C. islandica*, antimicrobial, antioxidative, anti-tumor as well as analgesic, anti-inflammatory and wound-healing activities being amongst them (Gómez-Serranillos, Fernández-Moriano, González-Burgos, Divakar, & Crespo, 2014; Xu et al., 2016).

Cynanchum paniculatum

Cynanchum paniculatum (Bunge) Kitag. ex H.Hara (Apocynaceae), is a herb used in traditional Chinese medicine (TCM) to invigorate blood, alleviate edema, relieve pain and toxicity. In recent studies anti-cancer, anti-inflammatory and anti-nociceptive activities have been reported (Ma, Weon, Lee, Lee, & Yun, 2014).

Cynanchum stauntonii

Cynanchum stauntonii (Decne.) Schltr. ex H.Lév (Apocynaceae), also known by its pinyin name "Bai-qjan" is also used in TCM to relieve cough and expel phlegm (C. B.-S. Lau et al., 2014).

Drynaria fortunei

Drynaria fortunei (Kunze ex Mett.) J.Sm. (Polypodiaceae), a herb used in traditional Chinese medicine, is used to treat ailments associated with kidney deficiency as well as bone-associated ailments such as bone and ligament injuries (Bensky, Clavey, Stöger, & Gamble, 2004).

In recent research, osteoprotective properties of *D. fortunei* on osteoporosis and other bone metabolic disorders have been investigated (Wang et al., 2011).

Eriobotrya japonica

Loquat, the common name of *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae), a fruit tree found in the subtropical reaches of China has been cultivated there for at least 2000 years and is regarded to be of high medicinal value. It has been used in TCM to treat diabetes, inflammatory ailments, chronic bronchitis as well as cough and even cancer. These applications have been reinforced by recent pharmacological studies, as anti-inflammatory, antidiabetic, anti-cancer, antioxidative as well as anti-thrombotic, antinociceptive and anti-allergic effects have been reported, among others (Yilong Liu, Zhang, Xu, & Li, 2016).

Euphrasia officinalis

Euphrasia officinalis L. (Scrophulariaceae), commonly known as Eyebright is a semi-parasitic, annual plant that has been in traditional use for centuries, mostly as a therapy of conjunctivitis, hordeolum and other eye disorders. In recent studies, extracts of *E. officinalis* exhibited a multitude of pharmacological effects, including antioxidant, anti-inflammatory, anti-microbial and anti-fungal, anticancer and antiviral as well as hepatoprotective, anti-catarrhal and antiepileptic activities (Paduch, Woźniak, Niedziela, & Rejdak, 2014).

Fomitopsis pinicola

The red banded polypore, or *Fomitopsis pinicola* (Sw.) P. Karst. (Fomitopsidaceae) has been traditionally used in Europe for centuries. Positive effects on ailments such as headache, nausea or hepatic dysfunction are told about the mushroom. The fruit bodies have astringent properties, being therefore used as an anti-inflammatory agent as well to stop bleedings. In modern studies, several of these effects and newer ones have been confirmed and reported, anti-inflammatory, antioxidative and even antibiotic effects being amongst them (Grienke et al., 2014).

Ganoderma lucidum

Reishi, the Chinese name for *Ganoderma lucidum* (Curtis) P. Karst. (*Ganodermatace*ae), is a wood-rot mushroom widely known and popular in Traditional Chinese Medicine for its health and longevity promoting effects. Due to the possibility of constant ingestion without side-effects, almost 2500 years ago it has gained the title of “superior herb” by herbalists of the Shu Dynasty. In TCM it is also used to prevent and alleviate chronic hepatitis, nephritis, bronchitis as well as high blood pressure and tumors. Its constituents have been reported to exhibit a wide range of biological activities, amongst which neuroprotective, anti-HIV as well as antioxidative, antimicrobial and antitumor properties are most noteworthy (Cör, Knez, & Hrnčič, 2018).

Gardenia jasminoides

Gardenia jasminoides J.Ellis (Rubiaceae) *is* an evergreen tree that is can be found in multiple areas of China where it is known as “Zhi Zi”. It is used in Traditional Chinese Medicine as well as a yellow dye. Its pharmacological activities have been reported to be antioxidative, antihypoglycemic, anti-inflammatory as well as antidepressant and properties to improve sleep quality have been observed as well (Xiao, Li, Wang, & Ho, 2017).

Gloeophyllum odoratum

Gloeophyllum odoratum (Wulfen) Imazeki (Gloeophyllaceae), a polypore mushroom from Central Europe commonly referred to as “Anise Mazegill”, has been reported to exhibit cytotoxic, immunomodulatory as well as anti-influenza-virus activities (Grienke et al,, 2018).

Humulus lupulus

Common hop, as *Humulus lupulus* L. (Cannabaceae) is known is a widely used European medicinal plant and a crucial ingredient in the beer brewing process. It has been in use for more than 2000 years, its applications in ancient times being a treatment for leprosy, foot odor, constipation, etc. In the middle ages, its anti-inflammatory, antimicrobial as well as diuretic and bile-increasing effects became known. More recently, hops has been in use to treat sleep disorders as well as anxiety. In recent studies, antioxidative, antimicrobial, sedative, anti-cancer, antidiabetic, anti-obesity as well as estrogenic and osteoporosis-related effects have been reported (Karabín, Hudcová, Jelínek, & Dostálek, 2016).

Inonotus obliquus

Inonotus obliquus (Ach. ex Pers.) Pilát (Hymenochaetaceae), commonly known in Russia as Chaga fungus, is a popular traditional medicinal mushroom around Russia, Belarus, Poland, Ukraine and Finland. Its traditional uses are gastrointestinal cancer, diabetes and cardiovascular disorders. Some of these effects can be linked to reports of recent studies, as anti-nociceptive, anti-inflammatory, as well as antitumor activities have been reported (Pomianek et al., 2018).

Lactuca sativa subsp. *capitate*

Lactuca sativa subsp. *capitate* (L.) Schübl. & G.Martens (Asteraceae), commonly known as lettuce, is not only a popular vegetable, it is used in certain countries as part of the regional traditional medicine. In Iran, *L. sativa* subsp. *capitate* is used to relieve inflammation, as well as osteodynia and gastrodynia. It is said to have anticonvulsant and sedative properties. A anti-inflammatory and antinociceptive effect has been reported in recent studies (Sayyah, Hadidi, & Kamalinejad, 2004).

Lignosus rhinocerus

Lignosus rhinocerus (Cooke) Ryvarden (Polyporaceae), known as the Tiger Milk Mushroom is widely used around South-East Asia, particularly Malaysia, where it is used both by the

indigenous as well as the urban population for a wide range of ailments such as asthma, cough, food poisoning, swollen breasts and joint pain, liver illness, general swelling as well as a general tonic. Anti-asthmatic, anti-coagulant, anti-inflammatory as well as anti-microbial and anti-obesity activities have been observed. Antiproliferative and immunomodulatory, neuritogenic, hepatoprotective and antioxidative properties have been observed as well (Nallathamby et al., 2018). Some of these effects, e.g. anti-tumor and immunomodulatory, have been associated with water-soluble polysaccharide-protein-complex as well as alkali-soluble β-glucans (Zaila et al., 2013). Among other compound classes that have already been identified are phenolics as well as terpenoids, which appear to be abundant. An extract fraction containing the highest amounts of these classes showed the highest radical-scavenging activity (H. Y. Y. Yap, Tan, Ng, Tan, & Fung, 2018). Lanostane-type triterpenoids have been observed, however their absolute structures remain to be elucidated (Aminudin et al., 2014).

The hot and cold aqueos extracts of *L. rhinocerus* sclerotia have been extensively investigated, with a multitude of active compounds mostly of the polysaccharide-class being elucidated in the last years. Ethanolic extracts however, offer room for new research, as these have not yet been as thoroughly studied. Ethanolic extracts have already been reported to have a significant neuritogenic effect in rat pheochromocytoma (PC-12) cells as well as positive effects on cell viability, however no active compounds have been presented (Seow et al., 2015). In other research papers, methanolic extracts have been reported to be high in phenolic content and have a strong antioxidative as well as radical-scavenging activity (Y. H. Yap et al., 2013). A methanolic fraction of a pressurized liquid extraction (PLE) extract has shown selective cytotoxic activity against human colorectal cancer cells. (Zaila et al., 2013).

Pimenta dioica

Pimenta dioica (L.) Merr (Myrtaceae), whose dried unripe berries are called Allspice or Newspice or Piment, is a Jamaican medicinal plant with a broad use as a spice as well as a medicinal herb. In the Caribbean, it is used as a folk medicine against dysmenorrhea, colds, dyspepsia as well as diabetes, sore joints and myalgia. It has been added into Ayurveda, the Indian traditional medicine, to cure toothaches and respiratory congestion. It is, too, used in modern herbal medicine to treat neuralgia, its essential oil has found an application as an agent against muscle cramps, to promote circulation as well as to relieve headache and stress

and even to combat depression. Extracts of *P. dioica* have been reported to have antioxidant activities, hypotensive and antiproliferative effects, as well as having properties that alleviate menopausal symptoms (Zhang & L. Lokeshwar, 2012).

Piptoporus betulinus

Piptoporus betulinus (Bull.) P. Karst. (Fomitopsidaceae), the birch polypore mushroom is one of the items found on the mummified body of the man found in a glacier, called "Ötzi". It has a long ethnomedicinal tradition among Northeast-European countries as well as Russia. It is traditionally used as a tea to strengthen immunity, relieve fatigue as well as against some types of cancer. Due to its astringent properties, the fruit bodies have been used externally as a haemostatic agent. Pharmacological studies could report a cytotoxic and anti-virus activities (Grienke, Zöll, Peintner, & Rollinger, 2014).

Plantago lanceolata

Ribwort plantain, the common name of *Plantago lanceolata* L. (Plantaginaceae), is widely used in traditional medicine to alleviate respiratory tract problems, viral infections, skin diseases and insect bites. Extracts isolated from *P. lanceolata* showed inhibitory activity on NF-κB and managed to activat PPAR-α and γ, as well as antioxidative and anti-inflammatory activity in recent studies (Vogl et al., 2013).

Polygonum aviculare

Polygonum aviculare L. (Polygonaceae), known as common knotweed is a herb used in Korean herbal medicine for mostly inflammatory diseases, i.e. arthritis and skin afflictions. Extracts of *P. aviculare* have been reported to exhibit immunoregulatory, anti-inflammatory and antioxidative properties (Park et al., 2018).

Potentilla aurea

The dwarf yellow cinquefoil, as *Potentilla aurea* L. (Asteraceae) is commonly known, has been used in traditional medicine for centuries. In combination with other species of the genus *Potentilla*, it is used to treat toothache, jaundice, inflammatory ailments, ulcera as well as a wound-healing agent. So far, only an antioxidant effect has been reported (Tomczyk & Latté, 2009).

Pterocarpus santalinus

Commonly known as Red Sandalwood, *Pterocarpus santalinus* L.f. (Leguminosae) is in has been traditionally used for centuries if not millennia in India, Taiwan, China and other Asian countries. It is used as a sacred wood in Hinduism as well as a traditional remedy for various ailments like diabetes, skin and eye diseases, ulcers and to induce vomiting (Soundararajan, G, Murugesan, & Chandrashekar, 2016). It is also used to treat severe cough as well as dysentery, pyrexia and diarrhea. Extracts from its heart wood, leaves and bark as well as isolated substances have been investigated for their biological activities and exhibited antimicrobial, anti-inflammatory, antioxidant, anti-diabetic, cytotoxic as well as hepatoprotective properties (Navada & Vittal, 2014).

Rhodiola rosea

Rhodiola rosea L. (Crassulaceae), commonly known as golden root or roseroot is a plant with a wide etnomedicinal tradition in Asia and Eastern Europe. Most well-known for its adaptogenic properties, it has been used as a tonic and antidepressant, to alleviate high-altitude sickness and stimulate mental and physical capabilities, to mention a few of its uses. It exhibits a wide range of biological activities; adaptogenic, anti-aging, antioxidative, anti-inflammatory, antinociceptive, immunostimulative, antidepressant, neuroprotective as well as hepato- and cardioprotective, cytotoxic, anti-hyperlipidemic and antiviral effects have been reported so far (Wu, Wen, Chen, Chiang, & Wu, 2015).

Saussurea costus

Saussurea costus (Falc.) Lipsch. (Asteraceae), an Indian Ayurvedic medicinal herb known simply as Costus or Kuth. Its roots are used to traditionally treat colds and cough, malaria, leprosy, rheumatic disorders as well as various forms of pain, cholera, gout and chronic skin diseases, to name a few. Immunomodulatory, hepatoprotective, anti-ulcer, cholagogic, anti-inflammatory as well as anti-tumor and antimicrobial effects, among others, have been reported (Pandey, Rastogi, Kumar, & Rawat, 2007).

Sida cordifolia

Sida cordifolia L. (Malvaceae), whose root is commonly referred to as “bala”, is a traditional herb used for centuries in Indian Ayurvedic medicine and as a medicinal plant in traditional medicine of Brazil, China and other countries. In these forms of medicine its applications are various, as it is used as an analgesic, anti-pyretic, anti-rheumatic as well as an anti-asthmatic,

laxative and diuretic, and many others. Recent pharmacological studies report a broad spectrum of pharmacological activities, i.e.: Anti-inflammatory, analgesic, antipyretic and antiulcerogenic, cardioprotective, antidiabetic, anti-oxidative and even sedative effects (Galal, Raman, & A. Khan, 2015).

Syzygium aromaticum

Syzygium aromaticum (L.) Merr. & L.M.Perry (Myrtaceae), the spice and medicinal herb commonly known as clove has been used as such for many centuries. Its unopened, dried flower buds are traditionally used as a carminative, stimulant as well as for its antiseptic, antiasthmatic and anaesthetic properties. It has been described by some sources as a natural antihelminthic. Various pharmacological activities have been reported, e.g. antibacterial and antifungal, antioxidant, antiinflammatory, anticarcinogenic, analgesic and anesthetic, antithrombotic as well as antiasthmatic and antidepressant effects (Mittal, Gupta, Parashar, Mehra, & Khatri, 2014).

Terminalia chebula

Terminalia chebula Retz. (Combretaceae) known in India as Harad, or "the king of medicinal plants" as some would have it. The genus *Terminalia* has a long tradition in the Indian Ayurvedic practice. It is used for a range of ailments, from abdominal and back pain, headaches and fevers, wounds and hemorrhages to coughs and colds, pneumonia, sexually transmitted diseases, worms and ulcers as well as leprosy and heart disorders. It is said to have anti-inflammatory properties and is used as a general tonic. Native *Terminalia* species are well known in African traditional medicinal practice as well. Its evidence-supported pharmacological properties have been reported to be a high antioxidant activity, antimicrobial activity, even against *Plasmodium falciparum*. Anti-inflammatory and immunomodulatory activities as well as antiviral properties have been reported along antidiabetic, neuroprotective and wound-healing properties (Cock, 2015).

Valeriana officinalis

Valeriana officinalis L. (Caprifoliaceae), also known as Valerian or Allheal, is a medicinal herb used in traditional medicine for centuries. It is used as a sedative, to treat cardiac arrhythmia as well as to relieve nervous unrest, promote sleep and help patients relax and reduce anxiety. Its properties have been largely confirmed in pharmacological studies, that report

acetylcholinesterase-inhibitory effects, sleep-promoting, anxiolytic as well as antioxidative, antibronchospastic and cytoprotective effects (Nandhini, Narayanan, & Ilango, 2018).

Zanthoxylum armatum

Nepal pepper or prickly ash, *Zanthoxylum armatum* DC. (Rutaceae), or otherwise known in Nepal as Timur, is a popular and important medicinal plant as well as spice. Various parts of the tree are used in indigenous traditional medicine as an agent against many ailments, such as dyspepsia and indigestion, fever, cold and cough, to relieve toothache as well as a treatment for cholera and tuberculosis. It is applied externally to repel leeches as well. Decoctions of the berries are said to have carminative and anti-spasm activities and are used to relieve abdominal pain, rheumatism, diabetes, and asthma. Some parts of the tree are used as an antihelminthic agent. Antimicrobial, antioxidative, as well as anti-inflammatory, antispasmodic, cytotoxic and phytotoxic activities have been reported along hepatoprotective, antiviral, skin-soothing and larvicidal properties (Phuyal, Jha, Prasad Raturi, & Rajbhandary, 2019).

3.2. Mycocemical Investigation of the Lifespan Prolonging Extract of *Lignosus rhinocerus*

3.2.1. Botanical Description and Definition of Mushrooms

A botanical line of division between fungi and mushrooms is not easily drawn, as Chang & Miles stated in 1992. By their definition mushrooms are all epigeous or hypogeous macrofungi with a distinctive fruiting body that is large enough to be picked by hand and can be seen by the naked eye. This means a mushroom can be, as opposed to older definitions, ascomycetes or basidiomycetes, aerial or hypogeous, can have a fleshy or non-fleshy texture and be edible or not edible.

3.2.2. Traditional Use of *Lignosus rhinocerus*

Lignosus rhinocerus (Cooke) Ryvarden, also known as Tiger Milk Mushroom is widely used around South-East Asia, particularly Malaysia, where it is used both by the indigenous as well as the urban population for a wide range of ailments such as asthma, cough, food poisoning, swollen breasts and joint pain, liver illness, general swelling as well as a general tonic. (B. F. Lau, Abdullah, Aminudin, Lee, & Tan, 2015)

3.2.3. Classification of *Lignosus rhinocerus*

The genus of *Lignosus* is defined by stipitate basiocarps while exhibiting a trimitic hyphal system with hyphae that are binding well differentiated (Sotome et al., 2008). *L. rhinocerus* is part of the Polyporaceae family and consists of a cap (pileus), stem (stipe) and tuber (sclerotium; see Figure 2). Its morphology deviates from most polypores, opposing to other polypores where the fruiting body raises from woody substrate, the fruiting body (pileus and stipe) of *L. rhinocerus* rises from the sclerotium from under the ground. While the pili and stipes are mostly woody and therefore less interesting for medicinal purposes, the sclerotia form a compacted mass of fungal mycelia that serves as a food reserve, therefore being the part that is most commonly used for medicinal purposes (Nallathamby et al., 2018).

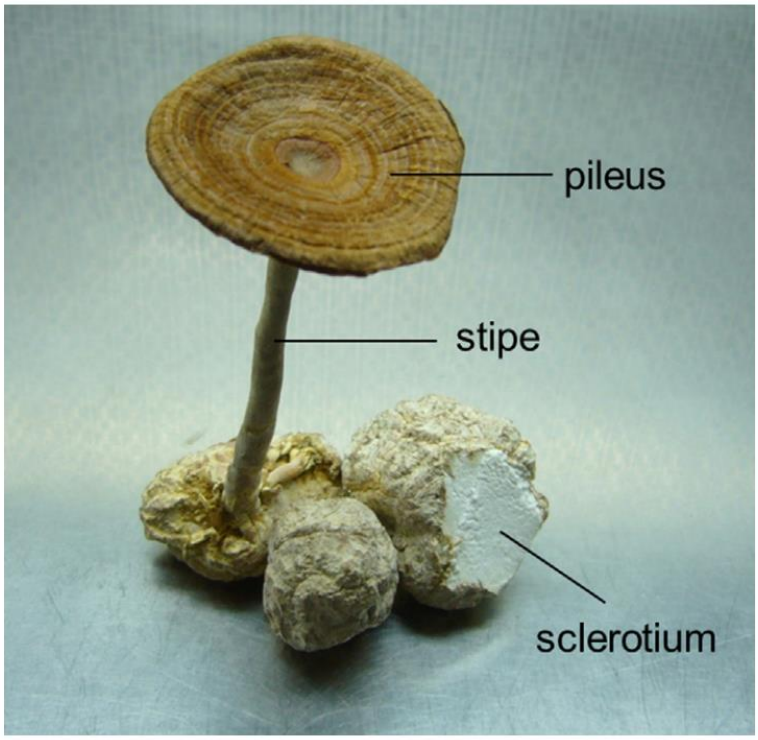


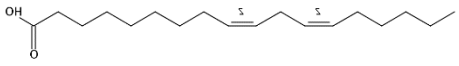
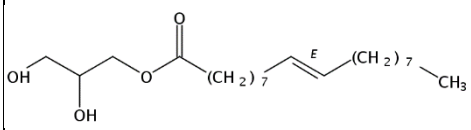
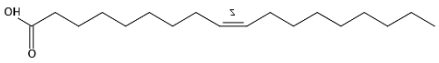
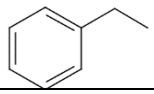
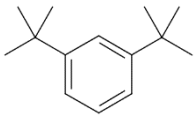
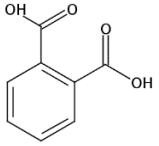
Figure 2 *L. rhinocerus* with its various parts (B. F. Lau et al., 2015).

Its sclerotia represent one of its stages in its life cycle consisting of hardened fungal mycelium forming a compact mass. This nutrient-rich aggregate of multiple hyphae is morphologically variable and possesses an ability to stay dormant until conditions for further growth are met (H.-Y. Y. Yap et al., 2014).

3.2.4. Known Constituents from Literature

Some of the attributed effects, e.g. anti-tumor and immunomodulatory, have been associated with water-soluble polysaccharide-protein-complex as well as alkali-soluble β-glucans (Zaila et al., 2013). Among other compound classes that have already been identified are phenolics as well as terpenoids, which appear to be abundant. Lanostane-type triterpenoids have been reported, however, their absolute structures remain to be elucidated (Aminudin et al., 2014). In addition, volatile compounds from *L. rhinoceurs* sclerotia were investigated by Johnathan, Gan, Ezumi, Faezahtul, & Nurul (2016) and revealed the presence of five major groups of compounds, i.e. alkanes (53 %), fatty acids (36 %), benzenes (7 %), phenols (3 %) and dicarboxylic acids (1 %). Table 1 gives an overview of the reported compounds sorted by substance class, CAS number, molecular weight and structure.

Table 1 - Known compounds from literature

Substance Class		CAS Number	Substance Name	Molecular Weight	Structure
Fatty acids	**1**	60-33-3	9,12-Octadecadienoic acid (Z,Z)-; Linoleic acid	280.45	
	2	2716-53-2	9-Octadecenoic acid, 2,3-dihydroxypropyl ester, (9E)-; 2,3-Dihydroxypropyl elaidate	356.54	
	3	112-80-1	9-Octadecenoic acid (9Z)-; Oleic acid	282.46	
Benzenes	**4**	100-41-4	α-Methyltoluene; Ethlybenzene	106.17	
	5	1014-60-4	Benzene, 1,3-bis(1,1-dimethylethyl)-; 1,3-Di-tert-butylbenzene	190.32	
Dicarboxylic acids	**6**	88-99-3	1,2-Benzenedicarboxylic acid; Phthalic acid	166.13	

3.2.5. Bioactivity of *Lignosus rhinocerus* Extracts and Isolated Compounds from Literature

Anti-asthmatic, anti-coagulant, anti-inflammatory as well as anti-microbial and anti-obesity activities have been reported. Antiproliferative and immunomodulatory, neuritogenic as well as hepatoprotective and antioxidative properties have been reported in extracts of *L. rhinocerus* as well (Nallathamby et al., 2018). The hot and cold aqueos extracts of *L. rhinocerus* sclerotia have been extensively investigated, with a multitude of active compounds mostly of the polysaccharide-class being elucidated in the last years. Ethanolic extracts however, offer room for new research, as these have not yet been as thoroughly studied. Ethanolic extracts have already been reported to have a significant neuritogenic effect in rat pheochromocytoma (PC-12) cells as well as positive effects on cell viability, however no active compounds have been presented (Seow et al., 2015). In other research papers, methanolic extracts have been reported to be high in phenolic content and have a strong antioxidative as well as radical-scavenging activity (Y. H. Yap et al., 2013). A methanolic fraction of a pressurized liquid extraction (PLE) extract has shown a selective cytotoxic activity against human colorectal cancer cells. (Zaila et al., 2013).

4. Results and Discussion

4.1. Phenotypic Screening of Selected Plant/Mushroom Extracts Prolonging the Lifespan of *Caenorhabditis elegans*

4.1.1. Lifespan Assay Results of 41 Small-Scale Extracts

In the scope of this diploma thesis 41 small-scale extracts (detailed information about extraction and sample preparation of all small-scale extracts is provided in the diploma thesis of Wieser, 2019), one large-scale extract of *Lignosus rhinocerus* and 14 thereof derived fractions (LR01_01 – LR01_14) were screened for their lifespan prolonging effect in the *C. elegans* lifespan assay. In chapter 6.1.2, the detailed information of all small-scale extracts is described. On pages 28 to 33, all results of the small-scale extract screening within the five experiments LS_E01_001 – LS_E01_005 (all tested in parallel triplicates) are shown. All small-scale extracts were tested at two concentrations, 25 and 100 µg/ml. For a better comparison of these five assays, all results were normalized to a DT_{50} value of 20 days for the vehicle control DMSO 1 %. Results with a significant lifespan increase (i.e. p-value < 0.05) are highlighted in green (e.g. EupoffHDMp at both concentrations, 25 and 100 µg/ml, see Table 2). DT_{50} is determined as the time when 50% of a worm population in one well is dead. For a better visualization of the lifespan increasing potential of all samples, the results were compared with the DT_{50} of the vehicle control (red line) and depicted as a bar chart (see Figure 3 to Figure 7).

Chapter 6.2.3 provides an overview of all lifespan assays that were performed in this diploma thesis, as well as information about the plant material, organ and sample label.

4.1.1.1. Results of LS_E01_001

In the first experiment, the extract of *Euphrasia officinalis* (EupoffHDMp), *Eriobotrya japonica* (ErijapFDMp), as well as *Gloeophyllum odoratum* (GloodoOE) showed a lifespan-prolonging activity in comparison to the vehicle control DMSO 1 %, as seen in Table 2. EupoffHDMp exhibited a significantly positive effect at both tested concentrations: at the lower concentration of 25 µg/ml it increased the lifespan by 9.76 % ($p < 0.01$), while at 100 µg/ml the effect climbed up to 16.46 % ($p < 0.01$). This may indicate a dose-dependency effect due

to lifespan-enhancing constituents. EupoffHDMp also presented a lifespan-enhancing effect at both tested concentrations, though tested in two experiments (25 µg/ml were screened in LS_E01_001, whereas 100 µg/ml were screened in LS_E01_002; results of LS_E01_002 are presented in Table 3). At a concentration of 25 µg/ml ErijapFDMp enhanced the lifespan of *C. elegans* by 12.20 % ($p < 0.05$), at 100 µg/ml the effect gained on intensity to 17.35 % ($p < 0.05$). This also suggests a dose-dependent effect similar to the one observed in EupoffHDMp. The tested ethanolic extract of the polypore fungus *G. odoratum* (GloodoOE) showed a statistically significant effect only at a concentration of 100 µg/ml, prolonging the lifespan of *C. elegans* by 13.41 % ($p < 0.01$). This could suggest that lifespan-enhancing constituents are not abundant enough in the extract to exhibit its effects at the lower concentration of 25 µg/ml.

Table 2 - DT_{50} of three parallel experiments LS_E01_001_A, LS_E01_001_B and LS_E01_001_C

Sample	Concentration	DT50			Mean	Stadev.	Data normalised	%	DT50 increase (%)	p-value	Activity
		LS_E01_001_A	LS_E01_001_B	LS_E01_001_C							
DMSO	1%	13,00	14,00	14,00	13,67		20,00	50,00	0,00		
Reserpine	30 µM	18,00	18,00	18,00	18,00	0,00	26,34	65,85	15,85	0,006	+
AzaindODMp	25 µg/mL	15,50	16,50	14,00	15,33	1,26	22,44	56,10	6,10	0,135	o
	100 µg/mL	11,00	11,00	21,00	14,33	5,77	20,98	52,44	2,44	0,860	o
SidcorHDMp	25 µg/mL	14,00	17,50	16,50	16,00	1,80	23,41	58,54	8,54	0,144	o
	100 µg/mL	14,00	16,00	15,50	15,17	1,04	22,20	55,49	5,49	0,113	o
GloodoOE	25 µg/mL	13,50	17,00	17,00	15,83	2,02	23,17	57,93	7,93	0,198	o
	100 µg/mL	16,50	18,00	17,50	17,33	0,76	25,37	63,41	13,41	0,003	+
PotaurHDMp	25 µg/mL	17,50	14,00	14,00	15,17	2,02	22,20	55,49	5,49	0,327	o
	100 µg/mL	12,00	12,50	16,50	13,67	2,47	20,00	50,00	0,00	1,000	o
CansatBCO2	25 µg/mL	14,50	18,00	12,50	15,00	2,78	21,95	54,88	4,88	0,496	o
	100 µg/mL	11,50	14,00	14,00	13,17	1,44	19,27	48,17	-1,83	0,621	o
EupoffHDMp	25 µg/mL	16,00	17,00	16,00	16,33	0,58	23,90	59,76	9,76	0,005	+
	100 µg/mL	18,50	17,00	19,00	18,17	1,04	26,59	66,46	16,46	0,006	+
ErijapFDMp	25 µg/mL		17,50	16,50	17,00	0,71	24,88	62,20	12,20	0,035	+

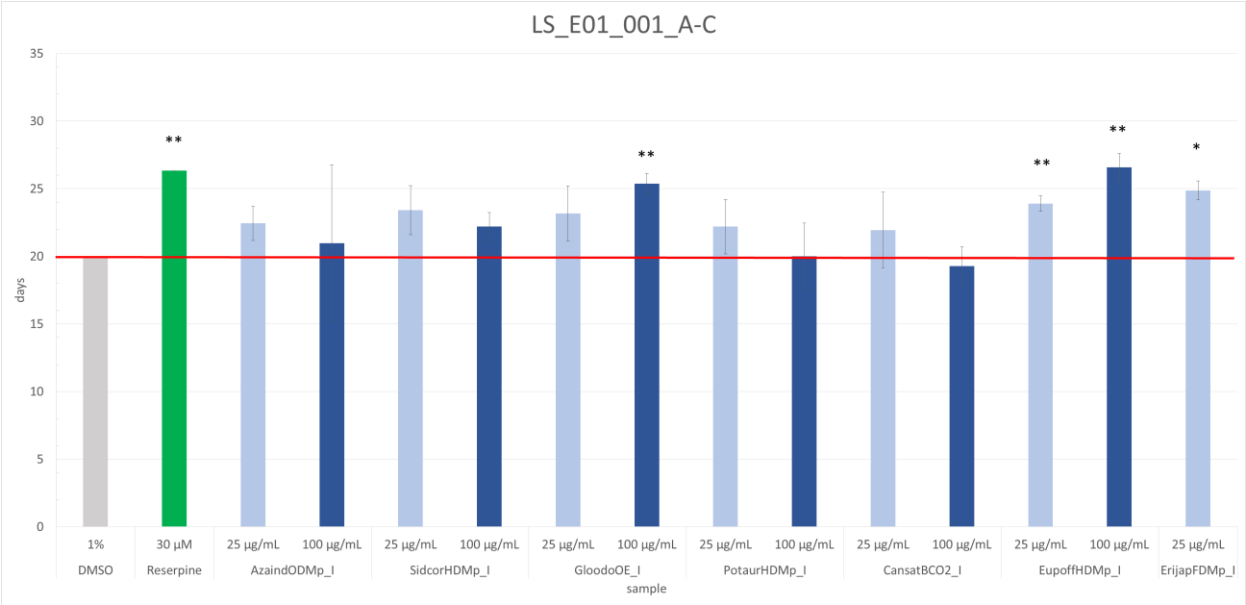


Figure 3 - LS_E01_001_A-C; Bar chart of the mean lifespan of the tested extracts AzaindODMp, SidcorHDMp, GloodoOE, PotaurHDMp, CansatBCO2 and EupoffHDMp (all tested at 25 µg/ml and 100 µg/ml), ErijapFDMp (tested at 25 µg/ml), the positive control (reserpine 30 µM) and the vehicle control (DMSO 1%; DT50 normalised to 20 days, red line). Statistical significance assessed by student's t-test (*p < 0.05, **p < 0.01, ***p <0.001). n = 3

4.1.1.2. Results of LS_E01_002

In the second experiment, besides the positive-control drug reserpine (15.31 %, $p < 0.05$), only ErijapFDMp at 100 µg/ml showed a statistically significant increase of the lifespan of *C. elegans* in comparison to the vehicle control, as mentioned before (17.35 %, $p < 0.05$).

Table 3 - DT_{50} of three parallel experiments LS_E01_002_A, LS_E01_002_B and LS_E01_002_C

Sample	Concentration	DT50			Mean	Stadev.	Data normalised	%	DT50 increase (%)	p-value	Activity
		LS_E01_002_A	LS_E01_002_B	LS_E01_002_C							
DMSO	1%	14,00	17,50	17,50	16,33		20,00	50,00	0,00		
Reserpine	30 µM	19,00	22,50	22,50	21,33	2,02	26,12	65,31	15,31	0,039	o
HumlupFDMp	25 µg/mL	16,50	23,50	23,50	21,17	4,04	25,92	64,80	14,80	0,163	o
	100 µg/mL	16,50	16,00	16,00	16,17	0,29	19,80	49,49	-0,51	0,900	o
PolaviHDMp	25 µg/mL	15,50	23,00	23,00	20,50	4,33	25,10	62,76	12,76	0,233	o
	100 µg/mL	14,50	23,00	23,00	20,17	4,91	24,69	61,73	11,73	0,310	o
PlalanHDMp	25 µg/mL	15,50	22,50	22,50	20,17	4,04	24,69	61,73	11,73	0,240	o
	100 µg/mL	13,00	22,00	22,00	19,00	5,20	23,27	58,16	8,16	0,477	o
AquvulHDMp	25 µg/mL	15,50	20,00	20,00	18,50	2,60	22,65	56,63	6,63	0,321	o
	100 µg/mL	13,00	20,00	20,00	17,67	4,04	21,63	54,08	4,08	0,645	o
LacsatFDMp	25 µg/mL	15,00	20,00	20,00	18,33	2,89	22,45	56,12	6,12	0,387	o
	100 µg/mL	14,50	21,00	21,00	18,83	3,75	23,06	57,65	7,65	0,383	o
SaucosRDMp	25 µg/mL	15,50	22,50	22,50	20,17	4,04	24,69	61,73	11,73	0,240	o
	100 µg/mL	15,00	15,00	15,00	15,00	0,00	18,37	45,92	-4,08	0,371	o
ErijapFDMp	100 µg/mL	-	22,00	22,00	22,00	0,00	26,94	67,35	17,35	0,040	+

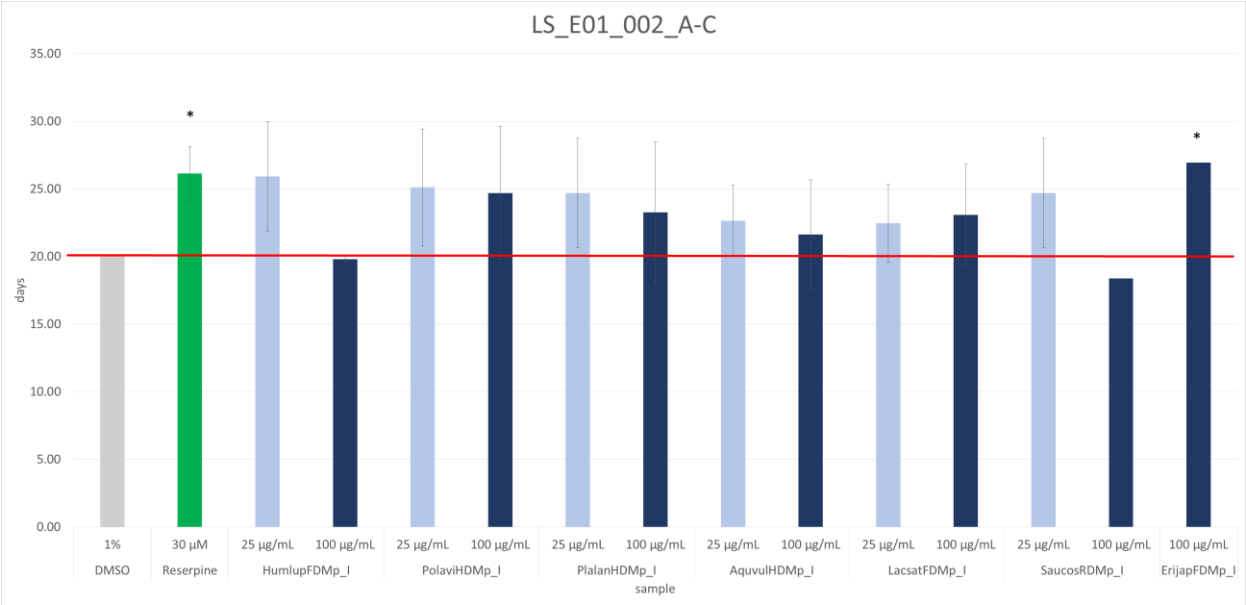


Figure 4 - LS_E01_002_A-C; Bar chart of the mean lifespan of the tested extracts HumlupFDMp, PolaviHDMp, PlalanHDMp, AquvulHDMp, LacsatFDMp, SaucosRDMp (all tested at 25 µg/ml and 100 µg/ml), ErijapFDMp (tested at 100 µg/ml), the positive control (reserpine 30 µM) and the vehicle control (DMSO 1%; DT50 normalised to 20 days, red line). Statistical significance assessed by student's t-test (*p < 0.05, **p < 0.01, ***p <0.001). n = 3

4.1.1.3. Results of LS_E01_003

The first screening of the three experiments LS_E01_003_A/B/C, LS_E01_004_A/B/C, and LS_E01_005_A/B/C could not be evaluated due to various reasons, either

(i) crystallization inside the wells of the microtiter plate for unknown reasons,

(ii) positive control less active than vehicle control DMSO 1 % or

(iii) a contaminated OP50 suspension.

Therefore, those experiments had to be repeated as LS_E01_003_D/E/F, LS_E01_004_ D/E/F and LS_E01_005_ D/E/F, respectively.

The second series of LS_E01_003 yielded promising results that proved to be statistically significant, as seen in Table 4. The extract of *Pimenta dioica* (PimdioODMp) significantly increased the lifespan of the nematodes by 10.22 % ($p < 0.05$) at a concentration of 100 µg/ml. The extract of *Gardenia jasminoides* (GarjasODMp) was shown to increase the lifespan of the

nematodes at both concentrations. However, only at 25 µg/ml significant results could be demonstrated (9.68 % DT_{50} increasement in comparison to DMSO 1 %). Similarly, the extract of *Pterocarpus santalinus* (PtesanXDMp) showed a significant lifespan increasing effect at the lower concentration of 25 µg/ml (15.59 %, $p < 0.01$). This could indicate a nematotoxic component that diminishes the effects of lifespan enhancing components at higher concentrations.

Table 4 - DT50 of three independent experiments LS_E01_003_D, LS_E01_003_E and LS_E01_003_F

Sample	Concentration	DT50			Mean	Stadev.	Data normalised	%	DT50 increase (%)	p-value	Activity
		LS_E01_003_D	LS_E01_003_E	LS_E01_003_F							
DMSO	1%	16,50	16,00	14,00	15,50		20,00	50,00	0,00		
Reserpine	30 µM	23,50	23,50	23,50	23,50	0,00	30,32	75,81	25,81	0,009	+
PimdioODMp	25 µg/mL	16,50	21,50	20,00	19,33	2,57	24,95	62,37	12,37	0,105	o
	100 µg/mL	19,50	19,50	17,00	18,67	1,44	24,09	60,22	10,22	0,049	+
PtesanXDMp	25 µg/mL	19,50	21,50	20,00	20,33	1,04	26,24	65,59	15,59	0,009	+
	100 µg/mL	18,50	19,50	16,50	18,17	1,53	23,44	58,60	8,60	0,086	o
InooblODM	25 µg/mL	17,50	21,00	13,00	17,17	4,01	22,15	55,38	5,38	0,554	o
	100 µg/mL	15,00	16,00	17,00	16,00	1,00	20,65	51,61	1,61	0,631	o
TercheODMp	25 µg/mL	18,00	20,00	13,00	17,00	3,61	21,94	54,84	4,84	0,555	o
	100 µg/mL	16,00	17,50	17,00	16,83	0,76	21,72	54,30	4,30	0,222	o
GarjasODMp	25 µg/mL	17,50	18,00	20,00	18,50	1,32	23,87	59,68	9,68	0,050	+
	100 µg/mL	17,00	20,50	20,00	19,17	1,89	24,73	61,83	11,83	0,058	o
RhorosE	25 µg/mL	18,00	18,00	21,00	19,00	1,73	24,52	61,29	11,29	0,054	o
	100 µg/mL	14,50	14,00	13,50	14,00	0,50	18,06	45,16	-4,84	0,179	o
InooblODM_Pakuso	25 µg/mL	15,50	18,00	21,00	18,17	2,75	23,44	58,60	8,60	0,232	o
	100 µg/mL	14,00	14,00	13,50	13,83	0,29	17,85	44,62	-5,38	0,156	o
InooblODM_EtOH15%	25 µg/mL	17,00	18,00	14,50	16,50	1,80	21,29	53,23	3,23	0,485	o
	100 µg/mL	14,00	14,00	14,00	14,00	0,00	18,06	45,16	-4,84	0,188	o

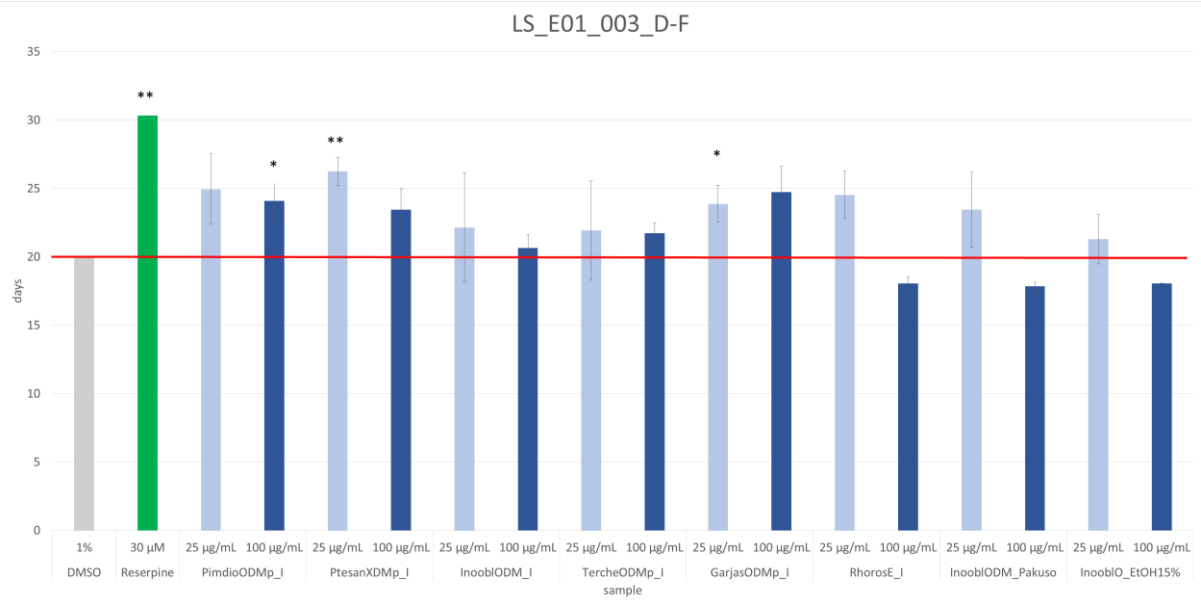


Figure 5 - LS_E01_003_D-F; Bar chart of the mean lifespan of the tested extracts PimdioODMp, PtesanXDMp, InooblODM, TercheODMp, GarjasODMp, RhorosE, InooblODM_Pakuso, InooblODM_EtOH15% (all tested at 25 µg/ml and 100 µg/ml), the positive control (reserpine 30 µM) and the vehicle control (DMSO 1%; DT50 normalised to 20 days, red line). Statistical significance assessed by student's t-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). n = 3

4.1.1.4. Results of LS_E01_004

The screening series LS_E01_004_D/E/F did only yield statistically significant data for the extract of *Syzygium aromatiucm* (SyzaroODMp) at the higher concentration of 100 µg/ml (5.24 % ($p < 0,05$) resulting in an increase of *C. elegans* lifespan. This effect seems to be dose-dependent, which points towards small amounts of lifespan enhancing components in the extract.

Table 5 - DT_{50} of three independent experiments LS_E01_004_D, LS_E01_004_E and LS_E01_004_F

Sample	Concentration	DT50			Mean	Stadev.	Data normalised	%	DT50 increase (%)	p-value	Activity
		LS_E01_004_D	LS_E01_004_E	LS_E01_004_F							
DMSO	1%	17,00	17,50	18,00	17,50		20,00	50,00	0,00		
Reserpine	30 µM	20,00	19,50	18,50	19,33	0,76	22,10	55,24	5,24	0,032	+
PipbetODM	25 µg/mL	17,00	17,00	16,50	16,83	0,29	19,24	48,10	-1,90	0,134	o
	100 µg/mL	18,50	16,00	14,50	16,33	2,02	18,67	46,67	-3,33	0,424	o
FompinODM	25 µg/mL	18,00	15,00	16,00	16,33	1,53	18,67	46,67	-3,33	0,316	o
	100 µg/mL	17,50	14,00	18,00	16,50	2,18	18,86	47,14	-2,86	0,513	o
GanlucDMp	25 µg/mL	17,00	17,00	15,00	16,33	1,15	18,67	46,67	-3,33	0,216	o
	100 µg/mL	18,00	16,50	18,50	17,67	1,04	20,19	50,48	0,48	0,819	o
CaloffBDMp	25 µg/mL	16,00	20,00	16,00	17,33	2,31	19,81	49,52	-0,48	0,913	o
	100 µg/mL	17,50	18,00	18,00	17,83	0,29	20,38	50,95	0,95	0,387	o
ValoffRDMp	25 µg/mL	16,50	16,50	15,00	16,00	0,87	18,29	45,71	-4,29	0,075	o
	100 µg/mL	16,50	16,00	18,00	16,83	1,04	19,24	48,10	-1,90	0,394	o
LicislODMp	25 µg/mL	18,50	18,00	0,00	12,17	10,54	13,90	34,76	-15,24	0,473	o
	100 µg/mL	16,50	16,00	17,50	16,67	0,76	19,05	47,62	-2,38	0,200	o
SyzaroODMp	25 µg/mL	15,00	19,50	17,00	17,17	2,25	19,62	49,05	-0,95	0,824	o
	100 µg/mL	20,00	19,50	18,50	19,33	0,76	22,10	55,24	5,24	0,032	+
AICAR	100 µM	17,50	18,50	19,00	18,33	0,76	20,95	52,38	2,38	0,200	o

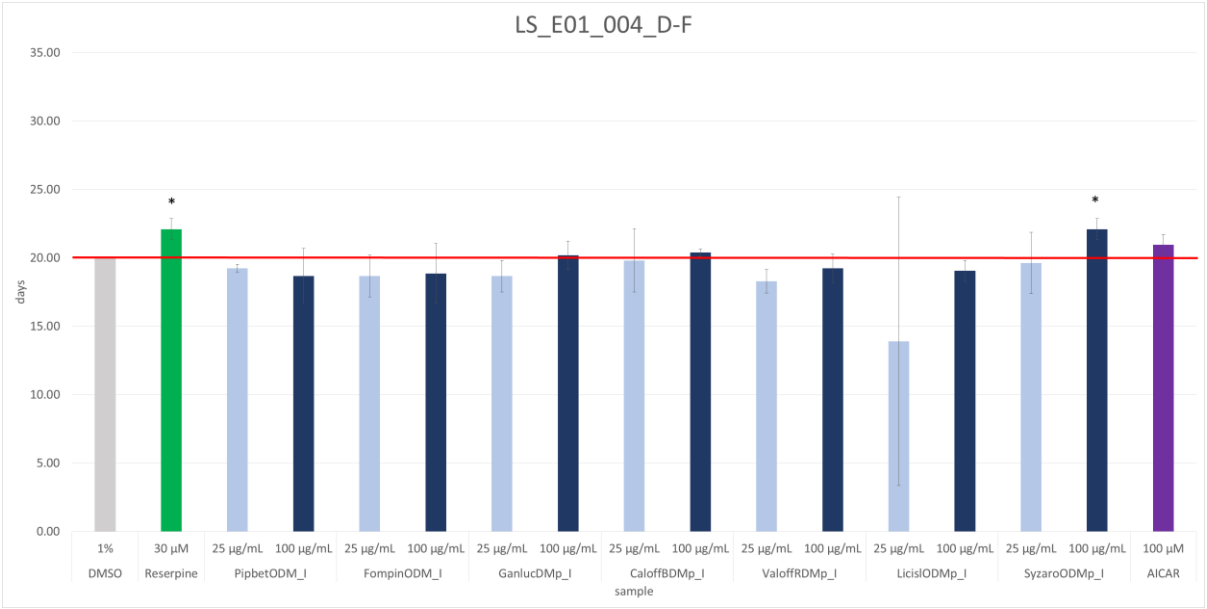


Figure 6 - LS_E01_004_D-F; Bar chart of the mean lifespan of the tested extracts PipbetODM, FompinODM, GanlucDMp, CaloffBDMp, ValoffRDMp, LicislODMp and SyzaroODMp (all tested at 25 µg/ml and 100 µg/ml), the adenosine analogue 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR), the positive control (reserpine 30 µM) and the vehicle control (DMSO 1%; DT50 normalised to 20 days, red line). Statistical significance assessed by student's t-test (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). n = 3

4.1.1.5. Results of LS_E01_005

Repetition of the extract series LS_E01_005 (D-F) revealed a statistically significant lifespan increasement for the screened extracts of *Cynanchum* paniculatum (CynpanRDMp), *Cynanchum stauntonii* (CynstaRDMp) as well as *L. rhinocerus* (LigrhiE_2018; see Table 6). CynstaRDMp increased the DT_{50}-Value by 8.67 % ($p < 0.01$) at a concentration of 100 µg/ml. LigrhiE_2018 and CynpanRDMp on the other hand showed an increase of lifespan at the lower concentration of 25 µg/mL by 11.73 % ($p < 0.01$) and 6.12 % ($p < 0.05$), respectively. It can be speculated that the effect of CynstaRDMp may be caused by compounds that are not as abundant in the plant and therefore need a higher concentration to show an effect. LigrhiE_2018 and CynpanRDMp on the other hand may contain nematotoxic constituents which mitigate the effects of possible lifespan-enhancing constituents at higher concentrations. No significance could be determined for the other tested extracts, as seen in Table 6.

Table 6 – DT_{50} of three independent experiments LS_E01_005_D, LS_E01_005_E and LS_E01_005_F

Sample	Concentration	DT50			Mean	Stadev.	Data normalised	%	DT50 increase (%)	p-value	Activity
		LS_E01_005_D	LS_E01_005_E	LS_E01_005_F							
DMSO	1%	16,00	16,00	17,00	16,33		20,00	50,00	0,00	1,000	
Reserpine	30 µM	19,00	18,50	20,50	19,33	1,04	23,67	59,18	9,18	0,020	+
DryforRDMp	25 µg/mL	19,00	17,00	18,50	18,17	1,04	22,24	55,61	5,61	0,073	o
	100 µg/mL	17,50	16,50	20,00	18,00	1,80	22,04	55,10	5,10	0,246	o
CynpanRDMp	25 µg/mL	19,00	18,00	18,00	18,33	0,58	22,45	56,12	6,12	0,013	+
	100 µg/mL	18,00	17,00	16,00	17,00	1,00	20,82	52,04	2,04	0,387	o
CynstaRDMp	25 µg/mL	16,00	19,00	19,50	18,17	1,89	22,24	55,61	5,61	0,230	o
	100 µg/mL	18,50	19,00	20,00	19,17	0,76	23,47	58,67	8,67	0,008	+
AndpanHDMp	25 µg/mL	19,00	16,00	16,00	17,00	1,73	20,82	52,04	2,04	0,581	o
	100 µg/mL	20,00	17,00	17,00	18,00	1,73	22,04	55,10	5,10	0,232	o
InooblODM_479	25 µg/mL	19,00	17,50	17,00	17,83	1,04	21,84	54,59	4,59	0,113	o
	100 µg/mL	16,50	17,00	20,00	17,83	1,89	21,84	54,59	4,59	0,302	o
LinrhiE_2018	25 µg/mL	20,00	20,50	20,00	20,17	0,29	24,69	61,73	11,73	0,002	+
	100 µg/mL	20,50	17,00	20,00	19,17	1,89	23,47	58,67	8,67	0,112	o
AntcinE_2018	25 µg/mL	19,00	16,00	19,00	18,00	1,73	22,04	55,10	5,10	0,232	o
	100 µg/mL	20,50	17,00	20,00	19,17	1,89	23,47	58,67	8,67	0,112	o
GloodoE0054_2018	25 µg/mL	17,00	17,50	19,50	18,00	1,32	22,04	55,10	5,10	0,148	o
	100 µg/mL	16,50	16,00	15,50	16,00	0,50	19,59	48,98	-1,02	0,493	o

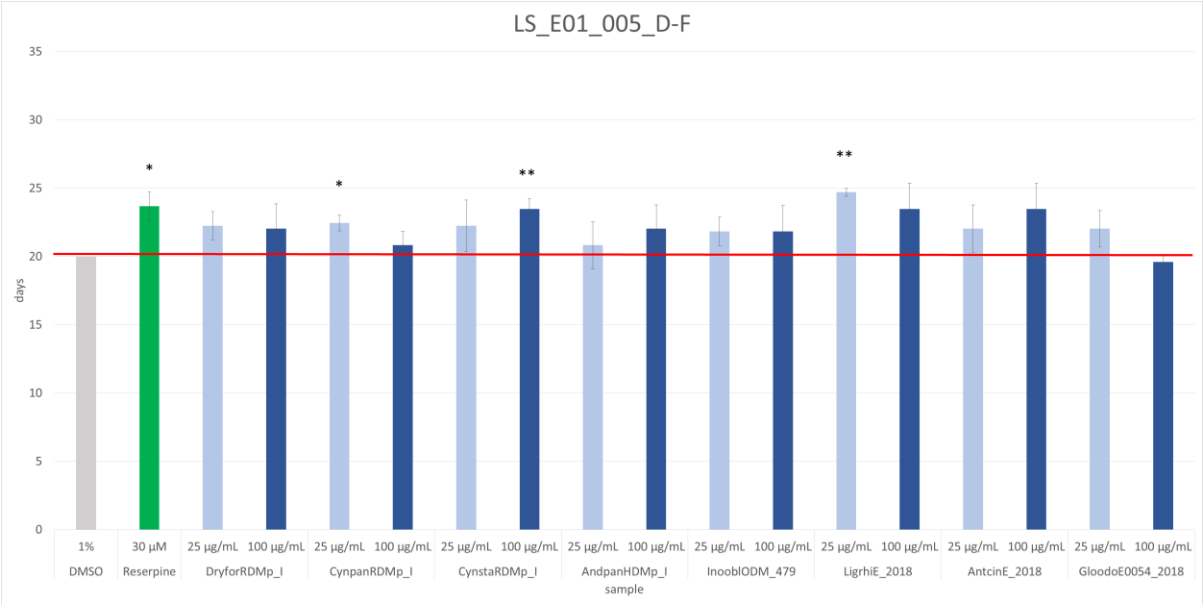


Figure 7 - LS_E01_005_D-F; Bar chart of the mean lifespan of the tested extracts DryforRDMp, CynpanRDMp, CynstaRDMp, AndpanHDMp, InooblODM_479, LinrhiE_2018, AntcinE_2018 and GloodoE0054_2018 (all tested at 25 µg/ml and 100 µg/ml), the positive control (reserpine 30 µM) and the vehicle control (DMSO 1%; DT50 normalised to 20 days, red line). Statistical significance assessed by student's t-test (*p < 0.05, **p < 0.01, ***p <0.001). n = 3

4.2. Mycochemical Investigation of the Lifespan Prolonging Extract of *Lignosus rhinocerus*

4.2.1. Large-Scale Extraction of *Lignosus rhinocerus*

Due to a statistically manifested lifespan increase of the ethanolic small-scale extract of *L. rhinocerus* sclerotia (LigrhiE_2018) a large-scale extraction of *L. rhinocerus* sclerotia was performed. Not only because the extract at 25 µg/ml increased the nematodes lifespan by 11.80 %, but also because a literature research of this mushroom species revealed that there is scarce information regarding the (bioactive) constituents of this mushroom species. Detailed information about extraction preparation is given in Chapter 6.1.1. The final extract yield of LigrhiScE is given in Chapter 6.1.5.

4.2.2. Mychochemical Workflow of the Large-Scale Extract of *Lignosus rhinocerus*

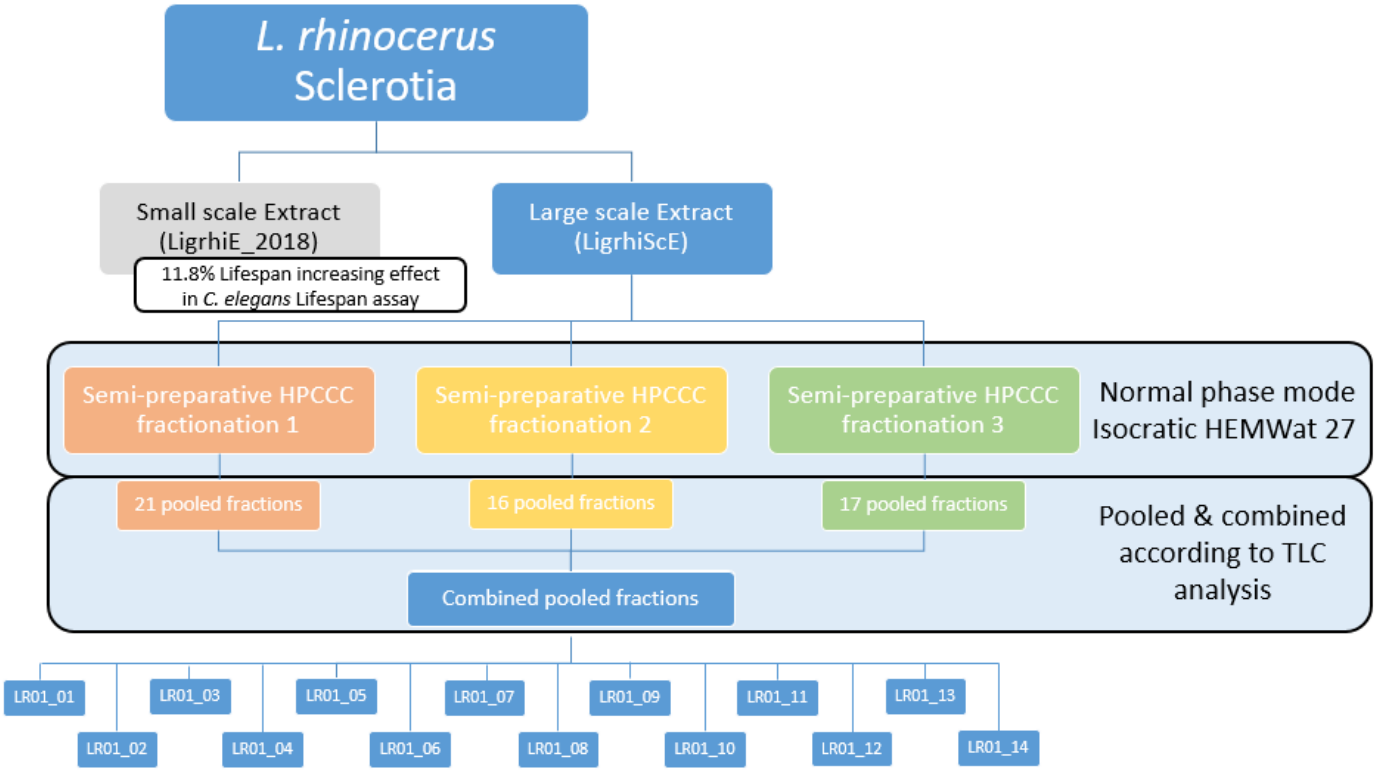


Figure 8 Fractionation tree of *L. rhinocerus* sclerotia

4.2.3. Fractionation of the Large-Scale Extract of *Lignosus rhinocerus* with High-Performance Counter-Current Chromatography

For a bioactivity guided isolation in the *C. elegans* lifespan assay, the large-scale extract of *L. rhinocerus* (LigrhiScE) was further fractioned by HPCCC (see **Chyba! Nenašiel sa žiaden zdroj**

odkazov.). HPCCC was the method of choice since it offers a multitude of advantages compared to HPLC, such as

(i) the possibility of up-scaling the process with ease as well as

(ii) larger sample volumes and

(iii) no loss of substance to adsorbtion to a stationary phase (i.e. column).

Initially, a suitable solvent system for the HPCCC fractionation for LigrhiScE was needed. Therefore the so-called HEMWat solvent system table was chosen (see Table 11, Chapter 6.1.3). Detailed information about the HEMWat solvent system screening procedure is given in chapter 6.1.3. After a comprehensive and detailed screening for an ideal binary solvent system for the HPCCC fractionation, about 250 mg of extract was fractionated with semi-preparative HPCCC. An isocratic, normal-phase mode using HEMWat solvent system No. 27 was used for the separation. All fractions were further analyzed by Thin-Layer Chromatography (TLC) and similar fractions were pooled together (21 pooled fractions of semi-preparative HPCCC fractionation 1). This process was performed in total three times (semi-preparative HPCCC fractionation 2 resulted in 16 pooled fractions and semi-preparative HPCCC fractionation 3 resulted in 17 pooled fractions). Those fractions were again analyzed by TLC and finally combined to 14 fractions, namely LR01_01 – LR01_14. All fraction yields are given in chapter 6.1.5.

4.2.4. TLC of LR01_01 to LR01_14

The resulting HPCCC fractionations LR01_01 – LR01_14 and the large-scale extract LigrhiScE were analyzed by collective-TLC (see Figure 9 - Figure 10). TLC parameters are described in Chapter 6.1.4.

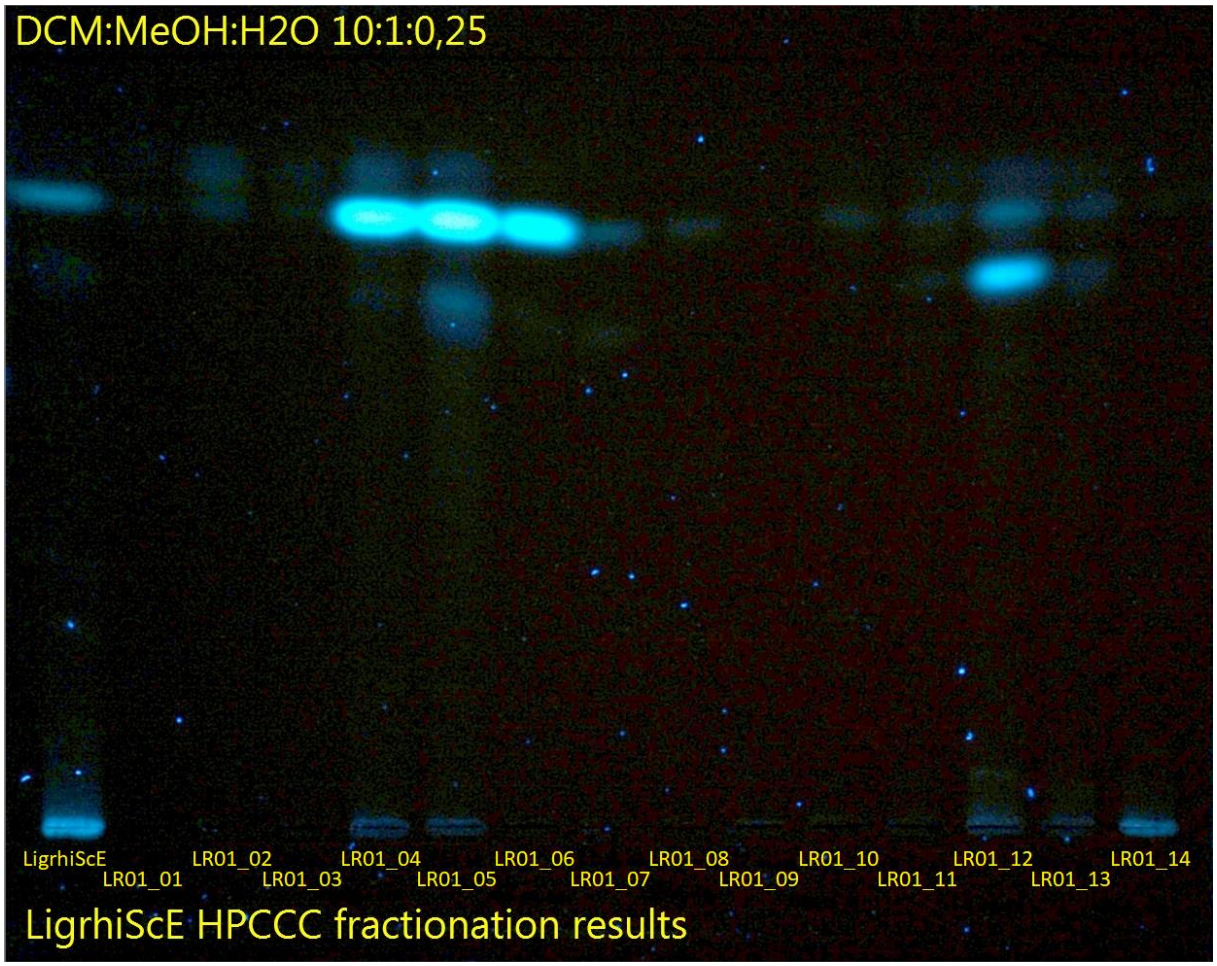


Figure 9 - Collective-TLC analysis of HPCCC fractions LR01_01 – LR01_14;
Detection at UV_{366} nm

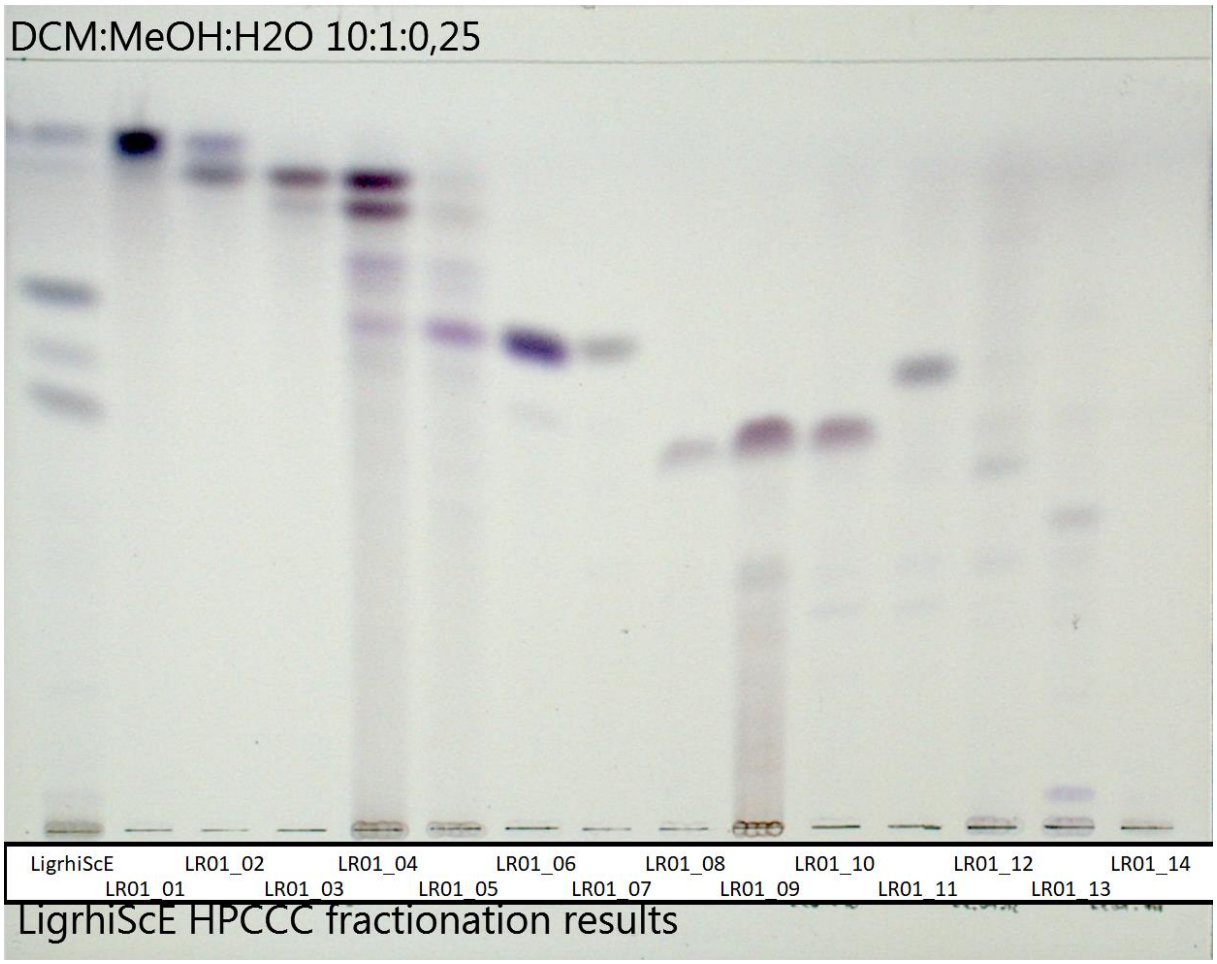


Figure 10 - Collective-TLC analysis of HPCCC fractions LR01_01 – LR01_14;
Detection in VIS after using spraying reagent Vanillin/Sulphuric acid.

After analyzing the HPCCC fractions LR01_01 – LR01_14 by TLC, it became evident that a complete isolation of single constituents could not be achieved. This remains to be a topic for further research at the department. LR01_01 to LR01_14 are mostly fractionated because of their polarity, therefore making a further fractionation and/or isolation easier. It allows for a more specific elucidation of the bioactivities of each respective fraction. In addition to that, a comprehensive analysis using Dionex HPLC connected to a Charged Aerosol Detector (CAD) and Mass-Spectrometer (MS) was performed. LC-MS-CAD parameters are given in 6.1.6.

Due to the low yield of fractions LR01_04, LR01_05 and LR01_12 these were combined with fractions LR01_03, LR01_06 and LR01_13, respectively.

4.2.5. Dereplication of LR01_01 to LR01_14

A dereplication of the generated HPCCC fractions LR01_01 – LR01_14 by UPLC-MS-CAD in both negative and positive mode was performed. The full list of results is provided in Table 7. It can be seen that the previous literature research corresponds to these results, e.g. an abundance of fatty acids, both saturated and unsaturated (i.e. tetracosanoic acid and 13-docosenoic acid) were found. As it is common in fungi, steroid-like compounds have been found as well, i.e. stigmast-4-en-3-one. It was not possible to identify the full secondary metabolite profile, which keeps the door open for certain novelty compounds to be identified in later research.

Table 7 - Dereplication results of HPCCC fractions LR01_01 to LR01_14.

Fraction	$[M+H]^+$	$[M-H]^-$	Rt [min]	Proposed constituents	MW	CAS-Registry Number	Chemical Formula	Reference
LR01_01		279,3	57,06	9,12-Octadecadienoic acid, (9*E*,12*E*)-	280,45	506-21-8		
LR01_02		279,3	57,06	9,12-Octadecadienoic acid, (9*E*,12*E*)-	280,45	506-21-8		
LR01_03	413,34		47,13	Stigmast-4-en-3-one	412,69	1058-61-3	$C_{29}H_{48}O$	Johnathan, Aa, Mf, Sh, & August, 2016
	300,34		37,84	Palmitoylethanolamid	299,49	544-31-0	$C_{18}H_{37}NO_2$	Johnathan, Aa, Mf, Sh, & August, 2016
LR01_04	393,47		53,29	Ergosta-4,6,8(14),22-tetraen-3-one, (22*E*)-	392,62	19254-69-4	$C_{28}H_{40}O$	Johnathan, Aa, Mf, Sh, & August, 2016
	305,44		53,29	5,8,11,14-Eicosatetraenoic acid, (5*Z*,8*Z*,11*Z*,14*Z*)-	304,47	506-32-1	$C_{20}H_{32}O_2$	B. F. Lau, Abdullah, & Aminudin, 2013
LR01_05		367,55	47,45	Tetracosanoic acid	368,64	557-59-5	$C_{24}H_{48}O_2$	B. F. Lau, Abdullah, & Aminudin, 2013
		634,37	50,49	-	-	-	-	-
LR01_06	634,38		51,9	-	-	-	-	-
LR01_07	411,45		46,77	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (6*E*,10*E*,14*E*,18*E*)- // **Squalene**	410,72	111-02-4	$C_{30}H_{50}$	Johnathan, Aa, Mf, Sh, & August, 2016
LR01_08		383,45	52,99	-	-	-	-	-
		397,48	55,88	Ergosta-5,8(14)-dien-3-ol	398,66	2231023-64-4	$C_{28}H_{46}O$	Johnathan, Aa, Mf, Sh, & August, 2016
	328,37		42,76	-	-	-	-	-
	323,22		40,89	-	-	-	-	-
		337,53	51,87	13-Docosenoic acid, (13*Z*)-	338,57	112-86-7	$C_{22}H_{42}O_2$	
LR01_09	281,29		40,78	9,12-Octadecadienoic acid (9*Z*,12*Z*)- // **Linoleic acid**	280,45	60-33-3	$C_{18}H_{32}O_2$	Johnathan, Aa, Mf, Sh, & August, 2016
				9,12-Octadecadienoic acid, (9*E*,12*E*)- // **Linelaidic acid**	280,45	506-21-8	$C_{18}H_{32}O_2$	Johnathan, Aa, Mf, Sh, & August, 2016
		383,41	52,95	-	-	-	-	-
LR01_10		355,37	48,74	-	-	-	-	-
LR01_11		327,42	46,04	4,7,10,13,16,19-Docosahexaenoic acid, (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-	328,49	6217-54-5	$C_{22}H_{32}O_2$	B. F. Lau, Abdullah, & Aminudin, 2013
LR01_12		253,3	42,13	9-Hexadecenoic acid, (9*Z*)-	254,41	373-49-9	$C_{16}H_{30}O_2$	
LR01_13	445	443	39,67	-	-	-	-	-
	380,37	378,4	39,18	-	-	-	-	-
LR01_14		271,76	13,85	Heptadecanoic acid	270,45	506-12-7	$C_{17}H_{34}O_2$	B. F. Lau, Abdullah, & Aminudin, 2013)

4.2.1. Bioactivity Investigations of Acquired Fractions in the *Caenorhabditis elegans* Lifespan Assay

The large-scale extract LigrhiScE as well as the HPCCC fractions LR01_01 – LR01_14 were investigated in the *C. elegans* lifespan assay LS_LR01_001_A/B/C (tested at 25 µg/ml) along with three samples of *Zanthoxylum armatum* DC. (tested at 25 and 100 µg/ml). The leaves, seeds and pericarp of *Z. armatum*, which grows in the medical plant garden of the Department of Pharmacognosy, University of Vienna, were collected and dried at 40 °C for 24 h. LLE extracts from the leaves (ZanarmFDMp_I), seeds (ZanarmSDMp_I) and the pericarp (ZanarmPDMp_I) of *Z. armatum* were produced in the same way as described in the diploma thesis of Wieser, 2019 (see 6.2.2). Extracts yields are given in Chapter 6.1.5. The lifespan assay LS_LR01_001_A/B/C screening yielded intriguing results, as shown in Figure 11. On one hand, the fractions LR01_03 and LR01_07 showed a statistically significant lifespan prolonging effect compared to the vehicle control DMSO 1 % (by 5.41 % and 9.07 %, respectively) as seen in Table 8. Unfortunately, LighriScE did not show any statistically significant activity, on the contrary, it even decreased the lifespan of the nematodes by – 4.90 % (n.s.).

Table 8 - DT_{50} results of LigrhiScE and LR01_01 - LR01_14 in the three parallel experiments LS_LR01_001_A, LS_LR01_001_B and LS_LR01_001_C

Sample	Concentration	DT50			Mean	Stadev.	Data normalised	%	DT50 increase (%)	p-value	Activity
		LS_R01_001_A	LS_R01_001_B	LS_R01_001_C							
DMSO	1%	19.00	20.40	19.30	19.57		20.00	50.00	0.00		
Reserpine	30 µM	24.50	24.33	23.00	23.94	0.82	24.47	61.18	11.18	0.002	+
LigrhiScE	25 µg/mL	22.00	19.95	11.00	17.65	5.85	18.04	45.10	-4.90	0.629	o
LR01_01	25 µg/mL	23.80	21.45	17.10	20.78	3.40	21.24	53.11	3.11	0.602	o
LR01_02	25 µg/mL	23.75	20.95	17.66	20.79	3.05	21.25	53.12	3.12	0.563	o
LR01_03	25 µg/mL	22.50	21.95	20.60	21.68	0.98	22.16	55.41	5.41	0.044	+
LR01_06	25 µg/mL	20.50	21.75	18.50	20.25	1.64	20.70	51.75	1.75	0.561	o
LR01_07	25 µg/mL	22.50	22.85	24.00	23.12	0.78	23.63	59.07	9.07	0.005	+
LR01_08	25 µg/mL	24.50	20.66	19.05	21.40	2.80	21.88	54.69	4.69	0.374	o
LR01_09	25 µg/mL	24.60	21.15	18.33	21.36	3.14	21.83	54.58	4.58	0.428	o
LR01_10	25 µg/mL	20.70	21.30	19.05	20.35	1.17	20.80	52.00	2.00	0.390	o
LR01_11	25 µg/mL	22.00	21.66	18.66	20.77	1.84	21.23	53.08	3.08	0.379	o
LR01_13	25 µg/mL	22.60	22.80	18.66	21.35	2.33	21.83	54.57	4.57	0.315	o
LR01_14	25 µg/mL	20.00	21.10	20.00	20.37	0.64	20.82	52.04	2.04	0.229	o

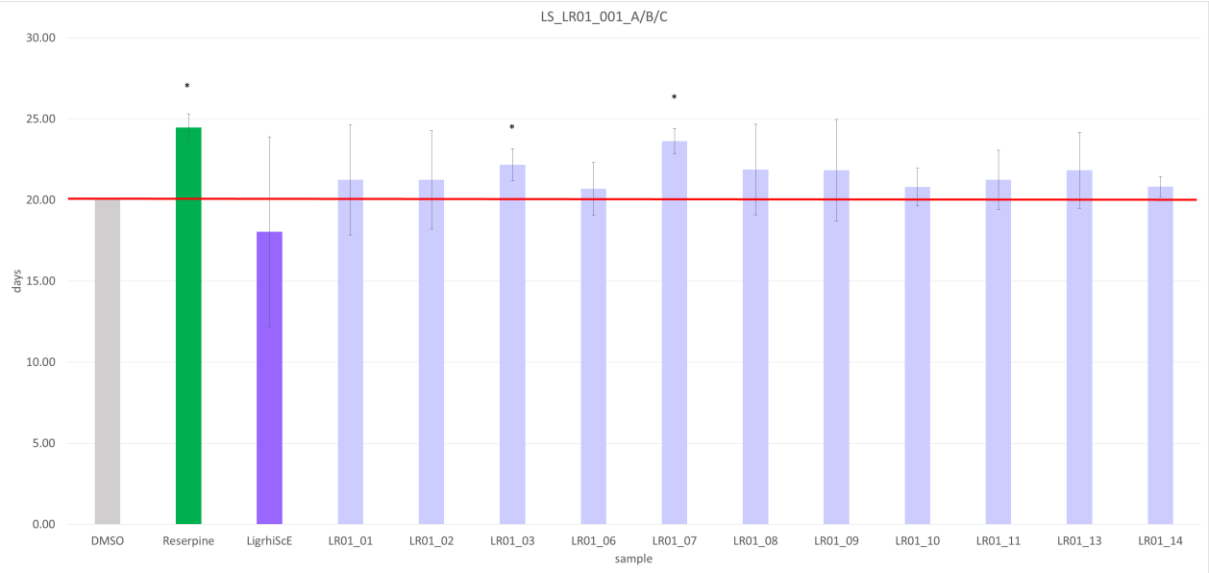


Figure 11 - Bar chart of the mean lifespan of the large-scale extract LigrhiScE, HPCCC fractions LR01_01 – LR01_14 (tested at 25µg/ml), vehicle control (DMSO 1%; DT50 normalised to 20 days, red line) and the positive control (reserpine 30 µM). Statistical significance assessed by student's t-test (*p < 0.05, **p < 0.01, ***p <0.001). n = 3

The results for the *Z. armatum* samples are presented in Table 9 and Figure 12. Both, the LLE extract of the pericarp (i.e. ZanarmPDMp_I) and the seeds (i.e. ZanarmSDMp_I) showed significant effects at 25 µg/ml by increasing the nematodes lifespan by 8.90 % ($p < 0.01$), and 7.28 % ($p < 0.01$), respectively. The leaves of *Z. armatum* (i.e. ZanarmFDMp_I) showed no (significant) effect on the lifespan of *C. elegans* at all.

Table 9 - DT_{50} results of ZanarmFDMp_I, ZanarmSDMp_I and ZanarmPDMp_I in the three parallel experiments LS_LR01_001_A, LS_LR01_001_B and LS_LR01_001_C

Sample	Concentration	DT50			Mean	Stadev.	Data normalised	%	DT50 increase (%)	p-value	Activity
		LS_R01_001_A	LS_R01_001_B	LS_R01_001_C							
DMSO	1%	19.00	20.40	19.30	19.57		20.00	50.00	0.00		
Reserpine	30 µM	24.50	24.33	23.00	23.94	0.82	24.47	61.18	11.18	0.002	+
ZanarmPDMp_I	25 µg/mL	24.00	23.05	22.10	23.05	0.95	23.56	58.90	8.90	0.009	+
	100 µg/mL	23.50	20.25	21.33	21.69	1.66	22.17	55.43	5.43	0.143	o
ZanarmFDMp_I	25 µg/mL	16.50	20.15	21.80	19.48	2.71	19.91	49.79	-0.21	0.963	o
	100 µg/mL	20.75	21.25	18.66	20.22	1.37	20.67	51.67	1.67	0.519	o
ZanarmSDMp_I	25 µg/mL	23.00	22.00	22.25	22.42	0.52	22.91	57.28	7.28	0.007	+
	100 µg/mL	22.15	19.66	18.50	20.10	1.86	20.55	51.37	1.37	0.679	o

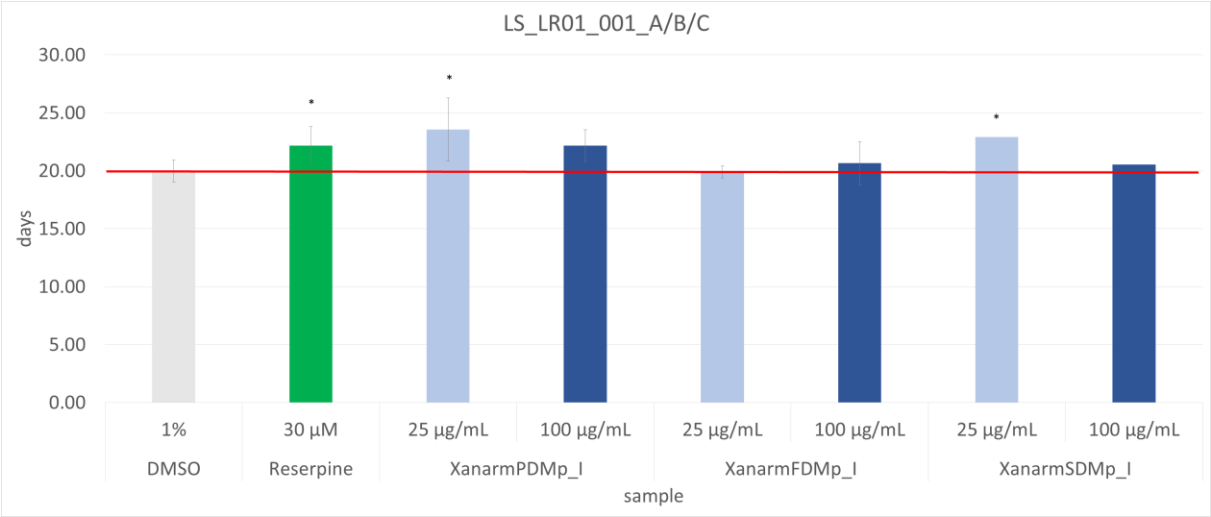


Figure 12 - Bar chart of the mean lifespan of the samples XanarmPDMp_I, XanarmFDMp_I and XanarmSDMp_I (25 and 100 µg/ml, respectively), vehicle control (DMSO 1%; DT50 normalised to 20 days, red line) and the positive control (reserpine 30 µM). Statistical significance assessed by student's t-test (*p < 0.05, **p < 0.01, ***p <0.001). n = 3

5. Conclusion

The phenotypic screening series LS_E01_001 – LS_E01_005 of selected medicinal plants and mushrooms on the lifespan of the nematode *C. elegans* yielded interesting results: out of the 41 tested samples, 11 showed a statistically significant increase of *C. elegans* lifespan as shown in Table 10.

Table 10 - Overview of Plant/Mushroom extracts that showed a statistically increased DT_{50} compared to vehicle control in the *C. elegans* lifespan assay

Species	Organ	Label	DT_{50} increase	p-value
Cynanchum stauntonii	rhizoma	CynstaRDMp	8,76% (100 µM)	p < 0.01
Cynanchum paniculatum	rhizoma	CynpanRDMp	6,12% (25 µM)	p < 0.05
Eriobotrya japonica	folium	PtesanXDMp	12.20 % (25 µM) 17.35 % (100 µM)	p < 0.05 p < 0.05
Euphrasia officinalis	herba	EupoffHDMp	9.76 % (25 µM) 16.46 % (100 µM)	p < 0.01 p < 0.01
Gardenia jasminoides	fructus	GarjasODMp	9.68 % (25 µM,)	p < 0.05
Gloeophyllum odoratum	sporocarpus	GloodoOE	13.41 % (100 µM)	p < 0.01
Lignosus rhinocerus	sclerotium	CynstaRDMp	11.73 % (25 µM)	p < 0.01
Pimenta dioica	fructus	PtesanXDMp	10.22 % (100 µM)	p < 0.05
Pterocarpus santalinus	lignum	PtesanXDMp	15.54 % (25 µM)	p < 0.01
Syzygium aromaticum	flos	SyzaroODMp	5.24 % (100 µM).	p < 0.05
Zanthoxylum armatum	pericarpus	XanarmPDMp	8.93 % (25 µM)	p < 0.05

After the comprehensive screening in the *C. elegans* lifespan assay, a literature research on the 11 plant/mushroom materials with significant lifespan-increasing activity was performed. This research resulted in enhanced curiosity on the medicinal mushroom *L. rhinocerus*, since

(i) the small-scale extract LigrhiE_2018 (screening result shown in LS_E01_005, Chapter 4.1.1.5) had a manifest lifespan increasing potential compared to the vehicle control DMSO 1 %. At 25 µg/ml it significantly increased the nematodes lifespan by 11.73 %. At the higher concentration of 100 µg/ml it increased the lifespan by 8.76 %, though not significantly;

(ii) and its secondary metabolite profile is scarcely investigated until now (effective August 2019).

A large-scale extraction of *L. rhinocerus* sclerotia resulted in 14.5 g of an ethanolic crude extract, labelled as LigrhiScE. Within the mycochemical investigation of LigrhiScE, the extract was further fractionated by HPCCC. The herby generated fractions LR01_01 – LR01_14 were analyzed by TLC and LC-MS-CAD, where it became evident that an isolation of single substances could not be achieved. However, the HPCCC fractions are already separated by similar polarity, making a further isolation of single, bioactive constituents more feasible. The bioactivity of the large-scale extract LigrhiScE and the HPCCC fractions LR01_01 – LR01_14 was examined in the *C. elegans* lifespan assay. A statistical manifested increase in lifespan could be observed for fraction LR01_07 by 9.13 % ($p < 0.05$) as well as for fraction LR01_14 by 2.01% ($p < 0.05$). Unfortunately, a lifespan-increasing effect of LigrhiScE could not be achieved. The abolished lifespan-increasing effect of the large-scale extract leaves room to speculate: this could attribute to

(i) unfortunate variances in the mushroom material, since there are (small) differences in the extraction protocol between the small-scale extract and the large-scale extract, which could influence the composition of the extract, e.g. the small-scale (LigrhiE_2018) was extracted in an ultrasound bath for 15 minutes in ethanol, while the large-scale extract (LigrhiScE) was extracted by means of shaking the material for several days on a nutator.

(ii) variability between the parallel assays LS_LR01_001_A, LS_LR01_001_B and LS_LR01_001_C, which could result on the one hand due to

   a. imprecise work during the set-up of the experiment (e.g. number of worms per well too high/low, non-homogenous samples preparation resulting in an inconsistent test concentration) and/or the analysis of the experiment itself (e.g. between-operator variability, inconsistent feeding of the worms),

   b. or on the other hand due to environmental influences on the assay plates, such as high room temperatures during the counting process or varying shaking-times on the plate shaker.

According to the results of the lifespan assay LS_LR01_001 (see Chapter 4.2.1), it is evident that the DT_{50}s of LigrhiScE varies strongly between 22.00 days (LS_LR01_001_A), 19.95 days (LS_LR01_001_B) and 11.00 days (LS_LR01_001_C), depicted by a big standard deviation of 5.85. A repetition of the assay is currently ongoing at the Department of Pharmacognosy, Vienna. In the subsequent dereplication step, some fatty acids and steroid-like compounds were identified and matched with findings in previous research, while some remained unidentified and are open to novel findings in future studies.

6. Materials and Methods

6.1. Mycochemical Investigation of the Lifespan Prolonging Extract of *Lignosus rhinocerus*

6.1.1. Large-Scale Extraction of *Lignosus rhinocerus*

For the large-scale extraction *of L. rhinocerus* ethanol 96 % was used as an extraction solvent. The sclerotia were ordered from "Tyroler Glückspilze®- Mushroom Production Center GmbH" in Innsbruck, Austria. The material was ground using a grinder. 1041 g of ground sclerotia were divided evenly into two 3000 ml Erlenmayer flasks and a sufficient amount of ethanol (2000 ml each) were used for the first extraction. The flasks were put on a shaker for two days, the suspension was then filtered with a Buchner funnel to obtain the liquid extract. By evaporating the extract on a rotary evaporator, the extraction solvent could be recycled and reused for the second and third extraction of the remaining material. Therefore, the material was divided evenly into three Erlenmayer flasks and the recycled ethanol was added (3000 ml in total) and put on a shaker for three days each. After three days on the shaker the suspension was filtered again using the method mentioned above. This step was repeated for the third extraction of *L. rhinocerus* sclerotia. The used material yielded 14.5 g of crude extract.

6.1.2. Labeling of Samples and Fractions

All small-scale extracts and the large-scale extract were labelled according to the following procedure: the first three letters of the genus and the first three letters of the epithet are used. If there would be an overlapping with other species the third letter is to be skipped and the fourth to be used. X stands for the organ used, e.g. H for Herba, F for Folium, R for Radix/rhizome. We added the abbreviation Sc for Sclerotia, and P for Pericarp. DM stands for the solvents dichloromethane-methanol, E for the solvent ethanol. The letter p stands for a polyamide gel solvent-phase-extraction (SPE). In this way we labelled

(i) the ethanolic extract of the sclerotia of *L. rhinocerus, i.e.* LigrhiScE, and

(ii) the combinded dichloromethane-methanol extract of the leaves, seeds and pericarp of *Z. armatum*, i.e. ZanarmFDMp_I, ZanarmSDMp_I and ZanarmPDMp_I.

The HPCCC fractionation have been labeled LR01_XX, where LR stands for the species *Lignosus rhinocerus*, 01 stands for the first step of fractionation and XX is replaced by each individual fraction in a chronological number.

6.1.3. HPCCC Solvent-System Screening and Method Development

For the screening of a suitable HEMWat system, the following approach was undertaken: An aliquot of the large-scale extract was dissolved in methanol (MeOH) at a concentration of 5 mg/ml (stock solution, SSo). 1.0 ml of the SSo is transferred to a test tube and the other proportions of the regarding HEMWat system were added, e.g. HEMWat system no. 25 consists of 6 parts hexane (*n*-hex), 1 part ethylacetate (EtOAc), 5 parts MeOH and 1 part water (H_2O). Hence, 6 parts *n*-hex, 1 part EtOAc, 5 parts MeOH and 1 part H2O is added to a test tube. One part of the SSo (dissolved in MeOH) is now added. The test tube is shaken to facilitate a distribution of the tested sample between the so generated two layers. 1.0 ml of the upper layer are then transferred to a 1.5 ml Eppendorf test tube for TLC and Ultra-Performance Liquid Chromatography (UPLC) analysis, the rest of the upper layer was removed, and a 1.0 ml of the lower layer was drawn in a similar fashion. For the initial screening, HEMWat Systems No. 10, 13, 15, 17, 19, 21, 24 were prepared according to the protocol described above. The layers of each HEMWat system were then analyzed by TLC to evaluate the distribution of constituents between the two layers. A more-or-less even distribution was desirable, and it became evident that a more non-polar HEMWat solvent system (i.e. HEMWat No. > 17) was needed, as only HEMWat No. 24 exhibited a marginally even distribution of the constituents in the upper and lower layer. For this reason, HEMWat systems No. 25, 26, 27, 28 (see Table 11) were selected for determination of their respective partition coefficients (K_D) for both normal-phase (lower layer serves as stationary phase) and reverse-phase (upper layer serves as stationary phase) on UHPLC.

Table 11 - HEMWat solvent systems 25 - 28

HEMWat No.	n-hex	EtOAc	MeOH	H_2O
25	6	1	6	1
26	9	1	9	1
27	19	1	19	1
28	1	-	1	-

In this manner, UHPLC chromatograms of the upper and lower layer of HEMWat system 25 - 28 of LigrhiScE were obtained, see Figure 13 - Figure 16. The used chromatographic method is described in Chapter 6.1.7.2. The most dominant peaks in the UV chromatogram at a wavelength of 210 nm were selected and numbered (see Figure 17). The difference between a potentially suitable and unsuitable HEMWat system can be visualized with mirrored chromatograms of the two layers of each system. In Figure 13, the separation of the constituents in LigrhiScE by HEMWat system No. 24 are shown. The peaks are unevenly distributed between the two layers, most of the hydrophilic substances are concentrated in the lower phase, while more hydrophobic substances accumulate in the upper layer. In contrast, in Figure 14 we can see HEMWat No. 28 with a more even distribution between the two layers, the more hydrophilic substances retain a higher affinity toward the lower layer, the ratio of distribution is however getting less pronounced. The more hydrophobic substances show a similar trend, pointing us toward a promising HEMWat System. The most promising HEMWat Systems to be selected were Numbers 26, 27 and 28.

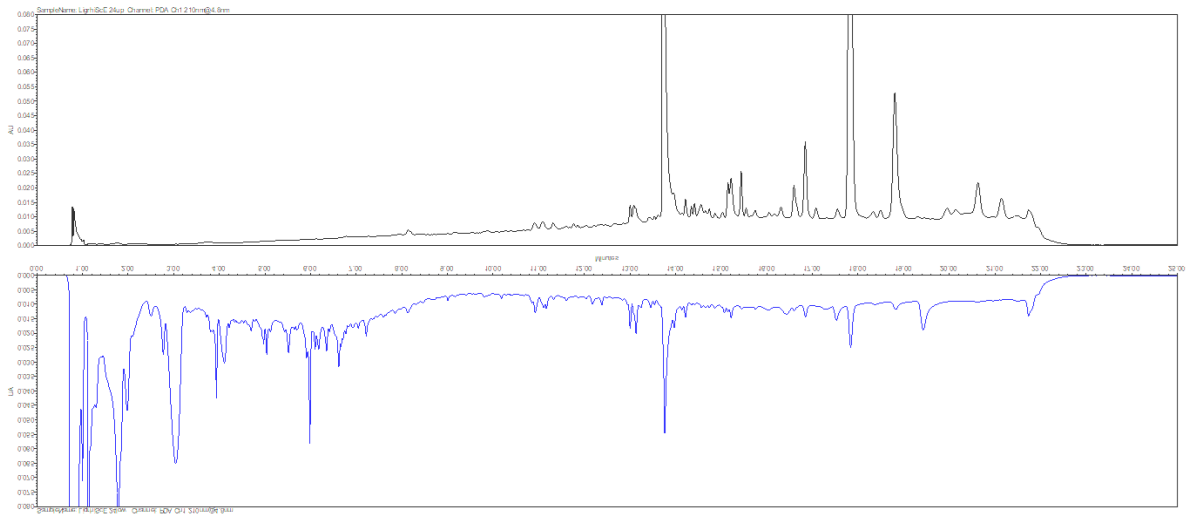


Figure 13 - UV chromatogram at 210nm of upper (black) and lower (blue) layer of HEMWat System No. 24. The chromatogram of the lower layer was flipped horizontally.

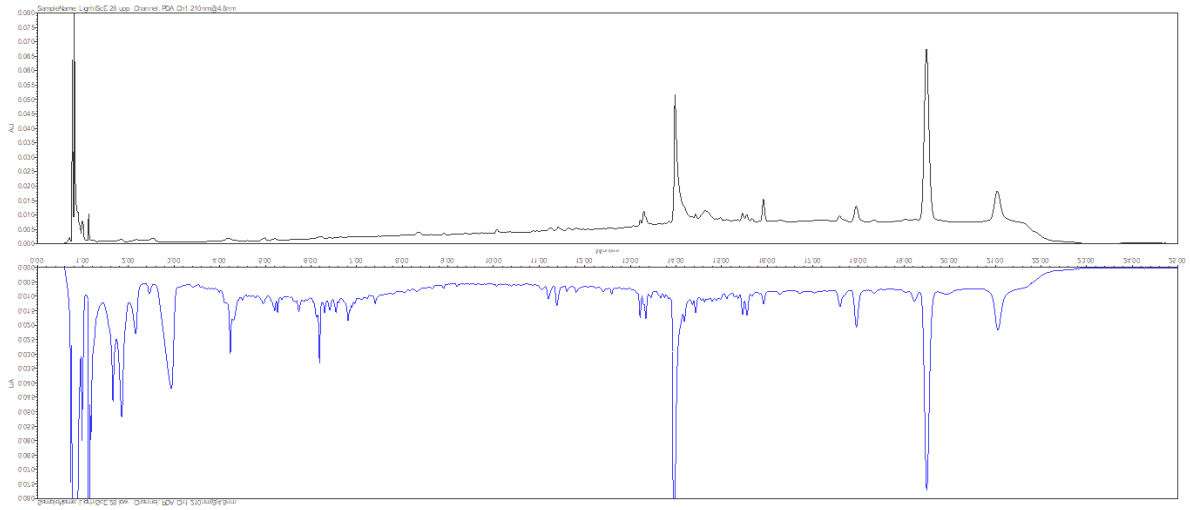


Figure 14 - UV chromatogram at 210nm of upper (black) and lower (blue) layer of HEMWat System No. 28. The chromatogram of the lower layer was flipped horizontally.

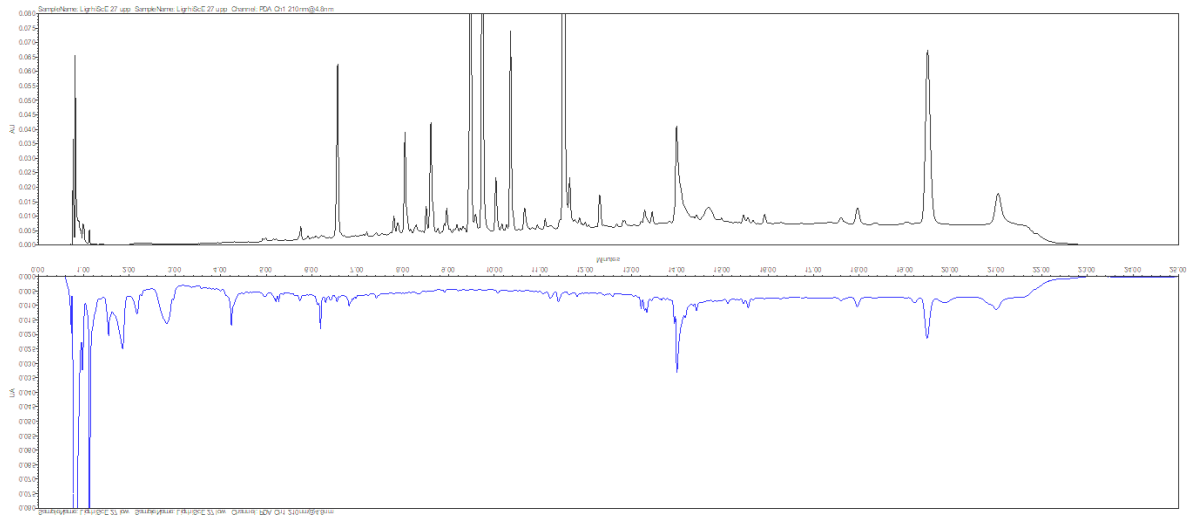


Figure 15 - UV chromatogram at 210nm of upper (black) and lower (blue) layer of HEMWat System No. 27. The sample appears to be contaminated, as is to be seen in the upper layer chromatogram. The chromatogram of the lower layer was flipped horizontally.

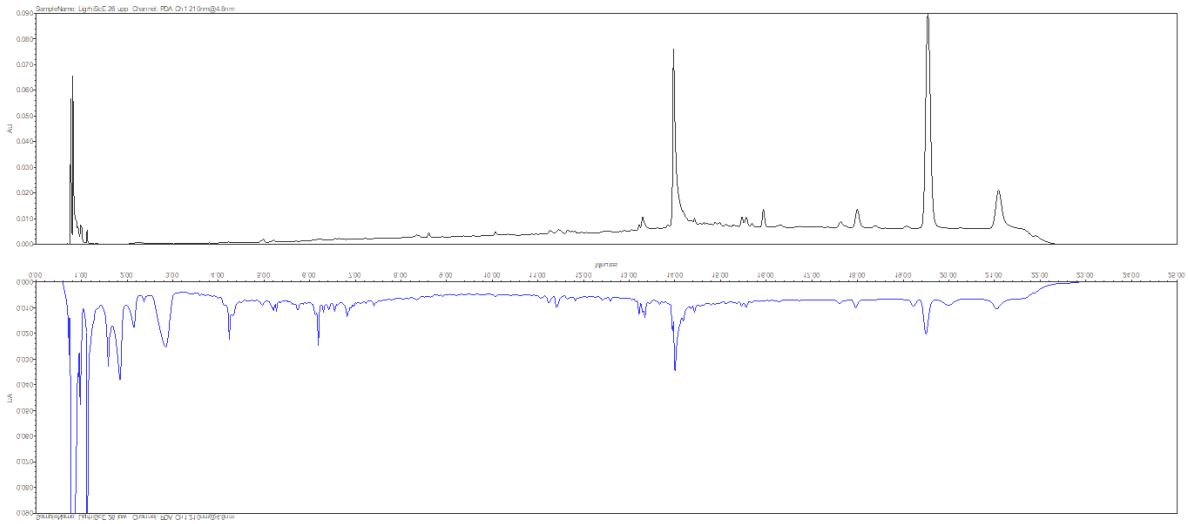


Figure 16 - UV chromatogram at 210nm of upper (black) and lower (blue) layer of HEMWat System No. 26. The chromatogram of the lower layer was flipped horizontally.

Table 12 and Table 13 presents the three HEMWat Systems selected for further analysis. The area under the peak of each of the prominent, chosen peaks was calculated for the upper and lower layer of each HEMWat system. The partition coefficient was then calculated for normal-phase (lower phase/upper phase) and reverse-phase (upper phase/lower phase) mode, respectively, see Table 12 and Table 13. A partition coefficient (K) between 0.6 and 16 ("sweet spot") is desirable for good results (Yang Liu et al., 2016). We opted for a "sweet spot" with values between 0.2 and 5, as well as "sweeter spot" with values between 0.5 and 2.

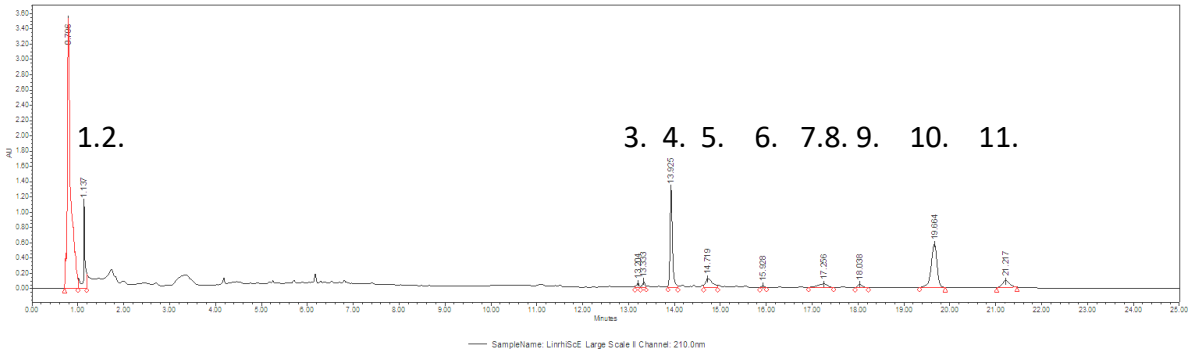


Figure 17 - UV chromatogram of LigrhiScE at 210nm, relevant peaks selected for analysis numbered 1-11.

Table 12 - K_D values of relevant peaks for normal-phase mode. Darker blue indicates better values

Partition coefficient *(K)* for distinct compounds **(normal-phase system)** stationary Phase: **Lower Layer**													
No.	HEMWat No.	HEMWat Solvent System	1	2	3	4	5	6	7	8	9	10	11
1	28	1:0:1:0	11.34	23.88	6.06	2.16	2.06	-	0.73	5.87	2.41	1.15	1.58
2	27	19:1:19:1	9.01	63.03	0.48	1.88	0.92	0.16	0.49	-	0.54	0.22	0.63
3	26	9:1:9:1	10.68	54.24	3.61	1.64	0.65	1.03	0.05	-	0.42	0.16	0.39

Table 13 - K_D values of relevant peaks for reverse-phase mode. Darker blue indicates better values

Partition coefficient *(K)* for distinct compounds **(reverse-phase system)** stationary Phase: **Upper Layer**													
	HEMWat No.	HEMWat Solvent System	1	2	3	4	5	6	7	8	9	10	11
1	28	1:0:1:0	0.09	0.04	0.16	0.46	0.49	-	1.38	0.17	0.41	0.87	0.63
2	27	19:1:19:1	0.11	0.02	2.11	0.53	1.08	6.08	2.04	-	1.84	4.48	1.58
3	26	9:1:9:1	0.09	0.02	0.28	0.61	1.54	0.97	19.65	-	2.37	6.33	2.58

With these findings a HPCCC gradient fractionation for LigrhiScE was developed, starting with HEMWat system No. 28 and gradually increasing the polarity over system No. 27 to 26. Since the resulting HPCCC fractions are easier to process when more volatile solvents are present, a normal-phase mode was used for the fractionation. Therefore the start was to fill the HPCCC coils with the lower, aqueous layer of HEMWat system 28 and after the equilibrium with the upper, organic layer of HEMWat system 28 was reached, the upper layer of system 28, followed by the upper layers of system 27 and 26, 150 ml each, were pumped through the system to facilitate gradient elution in normal-phase mode. After analyzing the procured fractions by TLC, it became obvious that an approach using isocratic elution in normal-phase mode was superior to a gradient elution. Therefore, an isocratic, normal-phase mode with HEMWat solvent system No. 27 was used for the fractionation of the large-scale extract LigrhiScE.

6.1.4. TLC-System for the Large-Scale Extract of *Lignosus rhinocerus* and HPCCC Fractions LR01_01 –LR01_14

For further analysis of the generated large-scale extract as well as the fractions produced by HPCCC fractionation, we used the following TLC System:

Mobile Phase: Dichloromethane/Methanol/Water (10+1+0.25 v/v/v/)

Stationary Phase: TLC Silica gel 60 F_{254}

Detection: Vanillin/Sulfuric acid

TLC plates were developed under chamber saturation, dried and analyzed at UV_{254}, UV_{366} and sprayed with a 1 % solution of vanillin in MeOH and fixed with a 5 % solution of sulphuric acid in MeOH. The plate was then heated to approx. 120 °C.

6.1.5. Extraction and Fraction Yields of *Lignosus rhinocerus*

The large-scale extraction of 1041g yielded 14.5 g of crude extract (LigrhiScE). The HPCCC fractionation of LigrhiScE yielded the following fraction weights (see Table 15).

Table 14 - Fraction yields of LR01_01 to LR01_14

Fraction name	Vial weight [g]	Yield [mg]
LR01_01	6.96045	19.20
LR01_02	6.96089	6.89
LR01_03	7.05214	3.31
LR01_04	7.08611	1.02
LR01_05	6.93763	0.52
LR01_06	6.87350	5.37
LR01_07	6.99076	42.11
LR01_08	6.90203	7.59
LR01_09	6.87143	25.52
LR01_10	7.04124	5.45
LR01_11	6.95990	2.13
LR01_12	6.86659	1.52
LR01_13	7.03110	12.50
LR01_14	6.95678	440.64

Table 15 Fraction yields of combined fractions after HPCCC fractionation

Fraction Name	**Vial Weight [g]**	**Fraction Yield [mg]**
LR01_01	6.96045	17.15
LR01_02	6.96089	5.98
LR01_03+04	7.05214	4.61
LR01_06	6.87350	4.00
LR01_07	6.99076	39.36
LR01_08	6.90203	6.31
LR01_09	6.87143	24.03
LR01_10	7.04124	3.21
LR01_11	6.95990	0.94
LR01_13+12	7.03110	13.19
LR01_14	6.95678	391.27

6.1.6. Method for UHPLC-CAD-MS Investigation of *Lignosus rhinocerus*

Method: GUS_5to98w2ppm_CAD_MS						
instrument	col.	temp. [°C]	flow rate [mL/min]	inj vol [µL]	config.	det. wavel. [nm]
Dionex HPLC-CAD	Agilent Zorbax SB-C18	40	1.0	10	CAD	210

time [min]	mobile phase A [%] H_2O	mobile phase C [%] ACN
0.00	95	5
45.00	2	98
55.00	2	98
66.00	95	5

LTQ XL™ Linear Ion Trap Mass Spectrometer, Thermo Fisher Scientific Inc. Bremen Deutschland.

6.1.7. Chromatographic Methods

6.1.7.1. High Performance Counter-Current Chromatography

Instrument	Mode	Column	Flow rate [ml/min]	Injection volume [ml]	rpm	pressure [psi]
Dynamic Extractions Spectrum	Normal-Phase	Analytical	6	2	1600	143

Instrument	Mode	Column	Flow rate [ml/min]	Injection volume [ml]	rpm	pressure [psi]
Dynamic Extractions Spectrum	Normal-Phase	Semi-Preparative	10	9	1600	187

6.1.7.2. Ultra-High Performance Liquid Chromatography

Method: LigrhiScE_PDA_ELSD_col2_25min

Method ID: 2962

instrument	col.	temp. [°C]	flow rate [mL/min]	inj vol [µL]	config.	det. wavel. [nm]
Waters Aquity H-Class	BEH C18, 100 mm	40	0.3	5	PDA ELSD	210

time [min]	mobile phase A [%] H_2O	mobile phase C [%] ACN
0.00	95	5
0.50	95	5
13.00	2	98
20.00	2	98
20.10	95	5
25.00	95	5

6.2. Phenotypic Screening of Selected Plant/Mushroom Extracts Prolonging the Lifespan of *Caenorhabditis elegans*

6.2.1. Origin of the natural materials

Most of the material for the *C. elegans* extract screening was obtained from Plantasia (wholesale company for Asian medicinal herbs in Oberndorf, Salzburg) or PADMA (wholesale company for Tibetan medicinal herbs in Switzerland), respectively. The major proportion of the mushroom raw material, was collected by Prof. Dr. Ursula Peintner, a collaboration partner at the Institute of Microbiology of the University of Innsbruck, Austria, i.e. sample GLoodoOE, PipbetODM, FompinODM, GanlucODMp, InooblODM and GloodoE0054_20180 (see Chapter 0). The CO_2 extract of *C. sativa*, i.e. CansatBCO2, was kindly provided by CannHelp GmbH, Vienna. The Rhizom of *P. ostruthium* was kindly provided by Kottas Pharma GmbH, Vienna. *I. obliquus* fruiting bodies, extracted and labelled as InooblODM_Pakuso and InooblODM_EtOH15%, were kindly provided by Pakuso LCC, Finland. The samples InooblODM_479, LigrhiE_2018, AntcinE_2018 and LigrhiScE were kindly provides by "Tyroler Glückspilze" - Mushroom Production Center GmbH, Innsbruck. And finally, the *Z. armatum* samples XanarmPDMp_I, XanarmFDMp_I and XanarmSDMp_I were collected by Mag. Julia Zwirchmayr at the medicinal garden of the University of Vienna, Austria.

6.2.2. Small-Scale Extraction

All the small-scale extracts screened in the *C. elegans* lifespan assay were prepared by former diploma student Agnes Wieser, according to the modified protocol of Camp, Davis, Campitelli, Ebdon, & Quinn (2012), with exception to the samples XanarmPDMp_I, XanarmFDMp_I and XanarmSDMp_I, that were generated in the scope of this thesis.

6.2.3. Overview of Investigated Extracts

Table 16 - Information to investigated herbal and fungal material

Species	Name/Pinying name	Organ	Label	Investigated in	Voucher/batch number
Andrographis paniculata (Burm.f.) Nees	Kalmegh, "King of Bitters"	Herba	AndpanHDMp	LS_E01_005	780672
Antrodia cinnamomea T.T. Chang & W.N. Chou	AC fungus	Sporocarpus	AntcinE_2018	LS_E01_005	provided by Mushroom Research Center (Tyrol/A)
Aquilegia vulgaris L.	European columbine	Herba	AquvulHDMp	LS_E01_002	21290300
Azadirachta indica A.Juss.	Neem	Fructus	AzaindODMp	LS_E01_001	21108301
Calendula officinalis L.	Common Marigold	Flos	CaloffBDMp	LS_E01_004	21348300
Cannabis sativa L.	Indian hemp	Flos	CansatBCO2	LS_E01_001	CO_2 extract provided by CannHelp
Cetraria islandica (L.) Ach.	Iceland moss	Lichen	LicislODMp	LS_E01_004	0041257#1
Cynanchum paniculatum (Bunge) Kitag. ex H.Hara	Xu-Chang-Qing	Rhizoma	CynpanRDMp	LS_E01_005	840274
Cynanchum stauntonii (Decne.) Schltr. ex H.Lév	Bai Qjan	Rhizoma	CynstaRDMp	LS_E01_005	310051
Drynaria fortunei (Kunze ex Mett.)	Gu-sui-bu	Rhizoma	DryforRDMp	LS_E01_005	030161
Eriobotrya japonica (Thunb.) Lindl.	Loquat	Folium	ErijapFDMp	LS_E01_001 LS_E01_002	030724
Euphrasia officinalis L.	Eyebright	Herba	EupoffHDMp	LS_E01_001	JR-20120801-B3
Fomitopsis pinicola (Sw.) P. Karst.	Red Belt Conk	Sporocarpus	FompinODM	LS_E01_004	FompinE0010
Ganoderma lucidum (Curtis) P. Karst.	Lingzhi mushroom	Sporocarpus	GanlucODM	LS_E01_004	680898
Gardenia jasminoides J.Ellis	Jasmine	Fructus	GarjasODMp	LS_E01_003	110284

Gloeophyllum odoratum (Wulfen) Imazeki	Anise mazegill	Sporocarpus	GloodoOE	LS_E01_001	JR-20140310-A1
Gloeophyllum odoratum (Wulfen) Imazeki	Anise mazegill	Sporocarpus	GloodoE0054_2018	LS_E01_005	JR-20140310-A1
Humulus lupulus L.	Common hop	Folium	Maceration with ethanol CO2 extract (Diploma thesis Michelle Schraudy)	LS_E01_002	Kottas W12203440
Inonotus obliquus (Ach. ex Pers.) Pilát	Chaga	Sporocarpus	InooblODM InooblODM_Pakuso InooblODM_EtOH15% InooblODM_479	LS_E01_003 LS_E01_003 LS_E01_003 LS_E01_005	UP-20121212-A1
Lactuca sativa subsp. *capitate* (L.) Schübl. & G.Martens	Butterhead lettuce	Folium	LacsatFDMp	LS_E01_002	21400300
Lignosus rhinocerus (Cooke) Ryvarden	Tiger Milk Mushroom	Sclerotia	LigrhiE_2018 LR01_01 – LR01_14 LigrhiScE_LS	LS_E01_005 LR01 Fraction screening	provided by Mushroom Research Center (Tyrol/A) Best before: 31.10.2021
Pimenta dioica (L.) Merr	Allspice	Fructus	PimdioODMp	LS_E01_003	21362100
Piptoporus betulinus (Bull.) P. Karst.	Birch Polypore	Sporocarpus	PipbetODM	LS_E01_004	PipbetE0039
Plantago lanceolata L.	Ribwort plantain	Herba	PlalanHDMp	LS_E01_002	21327101
Polygonum aviculare L.	Common knotgrass	Herba	PolaviHDMp	LS_E01_002	21322100
Potentilla aurea L.	Dwarf yellow cinquefoil	Herba	PotaurHDMp	LS_E01_001	21161301
Pterocarpus santalinus L.f.	Red Sandalwood	Lignum	PtesanXDMp	LS_E01_003	20712307
Rhodiola rosea L.	Golden Root	Radix	RhorosE (70%E)	LS_E01_003	JR_20180904_A1
Saussurea costus (Falc.) Lipsch.	Costus	Radix	SaucosRDMp	LS_E01_002	21280300
Sida cordifolia L.	Flannel weed	Herba	SidcorHDMp	LS_E01_001	20981300
Syzygium aromaticum (L.) Merr. & L.M.Perry	Clove	Flos	SyzaroODMp	LS_E01_004	21321101
Terminalia chebula Retz.	Black Myrobalan	Fructus	TercheODMp	LS_E01_003	21324301

Valeriana officinalis L.	Valerian	Radix	ValoffRDMp	LS_E01_004	KLA50083
Zanthoxylum armatum DC.	Winged prickly ash	Pericarpus Folium Semen	XanarmPDMp_I XanarmFDMp_I XanarmSDMp_I	LR01 Fraction screening	Medicinal plant garden/Dep. of Pharmacognosy UNIVIE, A

6.2.4. Lifespan Assay

For the lifespan assay, N2 strain worms are used. These wild-type nematodes are isolated from the area around Bristol. The assay is performed with some alterations according to the protocol of Solis and Petrascheck (2011), e.g. we perform the assay at 25 °C instead of 20 °C. Cultivation and preparation of media and agar plates are done according the protocol of Stiernagle (2006). Figure 19 and Figure 20 offer a pictorial representation of the workflow of the lifespan assay.

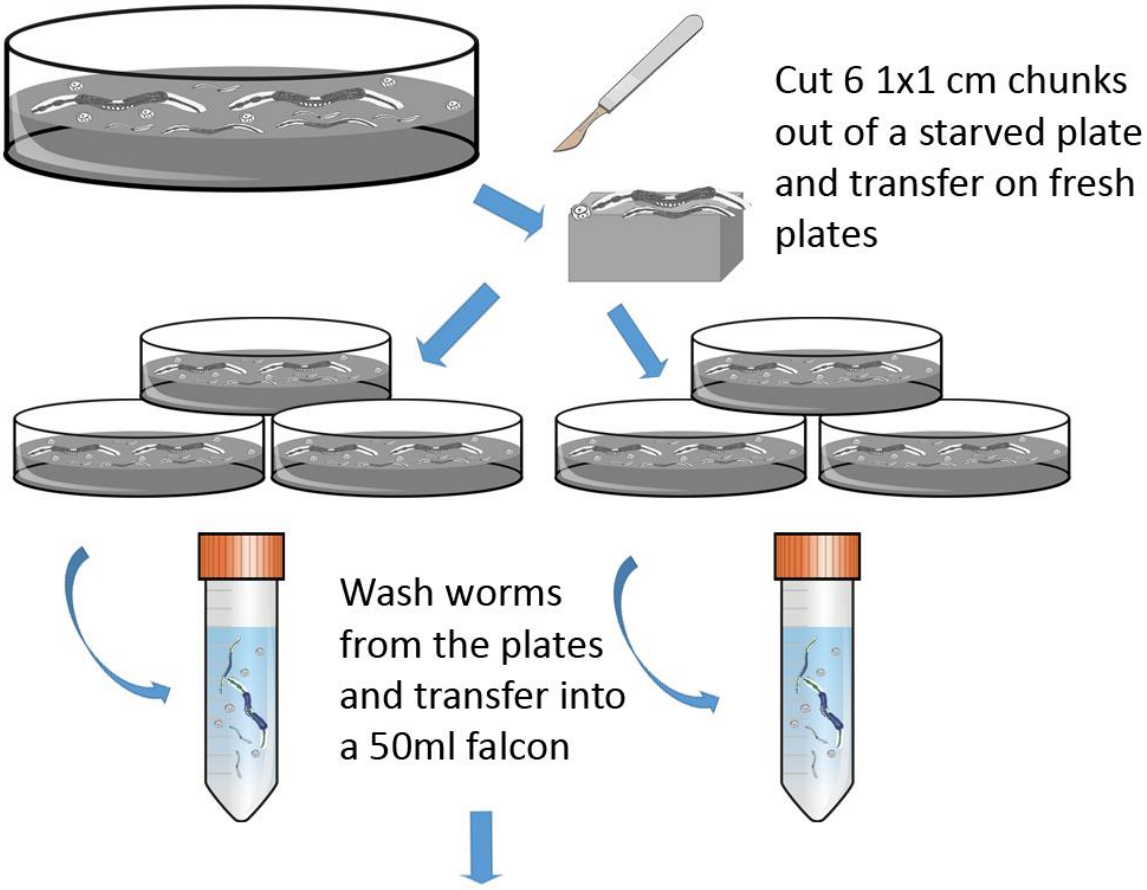


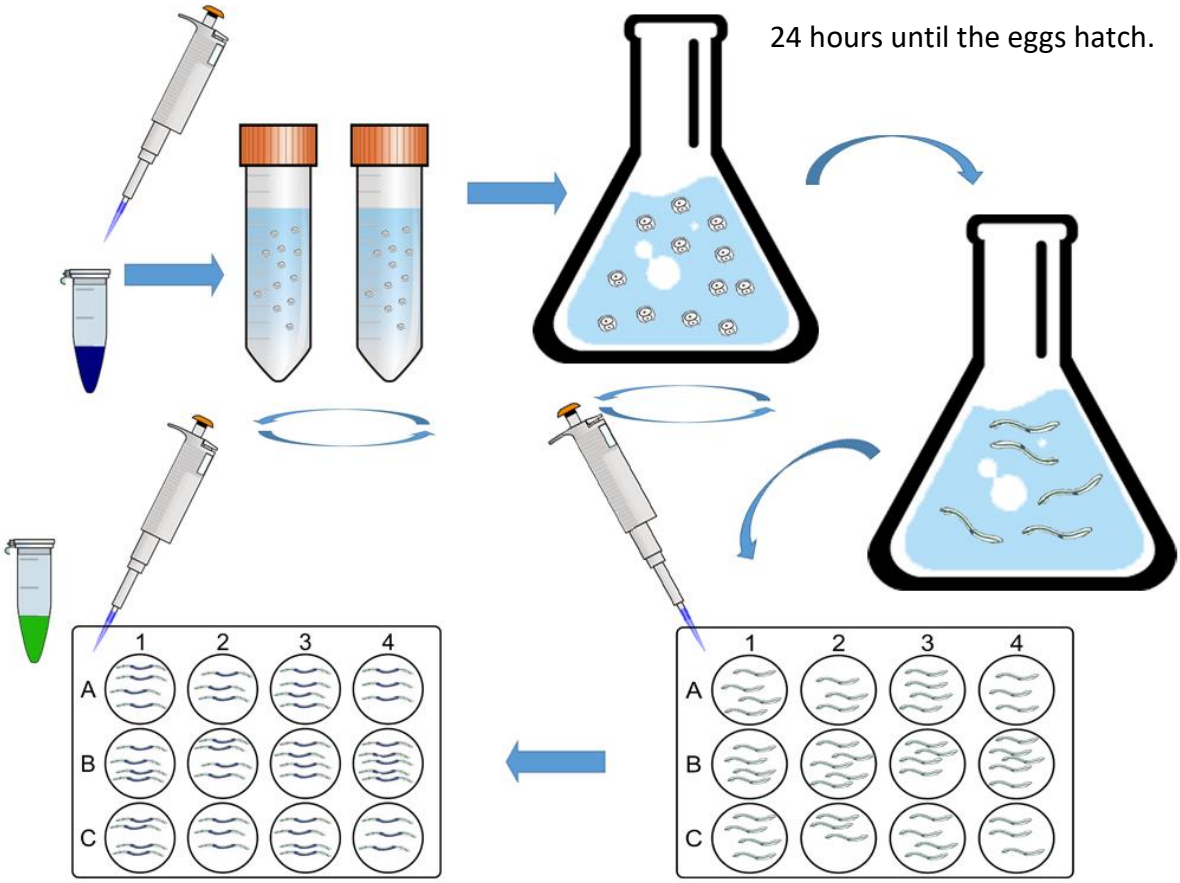
Figure 18 - Schematic diagram of worm cultivation; Workflow steps “chunk” and the start of step “sync”

To start the lifespan assay, on day -6 an older agar plate with a mixed population of worms that are partially starved is cut under sterile conditions into approx. one-by-one cm cubes that are transferred onto four to six fresh agar plates with a lawn of OP50 as a food source and incubated at 25°C for 48 hours (= chunk). On day -3 a mixed population of worms from the previously chunked agar plates is synchronized by incubation with a mixture of hypochlorite and NaOH to isolate the eggs (= sync). These are then incubated on a shaker for 48 h in S-

medium until all worms hatched (= L1 larvae). On day -1 L1 larvae are suspended at a density of 8-18 worms/well and seeded into 96-well plates with OP50 as food source (6 mg/ml). On day 0 L3 worms are sterilized with FUdR to keep the population synchronized.

Add „X-Reagent“ and vortex. Add M9-Buffer when 50% of the worms start to dissolve.

Transfer eggs into a chicane flask and shake at room temperature for approx. 24 hours until the eggs hatch.



At L3 stage add FUdR to keep the population syncronized

Transfer L1 worms into microtiter plates, a worm density of 3-18 worms/well is desired

Figure 19 - Schematic diagram of worm cultivation, end of step “sync” to step “FUdR”

The following day (day 1), test extracts/substances are added to the adult worm culture with DMSO as a carrier at a final concentration of 1 % in the well. Vehicle control worms are treated

with 1 % DMSO. The antipsychotic/anti-hypertensive drug reserpine is used as positive control at a concentration of 30 µM. The number of living worms in each well is counted until more than 50 % of the population is dead (DT_{50}). The worms are oxygenized regularly and bacteria is added on day 5 of adulthood to prevent starvation. The counting process is repeated three times per week.

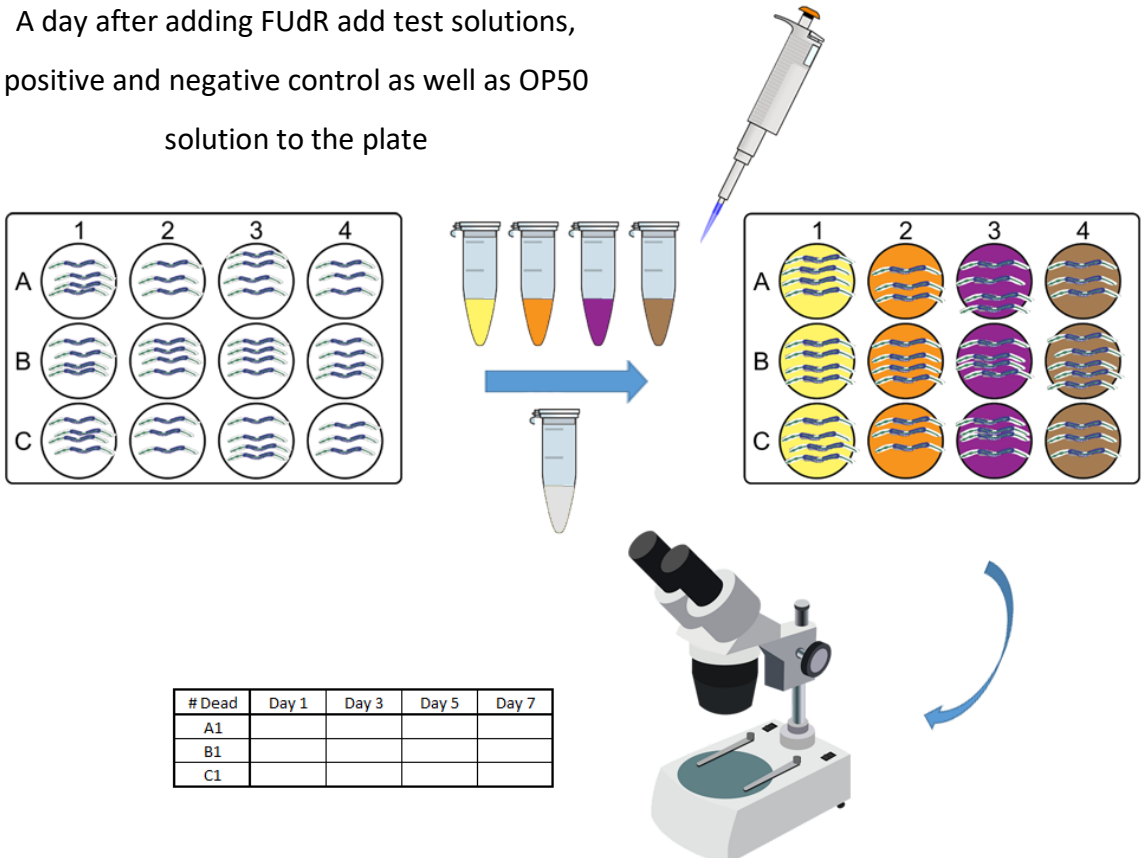


Figure 20 - Schematic diagram of worm cultivation and lifespan assay, steps “drugs” to counting of worms

6.2.5. Sample Preparation for the *Caenorhabditis elegans* Lifespan Assay

Dried extracts were dissolved in 100 % DMSO and diluted with S-Medium to concentrations of 100 and 25 µg/ml with a final concentration of 1 % DMSO in the assay.

6.2.6. Statistical Evaluation

Raw data of the life span assay are fed into an excel sheet to keep track of survival populations in each well. For each well the coordinates in the plate, strain, drug and the total number of animals alive on day 0 (X_0) are recorded 3 times a week. For generation of survival curves the fraction of living worms in each well is given as percentage at each time point. The average percentage is plotted against time starting with time point 0. The time, when 50% of worms per fraction were dead (DT_{50}), was used for the statistical evaluation of the assay. DT_{50}s are given based on the survival curves obtained from three independent experiments. Survival curves are analyzed using standard non-parametric survival statistics. The deviation of lifespan in comparison to the vehicle control is given as 'increase' in percentage including p-value. Significant activity is based on $p \leq 0.05$.

6.3. Instruments, Solvents and Reagents

6.3.1. Instruments

Device	Technical Features / Description
Analytical Balance	BP210D, Sartorius
Balance	Sartorius LC4801P
Centrifuge	Zentrifuge Heraeus Labofuge 4
Electronic Stirrer	Variomag; Electronicrührer MULTIPOINT HP
Grinder	Retsch ZM 100
HPCCC	Dynamic extractions Spectrum
Incubator	Memmert GTR0214
Incubator	Haereus
Laminar Air Flow	Steril Werkbank B10
Microscope	Nikon Labophot-2; Reichert-Jung Neovar 2
Microplate Shaker	IKA MS 3 digital
Ultrasonic Bath	Transsonic T 460, Elma

Mass spectrometer	LTQ XL™ Linear Ion Trap Mass Spectrometer, Thermo Fisher Scientific Inc. Bremen Deutschland.
UPLC	Waters Aquity H-Class
Vortex	Genius 3, IKA
Waterbath Product	Wasserbad GFL 1023 Description
Cell Culture Plate	Biologix 96-well, non-pyrogenic, non-cytotoxic; DNase/RNase/DANN-free; Sterile
Eppendorf Tube	500 Eppendorf Tubes; Safe-Lock Tubes
Falcons	Orange Scientific
Multichannel Pipette	Eppendorf Research (30-300 µL); StarLab (100-1200 µL)
Petri Dishes	Gosselin; Round Petri plate PS na 3 ventsH14
Pipette	Eppendorf Research (1000 µL, 100 µL, 10 µL)
Pipette Boy	Integra Pipetboy 2
Pipette Tips	Eppendorf; Sarstedt; StarLab
Reagent Reservoir	VWR North American Cat. No. 89094-680
Serological Pipette	Gosselin

6.3.2. Solvents and Reagents

Agar-Agar, bacteriological	BioScience; Art.-Nr. 6494.3
Calcium chloride anhydrous	Sigma-Aldrich; C1016-100G
Calciumchloride	Sigma; C1016-100G-D
Cholesterol	Sigma-Aldrich; C8503-25G
Citric acid monohydrate	Sigma; C1909-25G
Copper (II) sulfate pentahydrate	Sigma; C8027-500G
ddH_2O	purified by ion exchanger
DMSO p.a.	Emsure; Merck 1.02952.1000
Ethanol absolute	Sigma-Aldrich; 24194-2.5L-R
Ethylenediaminetetraacetic acid disodium salt dehydrate	E1644-250G
Iron(II) sulfate heptahydrate	Sigma; 215422-250G-D
5-Fluoro-2'-deoxyuridine (FUdR)	Sigma-Aldrich; F0503-250MG
K_2HPO_4	Sigma; P3786-100G-D
KH_2PO_4	Sigma; P5655-500G
Manganese(II) chloride tetrahydrate	Sigma; 63535-50G
Na_2HPO_4	Sigma; 57907-100G
Sodium hydroxide	Merck; 1.06462.1000
Sodium hypochloride	Colgate
Peptone from meat	Sigma-Aldrich; 91249-500G
Potassiium citrate tribasic monohydrate	Sigma; 60153-500G-F
Potassium phosphate dibasic	Sigma-Aldrich; P3786-100G
Potassium phosphate monobasic	Sigma-Aldrich; P5655-500G
Reserpine	Sigma-Aldrich; 83580-1G
Sodium chloride	Sigma-Aldrich; S7653-1KG
Sodium phosphate dibasic	Sigma-Aldrich; S7907-100G
Zinc sulfate heptahydrate	Sigma; Z4750-100G

6.4. Spraying Reagents for TLC Analysis

Vanillin, Sigma-Aldrich; V1104-500G

6.5. TLC-Plates

Merck TLC Silica Gel 60 F_{254} 20 x 20 cm TLC Plates

6.6. HPLC-Column for HPLC-CAD-MS Analysis

Agilent Zorbax SB-C18, 4.6 x 150 mm, 3.5 Micron

6.7. UPLC-Column

Waters ACQUITY UPLC© BEH Phenyl 1.7 µm, 2.1 x 100 mm Column, S/No: 01373502715781

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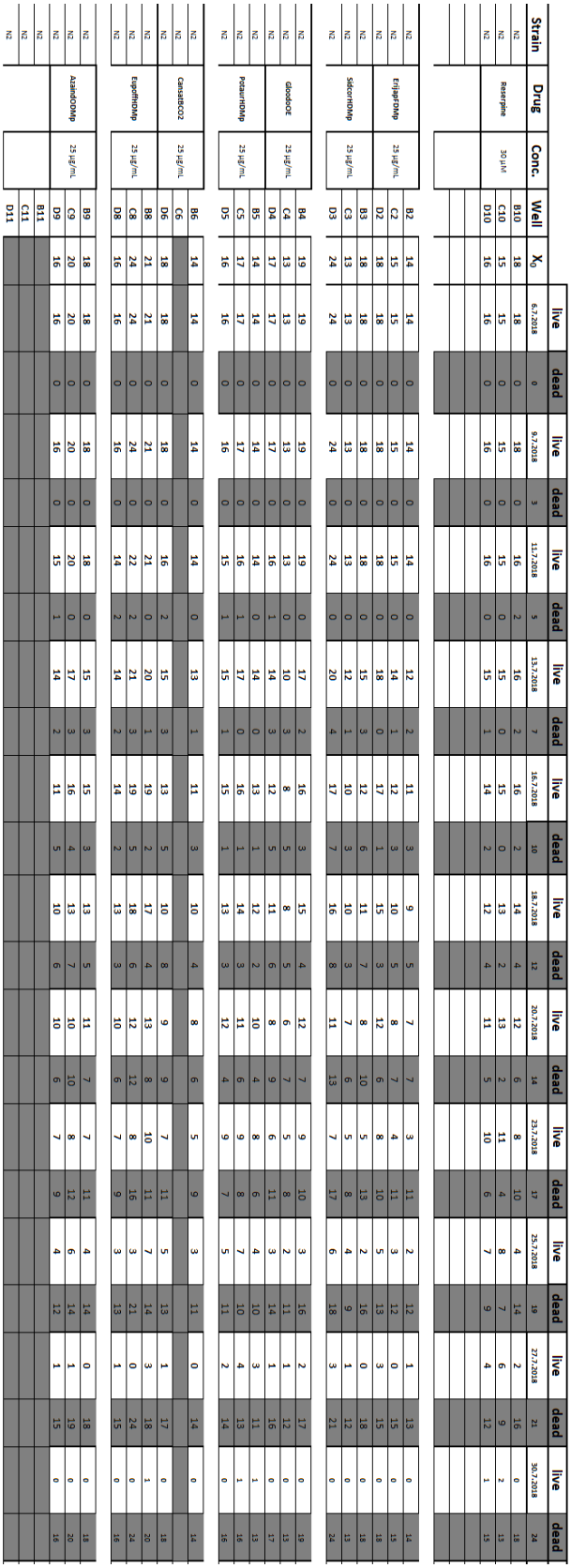
9. Appendix

Table 17 - Counting list of Experiment LS_E01_001 Plate A Part 1



Trial Number: LS_E01_001 A Experimentator: Date: 6.7.2018 Life Span Assay N2

Strain	Drug	Conc.	Well	X_0	Date: 6.7.2018		9.7.2018		11.7.2018		13.7.2018		16.7.2018		18.7.2018		20.7.2018		23.7.2018		25.7.2018		27.7.2018		30.7.2018	
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	Reserpine	30 µM	B10	18	18	0	18	0	16	2	16	2	16	2	14	4	12	6	8	10	4	14	2	16	0	18
N2			C10	15	15	0	15	0	15	0	15	0	13	2	13	2	13	2	11	4	8	7	6	9	2	13
N2			D10	16	16	0	16	0	16	0	15	1	14	2	12	4	11	5	10	6	7	9	4	12	1	15
N2	EnjjapFDMp	25 µg/mL	B2	14	14	0	14	0	14	0	12	2	11	3	9	5	7	7	3	11	2	12	1	13	0	14
N2			C2	15	15	0	15	0	15	0	14	1	12	3	10	5	8	7	4	11	3	12	0	15	0	15
N2			D2	18	18	0	18	0	18	0	18	0	17	1	15	3	12	6	8	10	5	13	3	15	0	18
N2	SdfcerfDMp	25 µg/mL	B3	18	18	0	18	0	18	0	15	3	12	6	11	7	8	10	5	13	2	16	0	18	0	18
N2			C3	13	13	0	13	0	13	0	12	1	10	3	10	3	7	6	5	8	4	9	1	12	0	13
N2			D3	24	24	0	24	0	24	0	20	4	17	7	16	8	11	13	7	17	6	18	3	21	0	24
N2	PmtaurfDMp	25 µg/mL	B5	14	14	0	14	0	14	0	13	1	11	3	10	4	8	6	5	9	3	11	0	14	0	14
N2			C5	17	17	0	17	0	16	1	15	1	15	1	13	3	12	4	9	7	5	11	2	14	0	16
N2			D5	16	16	0	16	0	15	1	15	1	15	1	13	3	12	4	9	7	5	11	2	14	0	16
N2	GlodoDCE	25 µg/mL	B4	19	19	0	19	0	19	0	17	2	16	3	15	4	12	7	9	10	3	16	2	17	0	19
N2			C4	13	13	0	13	0	13	0	10	3	8	5	8	5	6	7	5	8	2	11	1	12	0	15
N2			D4	17	17	0	17	0	16	1	14	3	12	5	11	6	8	9	6	11	3	14	1	16	0	17
N2			B6	14	14	0	14	0	14	0	13	1	11	3	10	4	8	6	5	9	3	11	0	14	0	14
N2	CamsatBSC02	25 µg/mL	C6																							
N2			D6	18	18	0	18	0	16	2	15	3	13	5	10	8	9	9	7	11	5	13	1	17	0	18
N2	EspgdfDMp	25 µg/mL	B8	21	21	0	21	0	21	0	20	1	19	2	17	4	13	8	10	11	7	14	3	18	1	20
N2			C8	24	24	0	24	0	22	2	21	3	19	5	18	6	12	12	8	16	3	21	0	24	0	24
N2			D8	16	16	0	16	0	14	2	14	2	14	2	13	3	10	6	7	9	3	13	1	15	0	16
N2	AzamDDMp	25 µg/mL	B9	18	18	0	18	0	18	0	15	3	15	3	13	5	11	7	7	11	4	14	0	18	0	18
N2			C9	20	20	0	20	0	20	0	17	3	16	4	13	7	10	10	8	12	6	14	1	19	0	20
N2			D9	16	16	0	16	0	15	1	14	2	11	5	10	6	10	6	7	9	4	12	1	15	0	16
N2			B11																							
N2			C11																							
N2			D11																							

Table 18 - Counting list of Experiment LS_E01_001 Plate A Part 2

Life Span Assay N2

Trial Number: LS_E01_001 **A** **Experimentator:** **Date:** 6.7.2018

					Date																							
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	DMSO	1%	E10	15	15	0	15	0	14	1	13	2	12	3	9	6	4	11	4	11	1	14	0	15	0	15
N2			F10	14	14	0	14	0	14	0	13	1	12	2	10	4	6	8	3	11	1	13	1	13	0	14
N2			G10	12	12	0	12	0	12	0	11	1	10	2	7	5	5	7	3	9	2	10	2	10	2	10
N2	SidcorHDMp	100 µg/mL	E3	18	18	0	18	0	18	0	16	2	14	4	11	7	8	10	4	14	1	17	0	18	0	18
N2			F3																							
N2			G3	13	13	0	13	0	13	0	12	1	11	2	8	5	7	6	3	10	0	13	0	13	0	13
N2	GloodoOE	100 µg/mL	E4	10	10	0	10	0	10	0	9	1	9	1	9	1	6	4	4	6	3	7	3	7	3	7
N2			F4	21	21	0	21	0	21	0	19	2	17	4	12	9	11	10	8	13	7	14	4	17	3	18
N2			G4	15	15	0	15	0	15	0	14	1	14	1	13	2	13	2	8	7	4	11	2	13	0	15
N2	PotaurHDMp	100 µg/mL	E5	15	15	0	15	0	15	0	14	1	13	2	8	7	5	10	1	14	0	15	0	15	0	15
N2			F5	25	25	0	25	0	23	2	22	3	19	6	13	12	9	16	5	20	1	24	1	24	0	25
N2			G5	19	19	0	19	0	18	1	14	5	12	7	9	10	6	13	3	16	1	18	0	19	0	19
N2	CansatBCO2	100 µg/mL	E6	18	18	0	18	0	18	0	17	1	16	2	12	6	10	8	8	10	7	11		18	0	18
N2			F6	11	11	0	11	0	8	3	8	3	6	5	3	8	3	8	1	10	0	11	0	11	0	11
N2			G6	17	17	0	17	0	17	0	14	3	13	4	7	10		17		17		17		17	0	17
N2			E7																							
N2			F7																							
N2			G7																							
N2	EupoffHDMp	100 µg/mL	E8	22	22	0	22	0	22	0	20	2	17	5	17	5	15	7	11	11	8	14	5	17	2	20
N2			F8	20	20	0	20	0	19	1	18	2	17	3	16	4	13	7	9	11	6	14	3	17	1	19
N2			G8	18	18	0	18	0	18	0	18	0	18	0	17	1	16	2	12	6	8	10	4	14	1	17
N2	AzaindODMp	100 µg/mL	E9	20	20	0	20	0	19	1	16	4	14	6	12	8	8	12	4	16	1	19	0	20	0	20
N2			F9	13	13	0	13	0	13	0	10	3	7	6	5	8	2	11	1	12	0	13	0	13	0	13
N2			G9	12	12	0	12	0	12	0	10	2	8	4	4	8	2	10	0	12	0	12	0	12	0	12
N2	DMSO	1%	E11	17	17	0	17	0	16	1	14	3	14	3	11	6	8	9	4	13	3	14	0	17	0	17
N2			F11	16	16	0	16	0	16	0	13	3	12	4	8	8	7	9	7	9	4	12	1	15	0	16
N2			G11	20	20	0	20	0	19	1	18	2	14	6	11	9	6	14	5	15	3	17	1	19	0	20

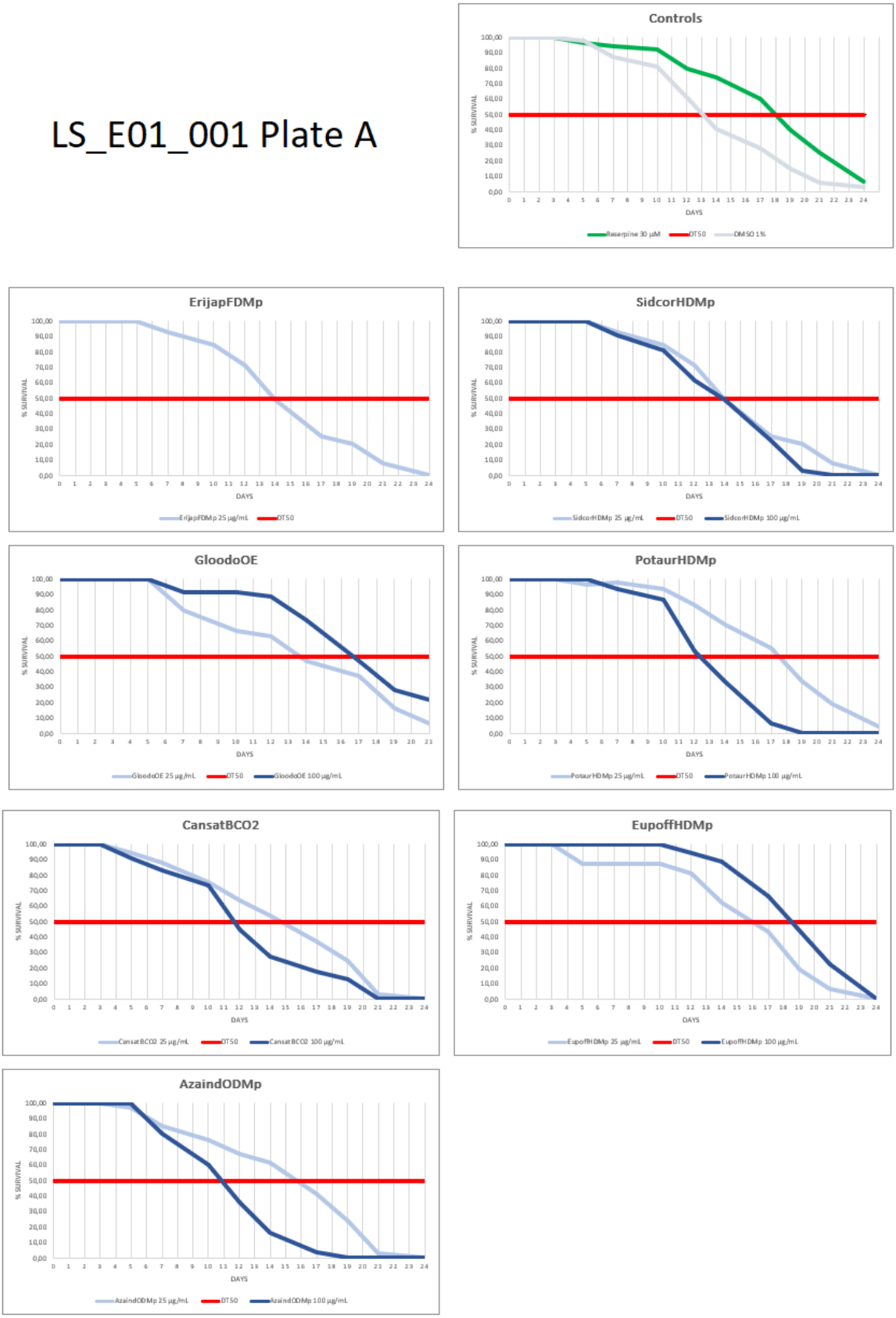


Figure 21 - Lifespan screening assay LS01_001 Plate A Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 19 - Counting list of Experiment LS_E01_001 Plate B Part 1

Life Span Assay N2

Trial Number: LS_E01_001 **B** **Experimentator:** **Date:** 6.7.2018

					Date																							
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	**Drug**	**Conc.**	**Well**	**X_0**	6.7.2018	0	9.7.2018	3	11.7.2018	5	13.7.2018	7	16.7.2018	10	18.7.2018	12	20.7.2018	14	23.7.2018	17	25.7.2018	19	27.7.2018	21	30.7.2018	24
N2			B10	17	17	0	17	0	17	0	17	0	16	1	16	1	14	3	11	6	8	9	5	12	1	16
N2	Reserpine	30 µM	C10	14	14	0	14	0	13	1	13	1	11	3	11	3	9	5	8	6	5	9	3	11	2	12
N2			D10	16	16	0	16	0	16	0	14	2	13	3	13	3	12	4	9	7	7	9	4	12	0	16
N2			B2	20	20	0	20	0	20	0	20	0	20	0	17	3	12	8	8	12	5	15	3	17	3	17
N2	ErijapFDMp	25 µg/mL	C2	13	13	0	13	0	13	0	12	1	11	2	11	2	10	3	8	5	3	10	3	10	2	11
N2			D2	24	24	0	24	0	24	0	22	2	22	2	18	6	16	8	11	13	6	18	6	18	5	19
N2			B3	15	15	0	15	0	14	1	13	2	11	4	8	7	5	10	2	13	0	15	0	15	0	15
N2	SidcorHDMp	25 µg/mL	C3	21	21	0	21	0	20	1	18	3	16	5	14	7	12	9	7	14	3	18	1	20	0	21
N2			D3	15	15	0	15	0	15	0	14	1	14	1	10	5	8	7	7	8	4	11	1	14	0	15
N2			B4	14	14	0	14	0	14	0	12	2	10	4	9	5	7	7	7	7	5	9	4	10	3	11
N2	GloodoOE	25 µg/mL	C4	22	22	0	22	0	22	0	21	1	20	2	15	7	13	9	9	13	6	16	2	20	0	22
N2			D4	25	25	0	25	0	25	0	22	3	21	4	15	10	14	11	11	14	8	17	6	19	3	22
N2			B5	23	23	0	23	0	23	0	21	2	20	3	17	6	12	11	12	11	5	18	3	20	0	23
N2	PotaurHDMp	25 µg/mL	C5	19	19	0	19	0	19	0	17	2	17	2	14	5	13	6	10	9	7	12	2	17	0	19
N2			D5	17	17	0	17	0	16	1	15	2	13	4	12	5	8	9	6	11	5	12	2	15	0	17
N2			B6	9	9	0	9	0	9	0	8	1	7	2	5	4	5	4	3	6	2	7	1	8	0	9
N2	CansatBCO2	25 µg/mL	C6	5	5	0	5	0	5	0	5	0	5	0	5	0	4	1	4	1	3	2	2	3	0	5
N2			D6	21	21	0	21	0	21	0	20	1	17	4	15	6	11	10	7	14	5	16	1	20	0	21
N2			B8	15	15	0	15	0	15	0	14	1	13	2	11	4	10	5	7	8	4	11	1	14	0	15
N2	EupoffHDMp	25 µg/mL	C8	14	14	0	14	0	14	0	11	3	9	5	9	5	8	6	8	6	3	11	2	12	0	14
N2			D8	22	22	0	22	0	22	0	20	2	17	5	15	7	11	11	7	15	5	17	2	20	0	22
N2			B9	12	12	0	12	0	12	0	11	1	11	1	10	2	8	4	7	5	7	5	3	9	0	12
N2	AzaindODMp	25 µg/mL	C9	20	20	0	20	0	20	0	17	3	14	6	12	8	9	11	6	14	4	16	3	17	1	19
N2			D9	18	18	0	18	0	18	0	16	2	14	4	13	5	10	8	7	11	5	13	1	17	0	18
N2			B11																									
N2			C11																									
N2			D11																									

Table 20 - Counting list of Experiment LS_E01_001 Plate B Part 2

Life Span Assay N2

Trial Number: LS_E01_001 **B** **Experimentator:** **Date:** 6.7.2018

					Date																							
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	DMSO	1%	E10	21	21	0	21	0	21	0	20	1	20	1	17	4	14	7	10	11	7	14	3	18	0	21			
N2			F10	16	16	0	16	0	16	0	14	2	14	2	13	3	11	5	8	8	4	12	1	15	0	16			
N2			G10	17	17	0	17	0	17	0	15	2	14	3	11	6	8	9	6	11	6	11	2	15	0	17			
N2	SidcorHDMp	100 µg/mL	E3	25	25	0	25	0	25	0	23	2	19	6	15	10	11	14	7	18	6	19	2	23	0	25			
N2			F3	14	14	0	14	0	14	0	14	0	13	1	13	1	12	2	9	5	7	7	1	13	0	14			
N2			G3	18	18	0	18	0	18	0	17	1	15	3	15	3	8	10	5	13	3	15	2	16	0	18			
N2	GloodoOE	100 µg/mL	E4	13	13	0	13	0	13	0	13	0	12	1	12	1	10	3	9	4	7	6	5	8	3	10			
N2			F4	17	17	0	17	0	17	0	16	1	15	2	14	3	11	6	8	9	8	9	6	11	4	13			
N2			G4	21	21	0	21	0	21	0	21	0	21	0	17	4	14	7	11	10	7	14	4	17	2	19			
N2	PotaurHDMp	100 µg/mL	E5	21	21	0	21	0	19	2	19	2	16	5	12	9	8	13	7	14	4	17	1	20	0	21			
N2			F5	21	21	0	21	0	21	0	19	2	17	4	14	7	11	10	9	12	6	15	2	19	0	21			
N2			G5	26	26	0	26	0	23	3	23	3	23	3	18	8	13	13	9	17	5	21	3	23	0	26			
N2	CansatBCO2	100 µg/mL	E6	18	18	0	18	0	18	0	18	0	16	2	13	5	8	10	5	13	5	13	2	16	0	18			
N2			F6	14	14	0	14	0	12	2	11	3	10	4	8	6	8	6	5	9	3	11	1	13	0	14			
N2			G6	20	20	0	20	0	20	0	18	2	14	6	11	9	9	11	7	13	4	16	1	19	0	20			
N2			E7																									
N2			F7																									
N2			G7																									
N2	EupoffHDMp	100 µg/mL	E8	15	15	0	15	0	15	0	13	2	12	3	9	6	9	6	7	8	5	10	5	10	2	13			
N2			F8	25	25	0	25	0	25	0	24	1	23	2	18	7	13	12	10	15	7	18	3	22	2	23			
N2			G8	16	16	0	16	0	16	0	16	0	16	0	15	1	13	3	8	8	6	10	3	13	2	14			
N2	AzaindODMp	100 µg/mL	E9	14	14	0	14	0	13	1	11	3	8	6	5	9	3	11	1	13	0	14	0	14	0	14			
N2			F9	11	11	0	11	0	11	0	10	1	5	6	4	7	3	8	2	9	1	10	1	10	0	11			
N2			G9	11	11	0	11	0	10	1	9	2	9	2	6	5	5	6	3	8	1	10	0	11	0	11			
N2	DMSO	1%	E11	20	20	0	20	0	20	0	18	2	17	3	15	5	11	9	5	15	2	18	1	19	0	20			
N2			F11	21	21	0	21	0	21	0	19	2	16	5	12	9	9	12	6	15	4	17	2	19	0	21			
N2			G11	18	18	0	18	0	18	0	15	3	13	5	9	9	7	11	4	14	4	14	1	17	0	18			

LS_E01_001 Plate B

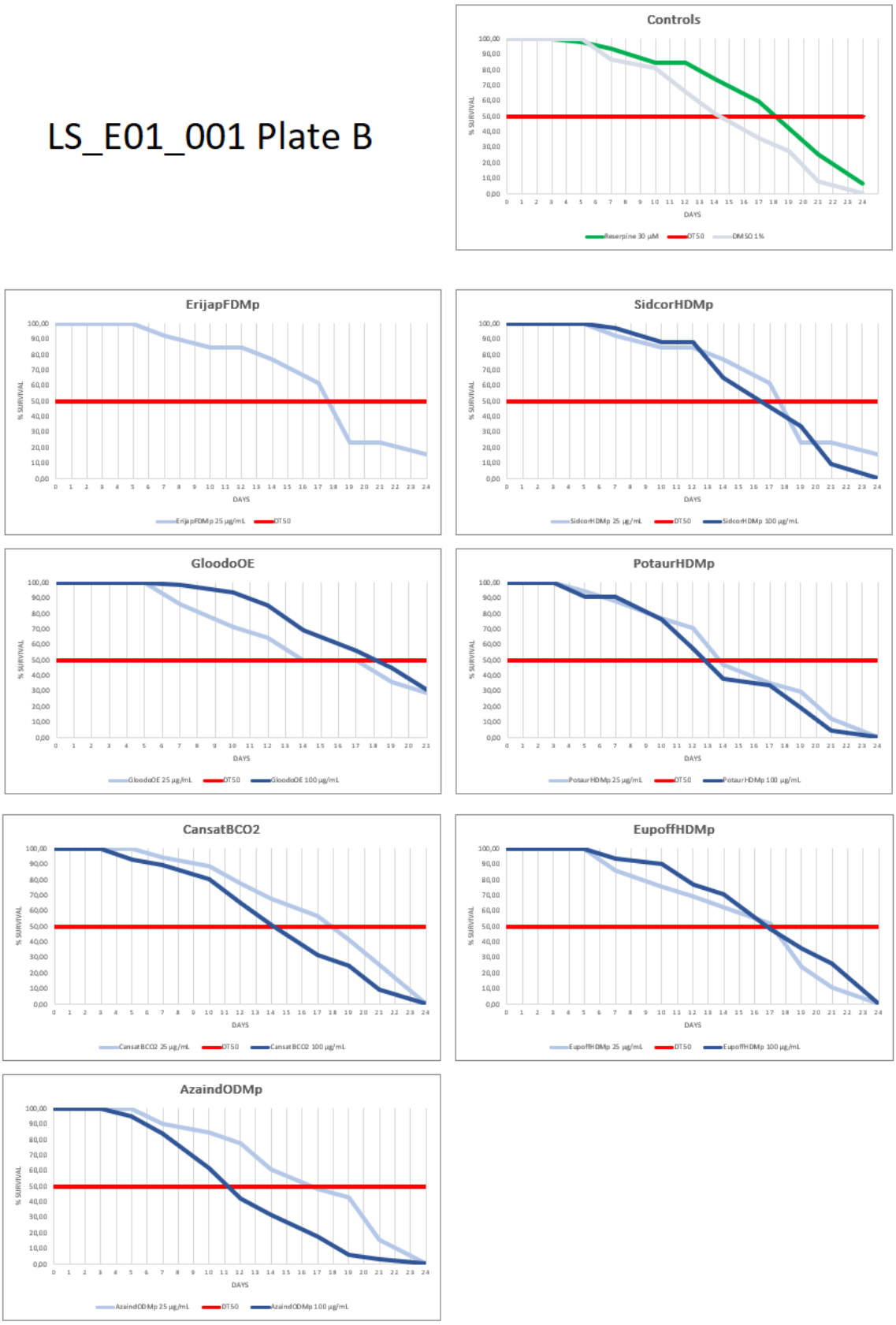


Figure 22 - Lifespan screening assay LS01_001 Plate B Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 21 - Counting list of Experiment LS_E01_001 Plate C Part 1

Trial Number: LS_E01_001 **Experimentator:** C **Date:** 6.7.2018

Life Span Assay N2

Strain	Drug	Conc.	Well	X_0	Date	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
						6.7.2018	0	9.7.2018	3	11.7.2018	5	13.7.2018	7	16.7.2018	10	18.7.2018	12	20.7.2018	14	23.7.2018	17	25.7.2018	19	27.7.2018	21	30.7.2018	24
N2	Reserpine	30 µM	B10	16		16	0	16	0	16	0	15	1	13	3	12	4	11	5	8	8	6	10	6	10	4	12
N2			C10	13		13	0	13	0	13	0	13	0	10	3	10	3	9	4	9	4	6	7	4	9	2	11
N2			D10	13		13	0	13	0	13	0	13	0	11	2	10	3	10	3	7	6	5	8	2	11	0	13
N2	EnlpgFDMp	25 µg/mL	B2	16		16	0	16	0	16	0	14	2	13	3	11	5	10	6	8	8	6	10	5	11	3	13
N2			C2	15		15	0	15	0	15	0	14	1	13	2	12	3	9	6	7	8	4	11	1	14	0	15
N2			D2	21		21	0	21	0	20	1	20	1	19	2	16	5	13	8	10	11	5	16	2	19	0	21
N2	SidcomFDMp	25 µg/mL	B3	19		19	0	19	0	18	1	17	2	15	4	12	7	10	9	6	13	5	14	2	17	1	18
N2			C3	16		16	0	16	0	16	0	15	1	13	3	11	5	7	9	3	13	1	15	1	15	1	15
N2			D3	16		16	0	16	0	16	0	16	0	14	2	11	2	7	5	7	9	5	11	3	13	1	15
N2	GlovidoDE	25 µg/mL	B4	15		15	0	15	0	15	0	15	0	14	1	14	1	13	2	9	6	7	8	2	13	0	15
N2			C4	13		13	0	13	0	13	0	12	1	10	3	9	4	8	5	4	9	1	12	1	12	0	13
N2			D4	15		15	0	15	0	15	0	15	0	14	1	14	1	12	3	9	6	6	9	0	15	0	15
N2	PotaurinFDMp	25 µg/mL	B5	17		17	0	17	0	17	0	16	1	13	4	10	7	6	11	2	15	2	15	0	17	0	17
N2			C5	17		17	0	17	0	16	1	15	2	14	3	14	3	11	6	5	12	2	15	2	15	0	17
N2			D5	20		20	0	20	0	19	1	18	2	17	3	16	4	15	5	7	13	2	18	1	19	0	20
N2	CansatBDO2	25 µg/mL	B6	17		17	0	17	0	17	0	15	2	12	5	10	7	6	11	2	15	2	15	0	17	0	17
N2			C6	8		8	0	8	0	8	0	8	0	7	1	5	3	2	6	1	7	0	8	0	8	0	8
N2			D6	20		20	0	20	0	20	0	19	1	16	4	9	11	8	12	3	17	1	19	1	19	0	20
N2	EtpoRhDMp	25 µg/mL	B8	14		14	0	14	0	14	0	14	0	12	2	11	3	8	6	6	8	2	12	1	13	0	14
N2			C8	17		17	0	17	0	17	0	17	0	16	1	14	3	10	7	8	9	6	11	2	15	0	17
N2			D8	14		14	0	14	0	14	0	14	0	12	2	10	4	9	5	7	7	3	11	1	13	0	14
N2	AzaInhSOMp	25 µg/mL	B9	18		18	0	18	0	16	2	16	2	13	5	12	6	10	8	5	13	3	15	1	17	0	18
N2			C9	6		6	0	6	0	5	1	5	1	4	2	3	3	2	4	1	5	0	6	0	6	0	6
N2			D9	16		16	0	16	0	16	0	15	1	13	3	13	3	11	5	7	9	3	13	1	15	0	16
N2			B11																								
N2			C11																								
N2			D11																								

Table 22 - Counting list of Experiment LS_E01_001 Plate C Part 2

Life Span Assay N2

Trial Number: LS_E01_001 **C** **Experimentator:** **Date:** 6.7.2018

					Date																									
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	DMSO	1%	E10	14	14	0	14	0	13	1	11	3	9	5	8	6	8	6	6	8	3	11	0	14	0	14				
N2			F10	17	17	0	17	0	15	2	14	3	13	4	10	7	7	10	4	13	2	15	1	16	0	17				
N2			G10													0		0		0		0		0		0				
N2	SidcorHDMp	100 µg/mL	E3	17	17	0	17	0	17	0	16	1	15	2	12	5	9	8	5	12	3	14	2	15	0	17				
N2			F3	13	13	0	13	0	13	0	13	0	12	1	9	4	7	6	5	8	4	9	1	12	0	13				
N2			G3	15	15	0	15	0	15	0	15	0	15	0	12	3	11	4	7	8	5	10	2	13	0	15				
N2	GloodeOE	100 µg/mL	E4	8	8	0	8	0	8	0	8	0	7	1	7	1	6	2	6	2	4	4	4	4	3	5				
N2			F4	14	14	0	14	0	14	0	14	0	14	0	11	3	9	5	7	7	5	9	3	11	3	11				
N2			G4	14	14	0	14	0	14	0	13	1	12	2	11	3	8	6	6	8	4	10	1	13	0	14				
N2	PotaurHDMp	100 µg/mL	E5	18	18	0	18	0	18	0	18	0	17	1	15	3	11	7	8	10	5	13	2	16	0	18				
N2			F5	14	14	0	14	0	14	0	14	0	13	1	12	2	12	2	7	7	4	10	1	13	0	14				
N2			G5	12	12	0	12	0	11	1	11	1	9	3	9	3	8	4	5	7	3	9	2	10	0	12				
N2	CansatBCO2	100 µg/mL	E6	16	16	0	16	0	14	2	14	2	12	4	11	5	8	8	5	11	5	11	2	14	0	16				
N2			F6	9	9	0	9	0	7	2	7	2	5	4	5	4	4	5	4	5	1	8	0	9	0	9				
N2			G6	15	15	0	15	0	14	1	14	1	14	1	12	3	9	6	7	8	3	12	2	13	0	15				
N2			E7													0		0		0		0		0		0				
N2			F7													0		0		0		0		0		0				
N2			G7													0		0		0		0		0		0				
N2	EupoffHDMp	100 µg/mL	E8	28	28	0	28	0	27	1	27	1	26	2	21	7	18	10	14	14	8	20	6	22	5	23				
N2			F8	14	14	0	14	0	14	0	13	1	11	3	11	3	11	3	8	6	7	7	3	11	1	13				
N2			G8	23	23	0	23	0	21	2	19	4	16	7	13	10	11	12	7	16	3	20	2	21	1	22				
N2	AzaindODMp	100 µg/mL	E9	18	18	0	18	0	15	3	15	3	15	3	12	6	9	9	6	12	3	15	3	15	0	18				
N2			F9	16	16	0	16	0	16	0	15	1	13	3	13	3	12	4	9	7	4	12	1	15	0	16				
N2			G9	20	20	0	20	0	19	1	19	1	18	2	15	5	11	9	8	12	5	15	2	18	0	20				
N2	DMSO	1%	E11	14	14	0	14	0	14	0	12	2	11	3	9	5	8	6	6	8	6	8	3	11	0	14				
N2			F11	16	16	0	16	0	16	0	14	2	14	2	11	5	8	8	5	11	3	13	1	15	0	16				
N2			G11	18	18	0	18	0	18	0	15	3	14	4	12	6	10	8	6	12	4	14	1	17	0	18				

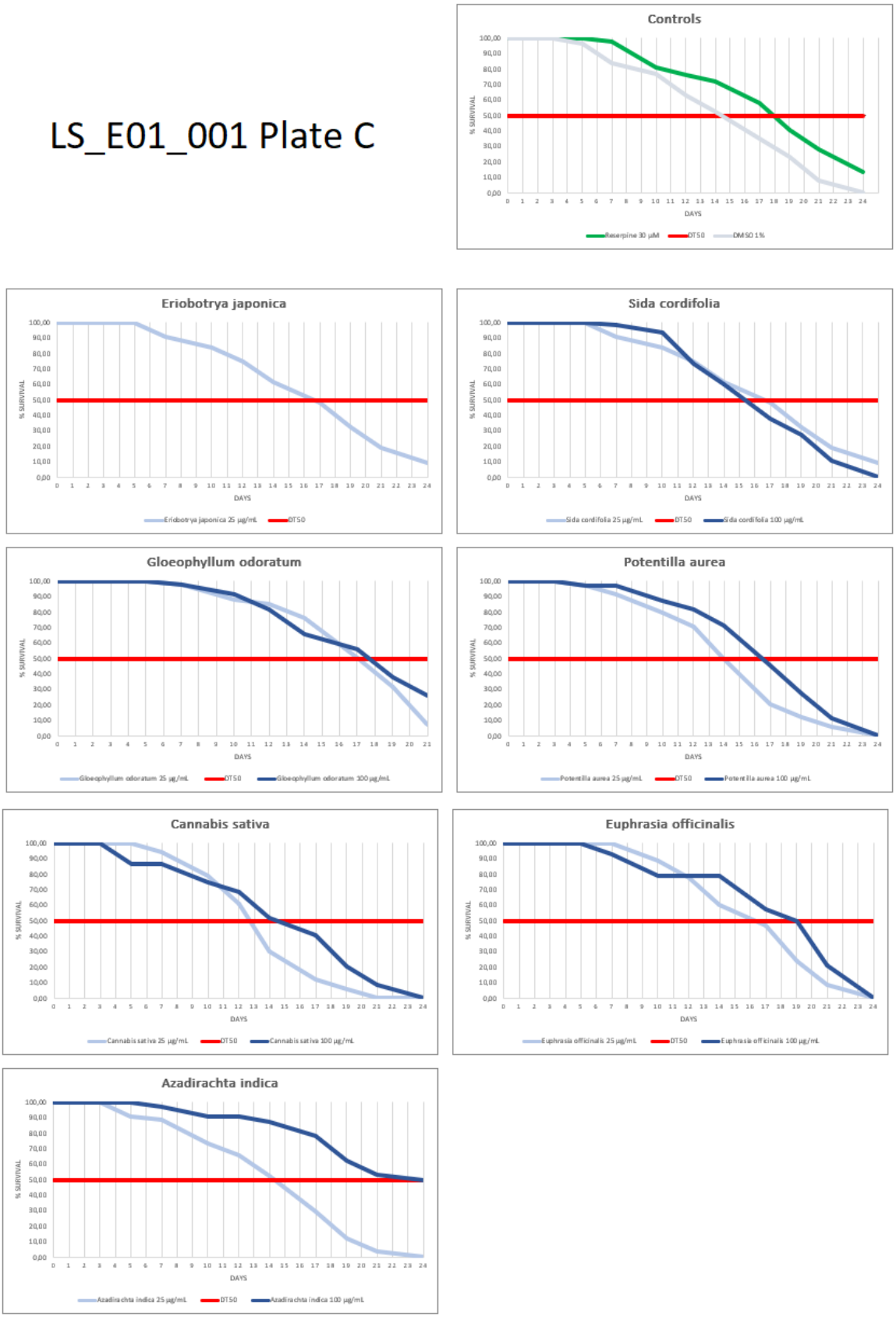


Figure 23 - Lifespan screening assay LS01_001 Plate C Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 23 - Counting list of Experiment LS_E01_002 Plate A Part 1

Life Span Assay N2

Trial Number: LS_E01_002 **A** **Experimentator:** **Date:** 21.9.2018

					Date													
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	Drug	Conc.	Well	X_0	21.9.2018	0	27.9.2018	6	1.10.2018	10	5.10.2018	14	8.10.2018	17	10.10.2018	19	12.10.2018	21
N2	Reserpine	30 µM	B2	12	12	0	12	0	12	0	9	3	6	6	4	8	4	8
N2			C2	24	24	0	23	1	23	1	14	10	12	12	9	15	7	17
N2			D2	13	13	0	13	0	13	0	10	2	6	7	6	7	4	9
N2	HumlupFDMp	25 µg/mL	B3			0	0	0	0	0	0	0	0	0	0	0	0	0
N2			C3	14	14	0	12	2	11	3	10	4	6	8	3	11	2	12
N2			D3	14	14	0	11	3	10	4	9	5	7	7	6	8	5	9
N2	PolaviHDMp	25 µg/mL	B4	16	16	0	13	3	13	3	11	5	10	6	4	12	4	12
N2			C4	20	20	0	19	1	15	5	13	7	8	12	4	16	4	16
N2			D4	17	17	0	13	4	10	7	7	10	4	13	1	16	1	16
N2	PlalanHDMp	25 µg/mL	B5	18	18	0	13	5	13	5	11	7	10	8	2	16	3	15
N2			C5	13	13	0	8	5	8	5	7	6	3	10	3	10	3	10
N2			D5	12	12	0	9	3	8	4	7	5	6	6	2	10	1	11
N2	AquvulHDMp	25 µg/mL	B6	13	13	0	13	0	10	3	9	4	7	6	3	10	2	11
N2			C6	14	14	0	14	0	12	2	8	6	3	11	3	11	3	11
N2			D6	10	10	0	10	0	8	2	6	4	3	7	3	7	3	7
N2	LacsatFDMp	25 µg/mL	B7	17	17	0	17	0	15	2	12	5	11	6	8	9	5	12
N2			C7	19	19	0	16	3	11	8	8	11	3	16	3	16	2	17
N2			D7	16	16	0	15	1	12	4	9	7	6	10	6	10	2	14
N2	SaucosRDMp	25 µg/mL	B8	21	21	0	21	0	18	3	12	9	7	14	4	17	3	18
N2			C8	18	18	0	18	0	13	5	10	8	7	11	6	12	5	13
N2			D8	13	13	0	12	1	10	3	9	4	5	8	5	8	3	10
N2	Reserpine	30 µM	B11	11	11	0	11	0	9	2	7	4	7	4	4	5	4	7
N2			C11	17	17	0	17	0	15	2	11	6	9	8	9	8	6	11
N2			D11	9	9	0	9	0	8	1	6	3	5	4	4	5	3	6

Table 24 - Counting list of Experiment LS_E01_002 Plate A Part 2

Life Span Assay N2

Trial Number: LS_E01_002 **A** **Experimentator:** **Date:** 21.9.2018

					Date													
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	DMSO	1%	E2	17	17	0	13	4	7	10	4	13	2	15	0	17	0	17
N2			F2	11	11	0	9	2	8	3	6	5	4	7	3	8	2	9
N2			G2	13	13	0	13	0	11	2	8	5	4	9	4	9	3	10
N2	HumlupFDMp	100 µg/mL	E3	10	10	0	8	2	8	2	8	2	5	5	5	5	2	8
N2			F3	16	16	0	14	2	13	3	11	5	5	11	4	12	3	13
N2			G3	9	9	0	7	2	7	2	5	4	5	4	1	8	1	8
N2	PolaviHDMp	100 µg/mL	E4	18	18	0	15	3	13	5	9	9	7	11	6	12	3	15
N2			F4	15	15	0	15	0	12	3	7	8	3	12	3	12	2	13
N2			G4	18	18	0	17	1	15	3	11	7	7	11	5	13	1	17
N2	PlalanHDMp	100 µg/mL	E5	13	13	0	11	2	8	5	5	8	2	11	0	13	0	13
N2			F5	13	13	0	13	0	12	1	7	6	3	10	2	11	1	12
N2			G5	14	14	0	10	4	8	6	6	8	2	12	1	13	1	13
N2	AquvulHDMp	100 µg/mL	B8	15	15	0	14	1	13	2	11	4	6	9	3	12	2	13
N2			C8	17	17	0	14	3	8	9	5	12	4	13	3	14	3	14
N2			D8	20	20	0	17	3	13	7	7	13	5	15	3	17	2	18
N2	LacsatFDMp	100 µg/mL	E8	14	14	0	14	0	11	3	8	6	5	9	1	13	1	13
N2			F8	16	16	0	14	2	12	4	8	8	4	12	2	14	1	15
N2			G8	7	7	0	6	1	6	1	4	3	3	4	3	4	2	5
N2	SaucosRDMp	100 µg/mL	E8	13	13	0	13	0	12	1	7	6	6	7	2	11	2	11
N2			F8	18	18	0	16	2	9	9	6	12	6	12	4	14	1	17
N2			G8	11	11	0	9	2	9	2	9	2	5	6	2	9	2	9
N2	DMSO	1%	E11	11	11	0	9	2	9	2	6	5	7	4	4	7	1	10
N2			F11	9	9	0	6	3	6	3	5	4	2	7	2	7	1	8
N2			G11	14	14	0	11	3	11	3	6	8	4	10	2	12	2	12

LS_E01_002 Plate A

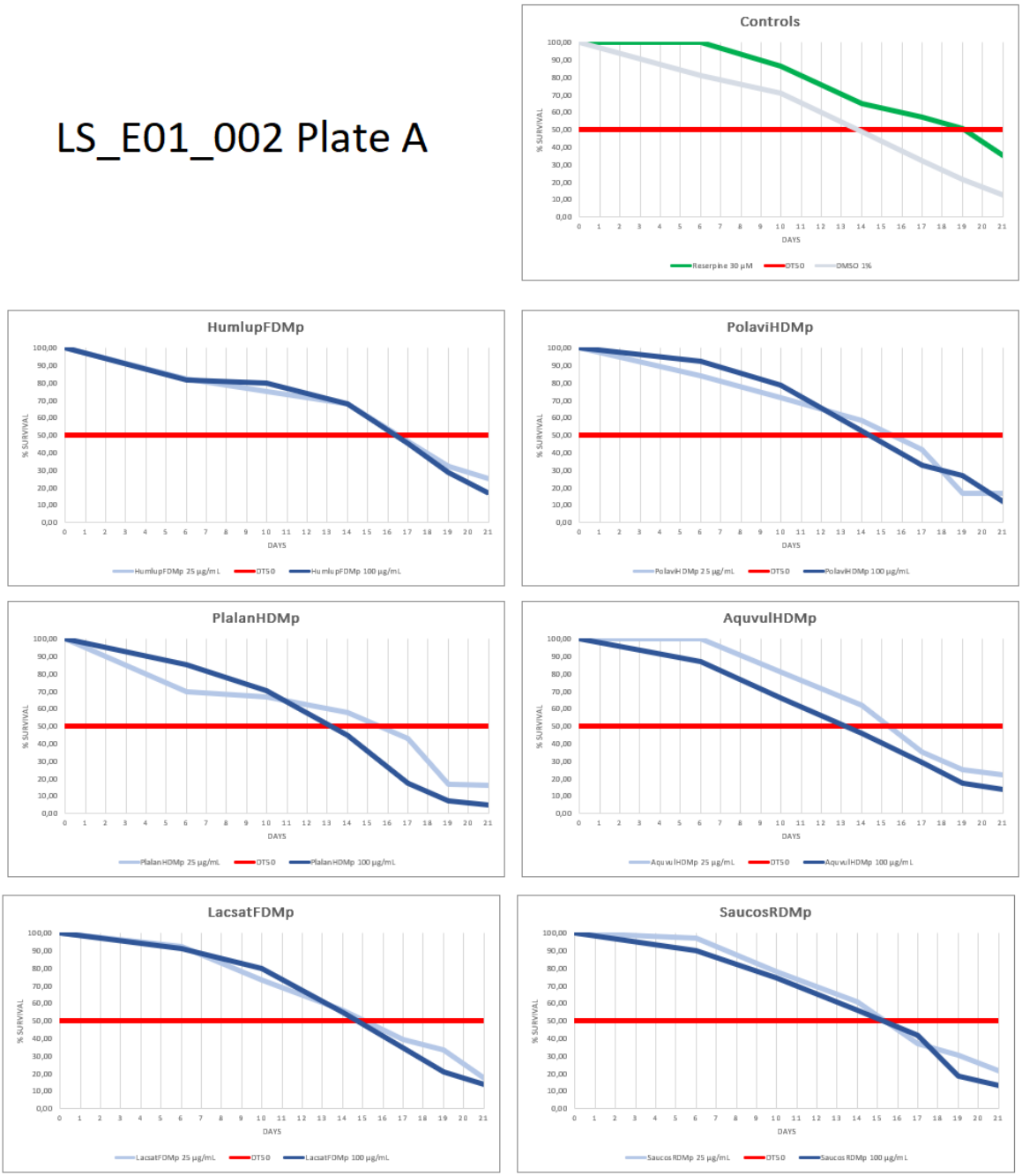


Figure 24 - Lifespan screening assay LS01_002 Plate A Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 25 - Counting list of Experiment LS_E01_002 Plate B Part 1

Life Span Assay N2

Trial Number: LS_E01_002 **B** **Experimentator:** **Date:** 13.7.2018

					Date																					
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	**Drug**	**Conc.**	**Well**	**X_0**	13.7.2018	0	16.7.2018	3	18.7.2018	5	23.7.2018	10	25.7.2018	12	27.7.2018	14	30.7.2018	17	1.8.2018	19	3.8.2018	21	6.8.2018	24	9.8.2018	27
N2			B2	11	11	0	11	0	11	0	11	0	10	1	10	1	8	3	8	3	6	5	6	5	0	11
N2	Reserpine	25 µg/mL	C2	17	17	0	17	0	17	0	17	0	16	1	16	1	15	2	15	2	14	3	7	10	3	14
N2			D2	12	12	0	12	0	12	0	12	0	12	0	12	0	12	0	11	1	9	3	5	7	2	10
N2			B3															0								
N2	HumlupFDMp	25 µg/mL	C3	12	12	0	12	0	12	0	12	0	11	1	11	1	10	2	10	2	10	2	6	6	2	10
N2			D3	12	12	0	12	0	12	0	12	0	12	0	11	1	10	2	9	3	8	4	4	8	2	10
N2			B4	11	11	0	11	0	11	0	11	0	11	0	10	1	10	1	10	1	9	2	7	4	1	10
N2	PolaviHDMp	25 µg/mL	C4	13	13	0	13	0	13	0	12	1	11	2	10	3	9	4	9	4	8	5	3	10	3	10
N2			D4	16	16	0	16	0	16	0	14	2	13	3	12	4	11	5	10	6	8	8	5	11	2	14
N2			B5	16	16	0	16	0	16	0	14	2	13	3	11	5	9	7	9	7	7	9	5	11	1	15
N2	PlalanHDMp	25 µg/mL	C5	11	11	0	11	0	11	0	10	1	9	2	9	2	7	4	7	4	6	5	3	8	1	10
N2			D5	13	13	0	13	0	13	0	11	2	10	3	7	6	5	8	5	8	5	8	4	9	0	13
N2			B6	10	10	0	10	0	10	0	10	0	10	0	8	2	7	3	7	3	5	5	4	6	0	10
N2	AquvulHDMp	25 µg/mL	C6	9	9	0	9	0	9	0	9	0	8	1	7	2	5	4	4	5	2	7	2	7	0	9
N2			D6	10	10	0	10	0	10	0	9	1	9	1	9	1	9	1	7	3	5	5	2	8	2	8
N2			B7															0								
N2	LacsatFDMp	25 µg/mL	C7	13	13	0	13	0	13	0	13	0	12	1	12	1	11	2	10	3	8	5	5	8	1	12
N2			D7	8	8	0	8	0	8	0	8	0	7	1	7	1	6	2	6	2	5	3	3	5	1	7
N2			B8	7	7	0	7	0	7	0	7	0	7	0	7	0	6	1	5	2	4	3	2	5	0	7
N2	SaucosRDMp	2 µg/mL	C8	17	17	0	17	0	17	0	17	0	15	2	13	4	12	5	11	6	10	7	4	13	0	17
N2			D8	11	11	0	11	0	11	0	9	2	8	3	8	3	7	4	5	6	2	9	2	9	0	11
N2			B9	16	16	0	16	0	16	0	14	2	13	3	13	3	13	3	12	4	9	7	4	12	2	14
N2		20 µg/mL	C9	12	12	0	12	0	12	0	12	0	12	0	11	1	8	4	7	5	5	7	3	9	1	11
N2			D9	10	10	0	10	0	10	0	8	2	8	2	8	2	6	4	4	6	3	7	2	8	1	9
N2			B10	13	13	0	13	0	13	0	12	1	11	2	11	2	10	3	10	3	9	4	5	8	0	13
N2		30 µm	C10	15	15	0	15	0	15	0	15	0	14	1	14	1	14	1	13	2	10	5	3	12	0	15
N2			D10	7	7	0	7	0	7	0	6	1	6	1	6	1	5	2	5	2	5	2	1	6	1	6
N2			B11	5	5	0	5	0	5	0	5	0	5	0	5	0	3	2	3	2	2	3	1	4	1	4
N2	Reserpine	100 µM	C11	15	15	0	15	0	15	0	14	1	14	1	13	2	12	3	7	8	7	8	2	13	2	13
N2			D11	11	11	0	11	0	11	0	11	0	10	1	9	2	8	3	6	5	6	5	2	9	0	11

Table 26 - Counting list of Experiment LS_E01_002 Plate B Part 2

Life Span Assay N2

Trial Number: LS_E01_002 **B** **Experimentator:** **Date:** 13.7.2018

					Date																					
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	Drug	Conc.	Well	X_0	13.7.2018	0	16.7.2018	3	18.7.2018	5	23.7.2018	10	25.7.2018	12	27.7.2018	14	30.7.2018	17	1.8.2018	19	3.8.2018	21	6.8.2018	24	9.8.2018	27
N2	DMSO	100 µg/mL	E2	11	11	0	11	0	11	0	9	2	9	2	8	3	6	5	6	5	4	7	2	9	1	10
N2			F2	9	9	0	9	0	9	0	7	2	7	2	5	4	3	6	3	6	2	7	1	8	0	9
N2			G2	6	6	0	6	0	6	0	5	1	5	1	4	2	3	3	3	3	2	4	0	6	0	6
N2	HumlupFDMp	100 µg/mL	E3	18	18	0	18	0	18	0	17	1	17	1	17	1	16	2	14	4	12	6	8	10	1	17
N2			F3	9	9	0	9	0	9	0	9	0	8	1	8	1	cont.	cont.	cont.	cont.	cont.	cont.	cont.	cont.	cont.	cont.
N2			G3	16	16	0	16	0	16	0	15	1	15	1	15	1	15	1	14	2	11	5	7	9	0	16
N2	PolaviHDMp	100 µg/mL	E4	18	18	0	18	0	18	0	17	1	17	1	17	1	16	2	13	5	11	7	5	13	0	18
N2			F4	10	10	0	10	0	10	0	10	0	10	0	9	1	7	3	6	4	5	5	1	9	1	9
N2			G4	10	10	0	10	0	10	0	10	0	10	0	10	0	10	0	9	1	7	3	3	7	0	10
N2	PlalanHDMp	100 µg/mL	E5	8	8	0	8	0	8	0	8	0	8	2	8	0	8	0	5	3	3	5	0	8	0	8
N2			F5	14	14	0	14	0	14	0	13	1	13	1	12	2	10	4	8	6	7	7	3	11	1	13
N2			G5	12	12	0	12	0	12	0	10	2	9	3	8	4	7	5	6	6	5	7	1	11	0	12
N2	AquvulHDMp	100 µg/mL	E6	13	13	0	13	0	13	0	13	0	12	1	10	3	7	6	7	6	5	8	2	11	1	12
N2			F6	19	19	0	19	0	19	0	17	2	17	2	15	4	14	5	12	7	10	9	6	13	2	17
N2			G6	17	17	0	17	0	17	0	17	0	17	0	17	0	15	2	13	4	11	6	5	12	0	17
N2	LacsatFDMp	100 µg/mL	E7	12	12	0	12	0	12	0	12	0	10	2	7	5	5	7	5	7	5	7	0	12	0	12
N2			F7	10	10	0	10	0	10	0	10	0	9	1	5	5	3	7	3	7	3	7	0	10	0	10
N2			G7	18	18	0	18	0	18	0	16	2	15	3	11	7	8	10	8	10	7	11	2	16	1	17
N2	SaucosRDMp	40 µg/mL	E8	15	15	0	15	0	15	0	15	0	15	0	13	2	12	3	10	5	7	8	0	15	0	15
N2			F8	11	11	0	11	0	11	0	11	0	11	0	10	1	8	3	8	3	8	3	1	10	1	10
N2			G8	4	4	0	4	0	4	0	3	1	3	1	3	1	3	1	3	1	2	2	1	3	0	4
N2		80 µg/mL	E9	11	11	0	11	0	11	0	10	1	9	2	6	5	5	6	5	6	5	6	0	11	0	11
N2			F9	10	10	0	10	0	10	0	10	0	8	2	6	4	5	5	4	6	4	6	1	9	1	9
N2			G9	7	7	0	7	0	7	0	6	1	5	2	4	3	3	4	3	4	2	5	1	6	1	6
N2	ErijapFDMp	100 µg/mL	E10	15	15	0	15	0	15	0	15	0	14	1	14	1	12	3	12	3	12	3	4	11	1	14
N2			F10	12	12	0	12	0	12	0	11	1	11	1	11	1	10	2	9	3	7	5	1	11	1	11
N2			G10	12	12	0	12	0	12	0	11	1	11	1	10	2	9	3	9	3	7	5	2	10	0	12
N2	DMSO	1%	E11	16	16	0	16	0	16	0	16	0	14	2	12	4	9	7	7	9	6	10	2	14	0	16
N2			F11	12	12	0	12	0	12	0	12	0	10	2	8	4	5	7	3	9	3	9	0	12	0	12
N2			G11	5	5	0	5	0	5	0	5	0	4	1	4	1	3	2	3	2	3	2	0	5	0	5

LS_E01_002 Plate B

Figure 25 - Lifespan screening assay LS01_002 Plate B Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 27 - Counting list of Experiment LS_E01_002 Plate C Part 1

Life Span Assay N2

Trial Number: LS_E01_002 **C** **Experimentator:** **Date:** 13.7.2018

					Date																					
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	Drug	Conc.	Well	X_0	13.7.2018	0	16.7.2018	3	18.7.2018	5	23.7.2018	10	25.7.2018	12	27.7.2018	14	30.7.2018	17	1.8.2018	19	3.8.2018	21	6.8.2018	24	9.8.2018	27
N2	Reserpine	25 µg/mL	B2	16	16	0	16	0	16	0	16	0	15	1	13	3	13	3	12	4	11	5	8	8	2	14
N2			C2	12	12	0	12	0	12	0	12	0	9	3	7	5	6	6	6	6	5	7	2	10	0	12
N2			D2															0		0		0		0		0
N2	HumlupFDMp	25 µg/mL	B3	12	12		12		12		12		11	1	9	3	5	7	5	7	5	7	3	9	2	10
N2			C3	10	10	0	10	0	10	0	10	0	9	1	8	2	7	3	6	4	4	6	4	6	1	9
N2			D3	17	17	0	17	0	17	0	17	0	16	1	14	3	13	4	13	4	13	4	8	9	2	15
N2	PolaviHDMp	25 µg/mL	B4	16	16	0	16	0	16	0	16	0	15	1	13	3	13	3	11	5	10	6	8	8	0	16
N2			C4	5	5	0	5	0	5	0	5	0	4	1	4	1	3	2	3	2	3	2	1	4	0	5
N2			D4	17	17	0	17	0	17	0	17	0	16	1	14	3	13	4	12	5	9	8	5	12	1	16
N2	PlalanHDMp	25 µg/mL	B5	10	10	0	10	0	10	0	10	0	9	1	8	2	6	4	6	4	6	4	3	7	0	10
N2			C5	11	11	0	11	0	11	0	11	0	10	1	10	1	10	1	10	1	10	1	8	3	2	9
N2			D5	19	19	0	19	0	19	0	19	0	17	2	17	2	17	2	17	2	15	4	8	11	2	17
N2	AquvulHDMp	25 µg/mL	B6	11	11	0	11	0	11	0	11	0	8	3	7	4	6	5	6	5	6	5	4	7	1	10
N2			C6	5	5	0	5	0	5	0	5	0	5	0	5	0	5	0	3	2	2	3	1	4	1	4
N2			D6	10	10	0	10	0	10	0	10	0	7	3	7	3	6	4	5	5	3	7	2	8	2	8
N2	LacsatFDMp	25 µg/mL	B7	7	7	0	7	0	7	0	7	0	6	1	5	2	5	2	5	2	3	4	2	5	1	6
N2			C7	14	14	0	14	0	14	0	14	0	11	3	11	3	9	5	9	5	7	7	3	11	1	13
N2			D7	12	12	0	12	0	12	0	12	0	11	1	11	1	8	4	8	4	8	4	4	8	0	12
N2	SaucosRDMp	2 µg/mL	B8	9	9	0	9	0	9	0	9	0	8	1	8	1	8	1	7	2	5	4	2	7	1	8
N2			C8	9	9	0	9	0	9	0	9	0	8	1	7	2	7	2	6	3	5	4	3	6	1	8
N2			D8	15	15	0	15	0	15	0	15	0	12	3	11	4	11	4	9	6	7	8	3	12	3	12
N2		20 µg/mL	B9	10	10	0	10	0	10	0	10	0	10	0	10	0	9	1	7	3	4	6	1	9	0	10
N2			C9	17	17	0	17	0	17	0	17	0	14	3	14	3	14	3	12	5	11	6	6	11	0	17
N2			D9	7	7	0	7	0	7	0	7	0	6	1	6	1	6	1	4	3	2	5	0	7	0	7
N2		100 µm	B10	12	12	0	12	0	12	0	12	0	11	1	10	2	9	3	9	3	9	3	3	9	1	11
N2			C10	12	12	0	12	0	12	0	12	0	11	1	11	1	9	3	9	3	9	3	4	8	2	10
N2			D10	10	10	0	10	0	10	0	10	0	9	1	7	3	7	3	7	3	3	7	0	10	0	10
N2	Reserpine	30 µM	B11	18	18	0	18	0	18	0	18	0	18	0	17	1	12	6	9	9	9	9	4	14	0	18
N2			C11	14	14	0	14	0	14	0	14	0	13	1	12	2	9	5	7	7	6	8	3	11	2	12
N2			D11	13	13	0	13	0	13	0	13	0	13	0	12	1	11	2	7	6	6	7	1	12	0	13

Table 28 - Counting list of Experiment LS_E01_002 Plate C Part 2

Life Span Assay N2

Trial Number: LS_E01_002 **C** **Experimentator:** **Date:** 13.7.2018

					Date																					
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	**Drug**	**Conc.**	**Well**	**X_0**	13.7.2018	0	16.7.2018	3	18.7.2018	5	23.7.2018	10	25.7.2018	12	27.7.2018	14	30.7.2018	17	1.8.2018	19	3.8.2018	21	6.8.2018	24	9.8.2018	27
N2	DMSO	100 µg/mL	E2	7	7	0	7	0	7	0	7	0	6	1	6	1	6	1	6	1	6	1	1	6	1	6
N2			F2	12	12	0	12	0	12	0	12	0	10	2	10	2	9	3	9	3	7	5	1	11	1	11
N2			G2	15	15	0	15	0	15	0	15	0	13	2	12	3	10	5	9	6	8	7	1	14	1	14
N2	HumlupFDMp	100 µg/mL	E3	10	10	0	10	0	10	0	10	0	9	1	9	1	6	4	6	4	6	4	0	10	0	10
N2			F3	8	8	0	8	0	8	0	8	0	7	1	6	2	5	3	4	4	3	5	1	7	0	8
N2			G3	15	15	0	15	0	15	0	15	0	14	1	13	2	10	5	9	6	5	10	2	13	1	14
N2	PolaviHDMp	100 µg/mL	E4	12	12	0	12	0	12	0	12	0	11	1	9	3	8	4	8	4	7	5	1	11	1	11
N2			F4	9	9	0	9	0	9	0	9	0	8	1	7	2	6	3	3	6	1	8	1	8	0	9
N2			G4	14	14	0	14	0	14	0	14	0	14	0	13	1	10	4	8	6	7	7	3	11	3	11
N2	PlalanHDMp	100 µg/mL	E5	8	8	0	8	0	8	0	8	0	7	1	7	1	6	2	5	3	5	3	1	7	1	7
N2			F5	7	7	0	7	0	7	0	7	0	7	0	7	0	5	2	5	2	4	3	1	6	1	6
N2			G5	5	5	0	5	0	5	0	5	0	4	1	4	1	4	1	4	1	4	1	0	5	0	5
N2	AquvulHDMp	100 µg/mL	E6	5	5	0	5	0	5	0	5	0	4	1	4	1	2	3	2	3	2	3	0	5	0	5
N2			F6	12	12	0	12	0	12	0	12	0	10	2	10	2	8	4	7	5	6	6	1	11	1	11
N2			G6	9	9	0	9	0	9	0	9	0	9	0	8	1	6	3	6	3	4	5	1	8	1	8
N2	LacsatFDMp	100 µg/mL	E7	10	10	0	10	0	10	0	10	0	10	0	10	0	8	2	7	3	6	4	0	10	0	10
N2			F7	8	8	0	8	0	8	0	8	0	7	1	7	1	7	1	5	3	3	5	1	7	0	8
N2			G7	12	12	0	12	0	12	0	12	0	11	1	10	2	9	3	8	4	7	5	2	10	0	12
N2	SaucosRDMp	40 µg/mL	E8	6	6	0	6	0	6	0	6	0	6	0	4	2	4	2	4	2	4	2	1	5	0	6
N2			F8	11	11	0	11	0	11	0	11	0	10	1	9	2	7	4	7	4	6	5	2	9	1	10
N2			G8	15	15	0	15	0	15	0	15	0	13	2	13	2	10	5	9	6	8	7	3	12	3	12
N2		80 µg/mL	E9	13	13	0	13	0	13	0	13	0	11	2	10	3	8	5	8	5	7	6	2	11	1	12
N2			F9	5	5	0	5	0	5	0	5	0	5	0	4	1	4	1	4	1	4	1	1	4	1	4
N2			G9	14	14	0	14	0	14	0	14	0	13	1	12	2	10	4	10	4	9	5	2	12	1	13
N2	ErijapFDMp	100 µg/mL	E10	12	12	0	12	0	12	0	12	0	11	1	9	3	7	5	6	6	5	7	1	11	0	12
N2			F10	13	13	0	13	0	13	0	13	0	11	2	10	3	10	3	9	4	5	8	2	11	0	13
N2			G10	15	15	0	15	0	15	0	15	0	15	0	14	1	12	3	10	5	6	9	2	13	1	14
N2	DMSO	1%	E11	13	13	0	13	0	13	0	13	0	12	1	11	2	7	6	6	7	4	9	2	11	0	13
N2			F11	15	15	0	15	0	15	0	15	0	14	1	12	3	9	6	6	9	5	10	3	12	0	15
N2			G11	16	16	0	16	0	16	0	16	0	13	3	13	3	11	5	7	9	4	12	3	13	0	16

Figure 26 - Lifespan screening assay LS01_002 Plate C Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 29 - Counting list of Experiment LS_E01_003 Plate D Part 1

Life Span Assay N2

Trial Number: LS_E01_003 **D** **Experimentator:** JZ **Date:** 24.10.2018

					Date													
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	Drug	Conc.	Well	X_0	25.10.2018	0	29.10.2018	4	31.10.2018	6	6.11.2018	12	9.11.2018	15	14.11.2018	20	16.11.2018	22
N2	Reserpine	30 µM	B2	15	15	0	15	0	15	0	13	2	9	6	6	9	6	9
N2			C2	8	8	0	8	0	8	0	8	0	7	1	7	1	7	1
N2			D2	5	5	0	5	0	5	0	4	1	4	1	4	1	3	2
N2	PimdioODMp	25 µg/mL	B3	10	10	0	10	0	10	0	8	2	6	4	3	7	3	7
N2			C3	10	10	0	10	0	10	0	6	4	6	4	3	7	2	8
N2			D3	15	15	0	15	0	15	0	10	5	10	5	2	13	1	14
N2	PtesanXDMp	25 µg/mL	B4	10	10	0	10	0	10	0	8	2	7	3	4	6	3	7
N2			C4	13	13	0	13	0	13	0	13	0	12	1	7	6	3	10
N2			D4	16	16	0	16	0	16	0	13	3	14	2	7	9	5	11
N2	InooblODM	25 µg/mL	B5	15	15	0	15	0	15	0	10	5	10	5	4	11	1	14
N2			C5	17	17	0	17	0	17	0	14	3	11	6	6	11	3	14
N2			D5	11	11	0	11	0	11	0	7	4	8	3	3	8	3	8
N2	TercheODMp	25 µg/mL	B6	18	18	0	18	0	18	0	14	4	10	8	4	14	3	15
N2			C6	7	7	0	7	0	7	0	6	1	5	2	4	3	1	6
N2			D6	15	15	0	15	0	15	0	11	4	10	5	7	8	4	11
N2	GarjasODMp	25 µg/mL	B7	10	10	0	10	0	10	0	10	0	7	3	6	4	3	7
N2			C7	14	14	0	14	0	14	0	13	1	5	9	3	11	3	11
N2			D7	7	7	0	7	0	7	0	5	2	4	3	4	3	4	3
N2	RhorosE	25 µg/mL	B8	16	16	0	16	0	16	0	11	5	10	6	8	8	2	14
N2			C8	11	11	0	11	0	11	0	8	3	7	4	4	7	3	8
N2			D8	14	14	0	14	0	14	0	12	2	8	6	5	9	5	9
N2	InooblODM_Pakuso	25 µg/mL	B9	12	12	0	12	0	12	0	9	3	6	6	3	9	1	11
N2			C9	10	10	0	10	0	10	0	7	3	5	5	3	7	3	7
N2			D9	7	6	1	6	1	6	1	5	2	4	3	3	4	3	4
N2	InooblODM_EtOH 15%	25 µg/mL	B10	15	15	0	15	0	15	0	12	3	9	6	6	9	5	10
N2			C10	10	10	0	10	0	10	0	9	1	6	4	2	8	1	9
N2			D10	13	13	0	13	0	13	0	11	2	8	5	5	8	2	11
N2	Reserpine	30 µM	B11	9	9	0	9	0	9	0	7	2	7	2	6	3	4	5
N2			C11	13	13	0	13	0	13	0	9	4	8	5	6	7	5	8
N2			D11	7	7	0	7	0	7	0	6	1	6	1	5	2	3	4

Table 30 - Counting list of Experiment LS_E01_003 Plate D Part 2

Life Span Assay N2

Trial Number: LS_E01_003 **D** **Experimentator:** JZ **Date:** 24.10.2018

					Date													
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	DMSO	1%	E2	11	11	0	11	0	11	0	8	3	7	4	5	6	3	8
N2			F2	4	4	0	4	0	4	0	3	1	3	1	3	1	3	1
N2			G2	11	11	0	11	0	11	0	8	3	6	5	4	7	4	7
N2	PimdioODMp	100 µg/mL	E3	10	10	0	10	0	10	0	8	2	8	2	5	5	5	5
N2			F3	9	9	0	9	0	9	0	7	2	7	2	5	4	4	5
N2			G3	13	13	0	13	0	13	0	11	2	10	3	4	9	2	11
N2	PtesanXDMp	100 µg/mL	E4	15	15	0	15	0	15	0	14	1	11	4	7	8	3	12
N2			F4	12	12	0	12	0	12	0	9	3	8	4	6	6	3	9
N2			G4	16	16	0	16	0	16	0	13	3	9	7	6	10	4	12
N2	InooblODM	100 µg/mL	E5	17	17	0	17	0	17	0	12	5	8	9	4	13	0	17
N2			F5	12	12	0	12	0	12	0	10	2	6	6	2	10	1	11
N2			G5	9	9	0	9	0	9	0	9	0	4	5	3	6	2	7
N2	TercheODMp	100 µg/mL	E6	17	17	0	17	0	17	0	11	6	8	9	6	11	2	15
N2			F6	12	12	0	12	0	12	0	9	3	7	5	6	6	2	10
N2			G6	10	10	0	10	0	10	0	9	1	5	5	4	6	4	6
N2	GarjasODMp	100 µg/mL	E7	10	10	0	10	0	10	0	10	0	5	5	5	5	4	6
N2			F7	15	15	0	15	0	15	0	12	3	9	6	5	10	4	11
N2			G7	15	15	0	15	0	15	0	11	4	9	6	5	10	5	10
N2	RhorosE	100 µg/mL	E8	14	14	0	14	0	14	0	12	2	7	7	4	10	3	11
N2			F8	12	12	0	12	0	12	0	7	5	4	8	1	11	1	11
N2			G8	18	18	0	18	0	18	0	12	6	8	10	3	15	3	15
N2	InooblODM_Pakuso	100 µg/mL	E9	10	10	0	10	0	10	0	9	1	5	5	4	6	2	8
N2			F9	18	18	0	18	0	18	0	15	3	7	11	4	14	4	14
N2			G9	16	16	0	16	0	16	0	15	1	8	8	4	12	2	14
N2	InooblODM_EtOH 15%	100 µg/mL	E10	16	16	0	16	0	16	0	12	4	5	11	1	15	1	15
N2			F10	14	14	0	14	0	14	0	10	4	4	10	1	13	0	14
N2			G10	18	18	0	18	0	18	0	16	2	8	10	6	12	6	12
N2	DMSO	1%	E11	19	19	0	19	0	19	0	15	4	9	10	2	17	1	18
N2			F11	11	11	0	11	0	11	0	9	2	5	6	4	7	3	8
N2			G11	10	10	0	10	0	10	0	6	4	4	6	4	6	3	7

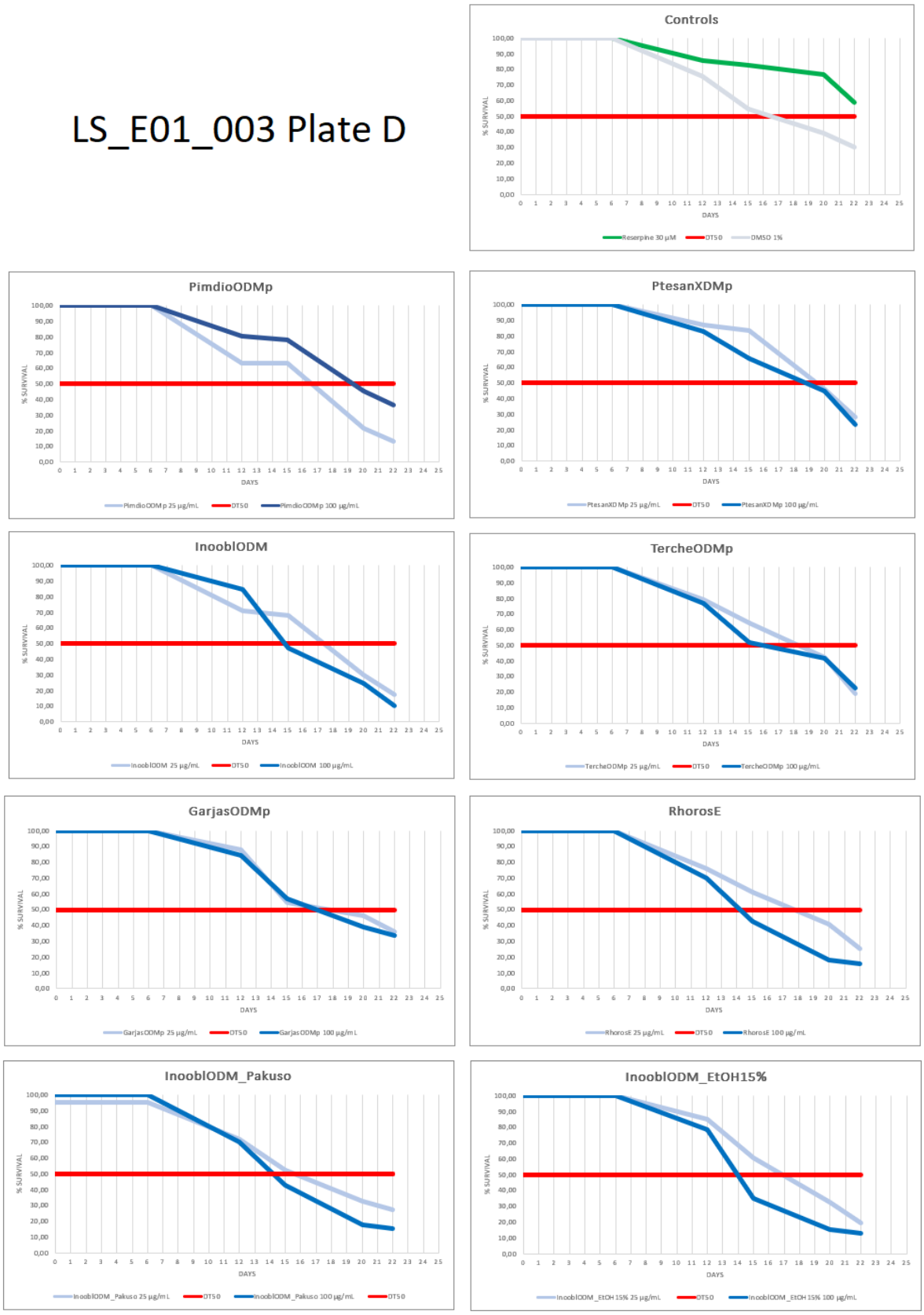


Figure 27 - Lifespan screening assay LS01_003 Plate D Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 31 - Counting list of Experiment LS_E01_003 Plate E Part 1

Life Span Assay N2

Trial Number: LS_E01_003 **E** **Experimentator:** JZ **Date:** 24.10.2018

					Date														
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	Drug	Conc.	Well	X_0	25.10.2018	0	29.10.2018	4	31.10.2018	6	6.11.2018	12	9.11.2018	15	14.11.2018	20	16.11.2018	22
N2	Reserpine	30 µM	B2	8	8	0	8	0	8	0	7	1	7	1	5	3	5	3
N2			C2	5	5	0	5	0	5	0	4	1	4	1	4	1	4	1
N2			D2	11	11	0	11	0	11	0	10	1	10	1	7	4	5	6
N2	PimdioODMp	25 µg/mL	B3	13	13	0	13	0	13	0	10	3	9	4	7	6	4	9
N2			C3	9	9	0	9	0	9	0	9	0	9	0	8	1	5	4
N2			D3	7	7	0	7	0	7	0	6	1	6	1	5	2	3	4
											0							
N2	PtesanXDMp	25 µg/mL	B4	12	12	0	12	0	12	0	10	2	10	2	9	3	5	7
N2			C4	15	15	0	15	0	15	0	10	5	9	6	9	6	3	12
N2			D4	13	13	0	13	0	13	0	12	1	10	3	7	6	2	11
N2	InooblODM	25 µg/mL	B5	8	8	0	8	0	8	0	6	2	5	3	5	3	4	4
N2			C5	19	18	1	18	1	18	1	12	7	8	11	4	15	4	15
N2			D5	8	8	0	8	0	8	0	5	3	5	3	5	3	2	6
N2	TercheODMp	25 µg/mL	B6	9	9	0	9	0	9	0	8	1	7	2	6	3	6	3
N2			C6															
N2			D6	6	6	0	6	0	6	0	5	1	4	2	2	4	1	5
N2	GarjasODMp	25 µg/mL	B7	14	14	0	14	0	14	0	11	3	9	5	7	7	4	10
N2			C7	13	13	0	13	0	13	0	10	3	8	5	6	7	4	9
N2			D7	14	14	0	14	0	14	0	10	4	8	6	5	9	3	11
N2	RhorosE	25 µg/mL	B8	7	7	0	7	0	7	0	6	1	4	3	2	5	2	5
N2			C8	13	13	0	13	0	13	0	12	1	10	3	7	6	5	8
N2			D8	12	12	0	12	0	12	0	8	4	7	5	4	8	3	9
N2	InooblODM_Pakuso	25 µg/mL	B9	9	9	0	9	0	9	0	8	1	7	2	5	4	4	5
N2			C9	12	12	0	12	0	12	0	9	3	5	7	2	10	0	12
N2			D9	10	10	0	10	0	10	0	9	1	7	3	5	5	3	7
N2	InooblODM_EtOH 15%	25 µg/mL	B10	7	7	0	7	0	7	0	7	0	6	1	4	3	3	4
N2			C10	14	14	0	14	0	14	0	10	4	7	7	4	10	2	12
N2			D10	9	9	0	9	0	9	0	8	1	5	4	3	6	3	6
N2	Reserpine	30 µM	B11	11	11	0	11	0	11	0	8	3	8	3	6	5	5	6
N2			C11	10	10	0	10	0	10	0	7	3	7	3	5	5	4	6
N2			D11	17	17	0	17	0	17	0	13	4	12	5	10	7	9	8

Table 32 - Counting list of Experiment LS_E01_003 Plate E Part 2

Life Span Assay N2

Trial Number: LS_E01_003 **E** **Experimentator:** JZ **Date:** 24.10.2018

					Date															
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	DMSO	1%	E2	9	9	0	9	0	9	0	6	3	5	4	5	4	3	6
N2			F2	11	11	0	11	0	11	0	10	1	7	4	7	4	4	7
N2			G2	11	11	0	11	0	11	0	9	2	7	4	6	5	5	6
N2	PimdioODMp	100 µg/mL	E3	12	12	0	12	0	12	0	9	3	5	7	3	9	2	10
N2			F3	6	6	0	6	0	6	0	6	0	5	1	4	2	2	4
N2			G3	8	8	0	8	0	8	0	6	2	6	2	4	4	2	6
N2	PtesanXDMp	100 µg/mL	E4	11	11	0	11	0	11	0	9	2	7	4	5	6	2	9
N2			F4	9	9	0	9	0	9	0	6	3	6	3	6	3	4	5
N2			G4	15	15	0	15	0	15	0	12	3	8	7	5	10	5	10
N2	InooblODM	100 µg/mL	E5	9	9	0	9	0	9	0	9	0	7	2	4	5	3	6
N2			F5	8	8	0	8	0	8	0	7	1	4	4	1	7	1	7
N2			G5	14	14	0	13	1	14	0	11	3	6	8	3	11	0	14
N2	TercheODMp	100 µg/mL	E6	10	10	0	10	0	10	0	9	1	7	3	5	5	2	8
N2			F6	17	17	0	17	0	17	0	11	6	7	10	5	12	2	15
N2			G6	10	10	0	10	0	10	0	8	2	6	4	5	5	4	6
N2	GarjasODMp	100 µg/mL	E7	21	21	0	21	0	21	0	15	6	11	10	8	13	6	15
N2			F7	11	11	0	11	0	11	0	10	1	9	2	6	5	4	7
N2			G7	11	11	0	11	0	11	0	8	3	8	3	6	5	3	8
N2	RhorosE	100 µg/mL	E8	16	16	0	16	0	16	0	12	4	7	9	4	12	3	13
N2			F8	11	11	0	11	0	11	0	7	4	3	8	2	9	2	9
N2			G8	15	15	0	15	0	15	0	11	4	6	9	1	14	2	13
N2	InooblODM_Pakuso	100 µg/mL	E9															
N2			F9	8	8	0	8	0	8	0	7	1	6	2	4	4	2	6
N2			G9	12	12	0	12	0	12	0	10	2	7	5	5	7	3	9
N2	InooblODM_EtOH 15%	100 µg/mL	E10	10	10	0	10	0	10	0	9	1	4	6	3	7	0	10
N2			F10	11	11	0	11	0	11	0	11	0	3	8	2	9	1	10
N2			G10	13	13	0	13	0	13	0	11	2	5	8	2	11	2	11
N2	DMSO	1%	E11	14	14	0	14	0	14	0	11	3	8	6	6	8	4	10
N2			F11	10	10	0	10	0	10	0	10	0	7	3	5	5	2	8
N2			G11	13	13	0	13	0	13	0	7	6	5	8	3	10	1	12

LS_E01_003 Plate E

Figure 28 - Lifespan screening assay LS01_003 Plate E Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 33 - Counting list of Experiment LS_E01_003 Plate F Part 1

Life Span Assay N2

Trial Number: LS_E01_003 **Plate F** **Experimentator:** JZ **Date:** 24.10.2018

					Date													
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	Drug	Conc.	Well	X_0	25.10.2018	0	29.10.2018	4	31.10.2018	6	6.11.2018	12	9.11.2018	15	14.11.2018	20	16.11.2018	22
N2	Reserpine	30 µM	B2	9	9	0	9	0	9	0	8	1	8	1	8	1	6	3
N2			C2	13	13	0	13	0	13	0	9	4	9	4	9	4	6	7
N2			D2															
N2	PimdioODMp	25 µg/mL	B3	17	17	0	17	0	17	0	13	4	11	6	10	7	5	12
N2			C3	15	15	0	15	0	15	0	10	5	8	7	6	9	3	12
N2			D3	11	11	0	11	0	11	0	8	3	7	4	6	5	3	8
N2	PtesanXDMp	25 µg/mL	B4	10	10	0	10	0	10	0	9	1	7	3	5	5	4	6
N2			C4	14	14	0	14	0	14	0	10	4	7	7	7	7	4	10
N2			D4															
N2	InooblODM	25 µg/mL	B5	15	15	0	15	0	15	0	8	7	4	11	1	14	1	14
N2			C5	11	11	0	11	0	11	0	8	3	6	5	4	7	2	9
N2			D5	17	17	0	16	1	17	0	12	5	5	12	2	15	1	16
N2	TercheODMp	25 µg/mL	B6	12	12	0	12	0	12	0	7	5	6	6	4	8	4	8
N2			C6	13	13		13		13		7	6	6	7	5	8	2	11
N2			D6	12	12	0	12	0	12	0	7	5	3	9	2	10	2	10
N2	GarjasODMp	25 µg/mL	B7	13	13	0	13	0	13	0	8	5	7	6	5	8	4	9
N2			C7	11	11	0	11	0	11	0	10	1	9	2	6	5	5	6
N2			D7	12	12	0	12	0	12	0	9	3	8	4	7	5	6	6
N2	RhorosE	25 µg/mL	B8	18	18	0	18	0	18	0	13	5	10	8	7	11	3	15
N2			C8	17	17	0	17	0	17	0	11	6	10	7	10	7	7	10
N2			D8	8	8	0	8	0	8	0	7	1	7	1	7	1	3	5
N2	InooblODM_Pakuso	25 µg/mL	B9	8	8	0	8	0	8	0	6	2	5	3	4	4	3	5
N2			C9	14	14	0	14	0	14	0	10	4	9	5	8	6	5	9
N2			D9	8	8	0	8	0	8	0	7	1	7	1	6	2	3	5
N2	InooblODM_EtOH 15%	25 µg/mL	B10	14	14	0	14	0	14	0	9	5	8	6	5	9	5	9
N2			C10	14	14	0	14	0	14	0	11	3	7	7	5	9	0	14
N2			D10	13	13	0	13	0	13	0	8	5	4	9	3	10	0	13
N2	Reserpine	30 µM	B11	16	16	0	16	0	16	0	11	5	11	5	9	7	9	7
N2			C11	12	12	0	12	0	12	0	9	3	9	3	7	5	7	5
N2			D11	12	12	0	12	0	12	0	8	4	7	5	6	6	6	6

Table 34 - Counting list of Experiment LS_E01_003 Plate F Part 2

Life Span Assay N2

Trial Number: LS_E01_003 **Plate F** **Experimentator:** JZ **Date:** 24.10.2018

																				Date	
					live	**dead**	**live**	**dead**	**live**	**dead**	**live**	**dead**	**live**	**dead**	**live**	**dead**	**live**	**dead**	**live**	**dead**
N2	DMSO	1%	E2	12	12	0	12	0	12	0	8	4	5	7	4	8	3	9
N2			F2	16	16	0	16	0	16	0	12	4	8	8	4	12	3	13
N2			G2	7	7	0	7	0	7	0	6	1	5	2	3	4	3	4
N2	PimdioODMp	100 µg/mL	E3	9	9	0	9	0	9	0	7	2	6	3	4	5	4	5
N2			F3	17	17	0	17	0	17	0	12	5	8	9	6	11	4	13
N2			G3	18	18	0	18	0	18	0	14	4	10	8	6	12	6	12
N2	PtesanXDMp	100 µg/mL	E4	18	18	0	18	0	18	0	13	5	8	10	7	11	4	14
N2			F4	16	16	0	16	0	16	0	14	2	9	7	4	12	3	13
N2			G4	8	8	0	8	0	8	0	6	2	5	3	5	3	5	3
N2	InooblODM	100 µg/mL	E5	9	9	0	9	0	9	0	8	1	7	2	4	5	3	6
N2			F5	16	16	0	16	0	16	0	9	7	6	10	3	13	0	16
N2			G5	12	12	0	12	0	12	0	9	3	7	5	6	6	2	10
N2	TercheODMp	100 µg/mL	E6	13	13	0	13	0	13	0	10	3	8	5	3	10	2	11
N2			F6	18	18	0	18	0	18	0	14	4	9	9	5	13	5	13
N2			G6	9	9	0	9	0	9	0	7	2	6	3	5	4	5	4
N2	GarjasODMp	100 µg/mL	E7	11	11	0	11	0	11	0	7	4	7	4	6	5	3	8
N2			F7															
N2			G7	14	14	0	14	0	14	0	11	3	8	6	7	7	5	9
N2	RhorosE	100 µg/mL	E8	14	14	0	14	0	14	0	9	5	4	10	1	13	0	14
N2			F8	16	16	0	16	0	16	0	9	7	6	10	3	13	3	13
N2			G8	11	11	0	11	0	11	0	7	4	5	6	3	8	2	9
N2	InooblODM_Pakuso	100 µg/mL	E9	13	13		13		13		11	2	9	4	7	6	5	8
N2			F9	11	11	0	11	0	11	0	10	1	8	3	5	6	3	8
N2			G9	14	14	0	14	0	14	0	9	5	9	5	9	5	2	12
N2	InooblODM_EtOH 15%	100 µg/mL	E10	10	10	0	10	0	10	0	8	2	4	6	3	7	1	9
N2			F10	16	16	0	16	0	16	0	10	6	5	11	2	14	0	16
N2			G10	13	13	0	13	0	13	0	10	3	4	9	3	10	3	10
N2	DMSO	1%	E11	18	18	0	18	0	18	0	9	9	7	11	7	11	5	13
N2			F11	17	17	0	17	0	17	0	14	3	6	11	5	12	5	12
N2			G11	14	14	0	14	0	14	0	9	5	7	7	6	8	6	8

Figure 29 - Lifespan screening assay LS01_003 Plate F Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 35 - Counting list of Experiment LS_E01_004 Plate E Part 1

Life Span Assay N2

Trial Number: LS_E01_004 **D** **Experimentator:** **Date:** 8.11.2018

					Date															
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	**Drug**	**Conc.**	**Well**	**X_0**	8.11.2018	0	11.11.2018	3	13.11.2018	5	16.11.2018	8	21.11.2018	13	23.11.2018	15	26.11.2018	18	28.11.2018	20
N2			B2	13	13	0	13	0	13	0	11	2	8	5	5	8	5	8	3	10
N2	Reserpine	30 µM	C2	16	16	0	16	0	16	0	13	3	12	4	12	4	12	4	11	5
N2			D2	17	17	0	17	0	17	0	15	2	10	7	9	8	7	10	4	13
N2			B3																	
N2	AICAR	100 µM	C3	17	17	0	17	0	17	0	15	2	13	4	10	7	8	9	4	13
N2			D3	14	14	0	14	0	14	0	14	0	12	2	12	2	6	8	4	10
N2			B4	17	17	0	17	0	17	0	14	3	13	4	12	5	6	11	2	15
N2	PipbetODM	25 µg/mL	C4	13	13	0	13	0	13	0	11	2	9	4	9	4	4	9	4	9
N2			D4	14	14	0	14	0	14	0	12	2	11	3	11	3	7	7	2	12
N2			B5	16	16	0	16	0	16	0	14	2	14	2	14	2	10	6	6	10
N2	FompinODM	25 µg/mL	C5																	
N2			D5	12	12	0	12	0	12	0	10	2	8	4	5	7	5	7	4	8
N2			B6	14	14	0	14	0	14	0	11	3	11	3	9	5	5	9	3	11
N2	GanlucDMp	25 µg/mL	C6	17	17	0	17	0	17	0	17	0	15	2	14	3	5	12	4	13
N2			D6	14	14	0	14	0	14	0	12	2	13	1	13	1	6	8	1	13
N2			B7	17	17	0	17	0	17	0	12	5	9	8	9	8	4	13	4	13
N2	CaloffBDMp	25 µg/mL	C7	17	17	0	17	0	17	0	14	3	13	4	12	5	10	7	9	8
N2			D7	13	13	0	13	0	13	0	9	4	8	5	6	7	4	9	4	9
N2			B8																	
N2	ValoffRDMp	25 µg/mL	C8																	
N2			D8	14	14	0	14	0	14	0	13	1	11	3	9	5	5	9	4	10
N2			B9	15	15	0	15	0	15	0	14	1	13	2	13	2	7	8	6	9
N2	LicislODMp	25 µg/mL	C9																	
N2			D9	15	15	0	15	0	15	0	15	0	14	1	14	1	10	5	6	9
N2			B10																	
N2	SyzaroODMp	25 µg/mL	C10	12	12	0	12	0	12	0	9	3	7	5	6	6	5	7	3	9
N2			D10																	
N2			B11	9	9	0	9	0	9	0	9	0	9	0	9	0	8	1	5	4
N2	Reserpine	30 µM	C11	9	9	0	9	0	9	0	5	4	4	5	4	5	4	5	3	6
N2			D11	12	12	0	12	0	12	0	9	3	7	5	7	5	7	5	4	8

Table 36 - Counting list of Experiment LS_E01_004 Plate D Part 2

Life Span Assay N2

Trial Number: LS_E01_004 **D** **Experimentator:** **Date:** 8.11.2018

				Date																	
N2	DMSO	1%	E2	15	15	0	15	0	15	0	13	2	11	4	8	7	6	9	3	12	
N2			F2																		
N2			G2	9	9	0	9	0	9	0	9	0	7	2	5	4	5	4	5	4	
N2		100 µg/mL	E3																		
N2			F3																		
N2			G3																		
N2	PipbetODM	100 µg/mL	E4	13	13	0	13	0	13	0	9	4	9	4	8	5	5	8	1	12	
N2			F4																		
N2			G4	8	8	0	8	0	8	0	7	1	6	2	6	2	6	2	1	7	
N2	FompinODM	100 µg/mL	E5	18	18	0	18	0	18	0	16	2	12	6	11	7	11	7	2	16	
N2			F5	9	9	0	9	0	9	0	8	1	8	1	8	1	4	5	1	8	
N2			G5	14	14	0	14	0	14	0	11	3	9	5	7	7	5	9	0	14	
N2	GanlucDMp	100 µg/mL	E6	18	18	0	18	0	18	0	14	4	13	5	12	6	5	13	4	14	
N2			F6	14	14	0	14	0	14	0	12	2	11	3	11	3	8	6	6	8	
N2			G6	13	13	0	13	0	13	0	13	0	11	2	11	2	8	5	4	9	
N2	CaloffBDMp	100 µg/mL	E7	12	12	0	12	0	12	0	11	1	9	3	9	3	5	7	4	8	
N2			F7	13	13	0	13	0	13	0	12	1	11	2	11	2	5	8	3	10	
N2			G7	14	14	0	14	0	14	0	12	2	12	2	12	2	7	7	2	12	
N2	ValoffRDMp	100 µg/mL	E8	14	14	0	14	0	14	0	11	3	8	6	8	6	3	11	2	12	
N2			F8	18	18	0	18	0	18	0	17	1	14	4	12	6	8	10	5	13	
N2			G8	18	18	0	18	0	18	0	16	2	12	6	11	7	8	10	5	13	
N2	LicisIODMp	100 µg/mL	E9	13	13	0	13	0	13	0	13	0	10	3	8	5	5	8	4	9	
N2			F9	12	12	0	12	0	12	0	8	4	7	5	5	7	5	7	1	11	
N2			G9																		
N2	SyzaroODMp	100 µg/mL	E10																		
N2			F10																		
N2			G10	16	16	0	16	0	16	0	14	2	14	2	13	3	9	7	9	7	
N2	DMSO	1%	E11	18	18	0	18	0	18	0	15	3	12	6	11	7	9	9	6	12	
N2			F11																		
N2			G11																		

LS_E01_004 D

Figure 30 - Lifespan screening assay LS01_004 Plate D Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 37 - Counting list of Experiment LS_E01_004 Plate E Part 1

Life Span Assay N2

Trial Number: LS_E01_004 **E** **Experimentator:** **Date:** 8.11.2018

					Date															
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	**Drug**	**Conc.**	**Well**	**X_0**	8.11.2018	0	11.11.2018	3	13.11.2018	5	16.11.2018	8	21.11.2018	13	23.11.2018	15	26.11.2018	18	28.11.2018	20
N2	Reserpine	30 µM	B2	13			13	0	13	0	10	3	7	6	6	7	6	7	4	9
N2			C2																	
N2			D2																	
N2	AICAR	25 µg/mL	B3	12			12	0	12	0	10	2	9	3	8	4	6	6	6	6
N2			C3	13			13	0	13	0	13	0	10	3	8	5	7	6	5	8
N2			D3																	
N2	PipbetODM	25 µg/mL	B4	14			14	0	14	0	14	0	13	1	10	4	8	6	3	11
N2			C4	17			17	0	17	0	16	1	14	3	9	8	7	10	6	11
N2			D4	18			18	0	18	0	16	2	12	6	11	7	6	12	6	12
N2	FompinODM	25 µg/mL	B5	16			16	0	16	0	11	5	10	6	8	8	8	8	5	11
N2			C5	17			17	0	17	0	14	3	12	5	9	8	6	11	5	12
N2			D5																	
N2	GanlucDMp	25 µg/mL	B6	15			15	0	15	0	12	3	10	5	9	6	8	7	5	10
N2			C6	16			16	0	16	0	15	1	12	4	10	6	6	10	5	11
N2			D6	17			17	0	17	0	14	3	13	4	10	7	8	9	5	12
N2	CaloffBDMp	25 µg/mL	B7	15			15	0	15	0	13	2	12	3	9	6	8	7	8	7
N2			C7	17			17	0	17	0	17	0	13	4	11	6	11	6	11	6
N2			D7	16			16	0	16	0	14	2	12	4	9	7	7	9	5	11
N2	ValoffRDMp	25 µg/mL	B8	15			15	0	15	0	13	2	9	6	8	7	7	8	3	12
N2			C8																	
N2			D8	13			13	0	13	0	11	2	10	3	7	6	6	7	4	9
N2	LicislODMp	25 µg/mL	B9	8			8	0	8	0	7	1	7	1	5	3	4	4	3	5
N2			C9	10			10	0	10	0	8	2	8	2	8	2	5	5	2	8
N2			D9	18			18	0	18	0	17	1	15	3	14	4	10	8	7	11
N2	SyzaroODMp	25 µg/mL	B10	16			16	0	16	0	14	2	14	2	9	7	8	8	8	8
N2			C10	12			12	0	12	0	10	2	10	2	10	2	8	4	7	5
N2			D10	11			11	0	11	0	11	0	9	2	5	6	5	6	4	7
N2	Reserpine	30 µM	B11	9			9	0	9	0	7	2	6	3	6	3	6	3	3	6
N2			C11	17			17	0	17	0	12	5	12	5	11	6	11	6	9	8
N2			D11	17			17	0	17	0	12	5	10	7	9	8	9	8	9	8

Table 38 - Counting list of Experiment LS_E01_004 Plate E Part 2

Life Span Assay N2

Trial Number: LS_E01_004 **E** **Experimentator:** **Date:** 8.11.2018

					Date																	
N2	DMSO	1%	E2	16			16	0	16	0	14	2	14	2	10	6	7	9	3	13
N2			F2	13			13	0	13	0	8	5	7	6	8	5	7	6	3	10
N2			G2	17			17	0	17	0	14	3	11	6	10	7	8	9	4	13
N2		100 µg/mL	E3																	
N2			F3																	
N2			G3																	
N2	PipbetODM	100 µg/mL	E4																	
N2			F4	17			17	0	17	0	14	3	12	5	10	7	6	11	3	14
N2			G4																	
N2	FompinODM	100 µg/mL	E5																	
N2			F5	16			16	0	16	0	12	4	8	8	6	10	3	13	2	14
N2			G5	15			15	0	15	0	13	2	11	4	6	9	4	11	4	11
N2	GanlucDMp	100 µg/mL	E6	13			13	0	13	0	11	2	9	4	6	7	4	9	2	11
N2			F6	15			15	0	15	0	11	4	11	4	10	5	6	9	4	11
N2			G6	12			12	0	12	0	10	2	8	4	8	4	7	5	4	8
N2	CaloffBDMp	100 µg/mL	E7	15			15	0	15	0	14	1	14	1	14	1	8	7	8	7
N2			F7																	
N2			G7	13			13	0	13	0	11	2	11	2	8	5	6	7	6	7
N2	ValoffRDMp	100 µg/mL	E8																	
N2			F8	16			16	0	16	0	14	2	12	4	9	7	5	11	5	11
N2			G8	16			16	0	16	0	15	1	12	4	9	7	6	10	6	10
N2	LicislODMp	100 µg/mL	E9	11			11	0	11	0	8	3	6	5	6	5	3	8	3	8
N2			F9	12			12	0	12	0	12	0	10	2	10	2	7	5	3	9
N2			G9																	
N2	SyzaroODMp	100 µg/mL	E10	13			13	0	13	0	10	3	10	3	10	3	9	4	7	6
N2			F10	16			16	0	16	0	15	1	15	1	13	3	9	7	7	9
N2			G10	10			10	0	10	0	9	1	6	4	6	4	5	5	4	6
N2	DMSO	1%	E11	17			17	0	17	0	15	2	13	4	11	6	10	7	4	13
N2			F11	16			16	0	16	0	15	1	10	6	11	5	10	6	5	11
N2			G11	18			18	0	18	0	17	1	12	6	11	7	11	7	6	12

LS_E01_004 E

Figure 31 - Lifespan screening assay LS01_004 Plate E Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 39 - Counting list of Experiment LS_E01_004 Plate F Part 1

Life Span Assay N2

Trial Number: LS_E01_004 **F** **Experimentator:** **Date:** 8.11.2018

					Date															
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	Drug	Conc.	Well	X_0	8.11.2018	0	11.11.2018	3	13.11.2018	5	16.11.2018	8	21.11.2018	13	23.11.2018	15	26.11.2018	18	28.11.2018	20
N2	Reserpine	30 µM	B2	18			18	0	18	0	11	7	10	8	7	11	7	11	4	14
N2			C2																	
N2			D2																	
N2	AICAR	25 µg/mL	B3	15			15	0	15	0	14	1	12	3	12	3	10	5	7	8
N2			C3	12			12	0	12	0	11	1	10	2	10	2	6	6	4	8
N2			D3	14			14	0	14	0	13	1	11	3	10	4	8	6	7	7
N2	PipbetODM	25 µg/mL	B4	16			16	0	16	0	12	4	9	7	9	7	5	11	3	13
N2			C4	15			15	0	15	0	11	4	10	5	10	5	6	9	5	10
N2			D4																	
N2	FompinODM	25 µg/mL	B5	16			16	0	16	0	15	1	11	5	10	6	4	12	2	14
N2			C5	12			12	0	12	0	8	4	6	6	6	6	5	7	2	10
N2			D5	13			13	0	13	0	10	3	9	4	8	5	6	7	5	8
N2	GanlucDMp	25 µg/mL	B6	14			14	0	14	0	10	4	10	4	8	6	8	6	5	9
N2			C6	17			17	0	17	0	14	3	13	4	7	10	7	10	6	11
N2			D6																	
N2	CaloffBDMp	25 µg/mL	B7	18			18	0	18	0	14	4	11	7	10	8	8	10	3	15
N2			C7	12			12	0	12	0	11	1	9	3	9	3	5	7	1	11
N2			D7	12			12	0	12	0	10	2	5	7	5	7	3	9	3	9
N2	ValoffRDMp	25 µg/mL	B8	14			14	0	14	0	12	2	11	3	8	6	6	8	3	11
N2			C8	17			17	0	17	0	11	6	8	9	8	9	5	12	4	13
N2			D8	16			16	0	16	0	15	1	13	3	7	9	6	10	2	14
N2	LicislODMp	25 µg/mL	B9																	
N2			C9																	
N2			D9																	
N2	SyzaroODMp	25 µg/mL	B10	17			17	0	17	0	15	2	13	4	11	6	8	9	5	12
N2			C10	15			15	0	15	0	12	3	9	6	8	7	6	9	4	11
N2			D10	9			9	0	9	0	8	1	7	2	7	2	4	5	2	7
N2	Reserpine	30 µM	B11																	
N2			C11	18			18	0	18	0	11	7	10	8	10	8	8	10	3	15
N2			D11	10			10	0	10	0	8	2	7	3	7	3	7	3	5	5

Table 40 - Counting list of Experiment LS_E01_004 Plate F Part 2

Life Span Assay N2

Trial Number: LS_E01_004 **F** **Experimentator:** **Date:** 8.11.2018

						Date																			
N2	DMSO	1%	E2	13			13	0	13	0	11	2	9	4	9	4	9	4	6	7
N2			F2																	
N2			G2	14			14	0	14	0	11	3	9	5	8	6	7	7	6	8
N2			E3																	
N2			F3																	
N2			G3																	
N2	PipbetODM	100 µg/mL	E4	16			16	0	16	0	14	2	14	2	3	13	3	13	2	14
N2			F4	14			14	0	14	0	12	2	10	4	8	6	7	7	7	7
N2			G4																	
N2	FompinODM	100 µg/mL	E5																	
N2			F5	17			17	0	17	0	13	4	12	5	10	7	8	9	8	9
N2			G5	17			17	0	17	0	15	2	13	4	11	6	9	8	6	11
N2	GanlucDMp	100 µg/mL	E6																	
N2			F6	12			12	0	12	0	8	4	7	5	7	5	6	6	3	9
N2			G6	18			18	0	18	0	16	2	15	3	13	5	13	5	7	11
N2	CaloffBDMp	100 µg/mL	E7	15			15	0	15	0	14	1	13	2	9	6	8	7	5	10
N2			F7	14			14	0	14	0	10	4	10	4	8	6	7	7	6	8
N2			G7																	
N2	ValoffRDMp	100 µg/mL	E8																	
N2			F8																	
N2			G8	13			13	0	13	0	11	2	10	3	10	3	6	7	5	8
N2	LicislODMp	100 µg/mL	E9	11			11	0	11	0	7	4	4	7	4	7	1	10	0	11
N2			F9																	
N2			G9	17			17	0	17	0	13	4	12	5	12	5	11	6	8	9
N2	SyzaroODMp	100 µg/mL	E10																	
N2			F10																	
N2			G10	14			14	0	14	0	13	1	11	3	9	5	8	6	5	9
N2	DMSO	1%	E11	8			8	0	8	0	7	1	5	3	5	3	4	4	3	5
N2			F11	8			8	0	8	0	6	2	6	2	6	2	6	2	2	6
N2			G11	6			6	0	6	0	5	1	3	3	3	3	3	3	1	5

LS_E01_004 F

Figure 32 - Lifespan screening assay LS01_004 Plate F Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 41 - Counting list of Experiment LS_E01_005 Plate A Part 1

Life Span Assay N2

Trial Number: LS_E01_005 **A** **Experimentator:** **Date:** 12.10.2018

					Date																	
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	**Drug**	**Conc.**	**Well**	**X_0**	12.10.2018	0	15.10.2018	3	17.10.2018	5	19.10.2018	7	22.10.2018	10	25.10.2018	13	29.10.2018	17	31.10.2018	19	5.11.2018	24
N2	Reserpine	30 µM	B2	4		0	4	0	4	0	3	1	3	1	2	2	0	4	0	4	0	4
N2			C2	11		0	11	0	11	0	11	0	10	1	10	1	7	4	6	5	2	9
N2			D2	14		0	14	0	13	1	13	1	11	3	11	3	8	6	7	7	4	10
N2	DryforRDMp	25 µg/mL	B3	9		0	9	0	9	0	9	0	7	2	7	2	5	4	5	4	2	7
N2			C3	12		0	12	0	12	0	12	0	10	2	9	3	7	5	7	5	2	10
N2			D3	11		0	11	0	10	1	10	1	10	1	8	3	6	5	4	7	2	9
N2	CynpanRDMp	25 µg/mL	B4	6		0	6	0	6	0	6	0	6	0	6	0	4	2	4	2	2	4
N2			C4	12		0	12	0	12	0	12	0	11	1	10	2	7	5	6	6	2	10
N2			D4	13		0	13	0	11	2	11	2	10	3	10	3	7	6	5	8	1	12
N2	CynstaRDMp	25 µg/mL	B5	7		0	7	0	6	1	6	1	6	1	5	2	2	5	2	5	0	7
N2			C5	11		0	11	0	10	1	10	1	10	1	8	3	8	3	5	6	2	9
N2			D5	11		0	11	0	9	2	9	2	8	3	6	5	4	7	3	8	1	10
N2	AndpanHDMp	25 µg/mL	B6	5		0	5	0	5	0	5	0	5	0	5	0	5	0	3	2	2	3
N2			C6	11		0	11	0	11	0	11	0	10	1	7	4	7	4	6	5	2	9
N2			D6	7		0	7	0	5	2	5	2	5	2	5	2	3	4	2	5	1	6
N2	InooblODM_479	25 µg/mL	B7	10		0	10	0	8	2	8	2	8	2	7	3	5	5	4	6	4	6
N2			C7	9		0	9	0	9	0	9	0	7	2	7	2	7	2	5	4	1	8
N2			D7	8		0	8	0	8	0	8	0	8	0	6	2	7	1	4	4	3	5
N2	LinrhiE_2018	25 µg/mL	B8	15		0	15	0	14	1	14	1	14	1	13	2	9	6	5	10	4	11
N2			C8	17		0	16	1	17	0	17	0	14	3	14	3	9	8	10	7	6	11
N2			D8	12		0	12	0	11	1	11	1	11	1	10	2	9	3	8	4	6	6
N2	AntcinE_2018	25 µg/mL	B9	18		0	18	0	16	2	16	2	15	3	15	3	8	10	9	9	7	11
N2			C9	11		0	11	0	10	1	10	1	8	3	8	3	5	6	5	6	3	8
N2			D9	9		0	9	0	9	0	9	0	9	0	8	1	4	5	5	4	2	7
N2	GloodoE0054_2018	25 µg/mL	B10	12		0	12	0	11	1	11	1	9	3	9	3	4	8	5	7	0	12
N2			C10	3		0	3	0	3	0	3	0	2	1	2	1	0	3	1	2	0	3
N2			D10	6		0	6	0	5	1	5	1	5	1	5	1	4	2	2	4	1	5
N2	Reserpine	30 µM	B11	10		0	10	0	10	0	10	0	10	0	10	0	6	4	5	5	0	10
N2			C11	10		0	10	0	8	2	8	2	8	2	8	2	6	4	5	5	2	8
N2			D11	7		0	7	0	7	0	7	0	6	1	5	2	4	3	2	5	2	5

Table 42 - Counting list of Experiment LS_E01_005 Plate A Part 2

Life Span Assay N2

Trial Number: LS_E01_005 **A** **Experimentator:** **Date:** 12.10.2018

						Date																					
N2	DMSO	1%	E2	11		0	11	0	9	2	9	2	8	3	7	4	7	4	7	4	2	9
N2			F2	10		0	10	0	9	1	9	1	8	2	8	2	5	5	5	5	2	8
N2			G2	6		0	6	0	6	0	6	0	4	2	4	2	2	4	1	5	0	6
N2	DryforRDMp	100 µg/mL	E3	5		0	5	0	5	0	5	0	4	1	4	1	2	3	0	5	0	5
N2			F3	9		0	9	0	9	0	9	0	9	0	9	0	7	2	5	4	3	6
N2			G3	17		0	17	0	16	1	16	1	14	3	14	3	9	8	9	8	5	12
N2	CynpanRDMp	100 µg/mL	E4	11		0	11	0	11	0	11	0	11	0	11	0	8	3	5	6	0	11
N2			F4	8		0	8	0	7	1	7	1	7	1	6	2	4	4	2	6	0	8
N2			G4	11		0	11	0	10	1	10	1	10	1	10	1	5	6	5	6	2	9
N2	CynstaRDMp	100 µg/mL	E5	11		0	11	0	11	0	11	0	9	2	8	3	6	5	5	6	0	11
N2			F5	9		0	9	0	9	0	9	0	9	0	6	3	7	2	4	5	0	9
N2			G5	9		0	9	0	6	3	6	3	6	3	6	3	5	4	4	5	1	8
N2	AndpanHDMp	100 µg/mL	E6	9		0	9	0	9	0	9	0	9	0	7	2	7	2	4	5	1	8
N2			F6	13		0	13	0	13	0	13	0	12	1	12	1	7	6	8	5	6	7
N2			G6	14		0	14	0	13	1	13	1	12	2	11	3	9	5	9	5	3	11
N2	InooblODM_479	100 µg/mL	E7	11		0	11	0	10	1	10	1	9	2	9	2	8	3	6	5	2	9
N2			F7	9		0	9	0	9	0	9	0	9	0	8	1	3	6	4	5	2	7
N2			G7	9		0	9	0	7	2	7	2	6	3	5	4	3	6	3	6	0	9
N2	LinrhiE_2018	100 µg/mL	E8	11		0	11	0	11	0	11	0	10	1	10	1	9	2	7	4	4	7
N2			F8	12		0	12	0	11	1	11	1	10	2	10	2	9	3	8	4	5	7
N2			G8	8		0	8	0	8	0	8	0	8	0	7	1	4	4	3	5	3	5
N2	AntcinE_2018	100 µg/mL	E9	6		0	5	1	4	2	4	2	4	2	3	3	3	3	2	4	1	5
N2			F9	13		0	13	0	12	1	12	1	9	4	9	4	4	9	6	7	1	12
N2			G9	8		0	8	0	6	2	6	2	6	2	3	5	5	3	3	5	1	7
N2	GloodoE0054_2018	100 µg/mL	E10	8		0	8	0	8	0	8	0	8	0	8	0	4	4	2	6	1	7
N2			F10	10		0	10	0	9	1	9	1	9	1	8	2	5	5	6	4	1	9
N2			G10	12		0	12	0	11	1	11	1	11	1	11	1	4	8	3	9	0	12
N2	DMSO	1%	E11	9		0	9	0	7	2	7	2	6	3	5	4	5	4	4	5	1	8
N2			F11	10		0	10	0	10	0	10	0	9	1	8	2	7	3	5	5	1	9
N2			G11	10		0	10	0	8	2	8	2	7	3	7	3	5	5	4	6	1	9

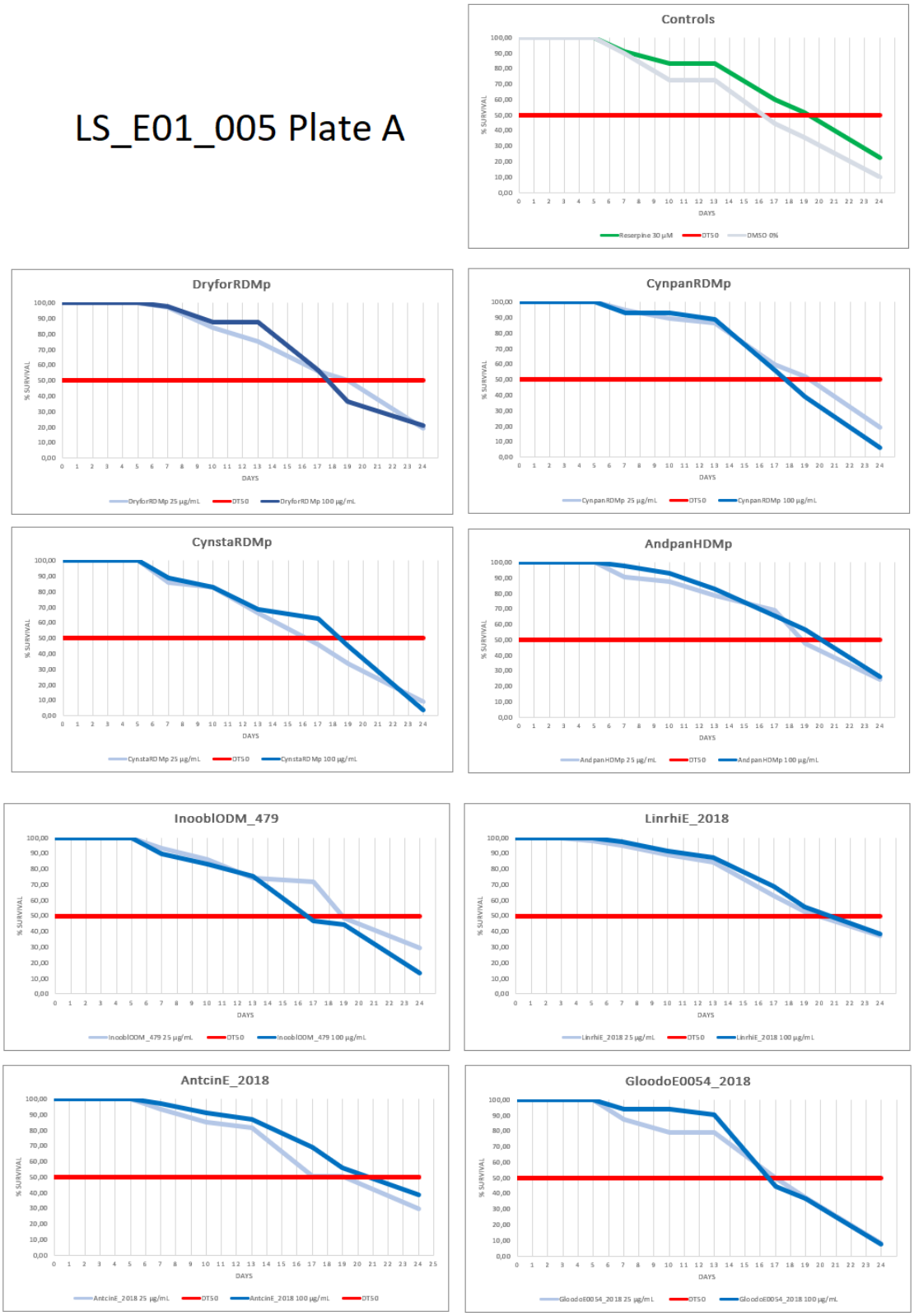


Figure 33 - Lifespan screening assay LS01_005 Plate A Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 43 - Counting list of Experiment LS_E01_005 Plate B Part 1

Life Span Assay N2

Trial Number: LS_E01_005 **B** **Experimentator:** **Date:** 12.10.2018

					Date																	
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	**Drug**	**Conc.**	**Well**	**X_0**	12.10.2018	0	15.10.2018	3	17.10.2018	5	19.10.2018	7	22.10.2018	10	25.10.2018	13	29.10.2018	17	31.10.2018	19	5.11.2018	24
N2	Reserpine	30 µM	B2	7			7	0	7	0	7	0	7	0	4	3	5	2	4	3	3	4
N2			C2	6			6	0	6	0	6	0	6	0	6	0	4	2	4	2	2	4
N2			D2	10			10	0	10	0	8	2	7	3	7	3	4	6	4	6	2	8
N2	DryforRDMp	25 µg/mL	B3	13			13	0	13	0	13	0	12	1	12	1	6	7	5	8	3	10
N2			C3	8			8	0	8	0	8	0	7	1	7	1	3	5	3	5	2	6
N2			D3	13			13	0	13	0	13	0	12	1	10	3	9	4	7	6	2	11
N2	CynpanRDMp	25 µg/mL	B4	8			8	0	8	0	8	0	7	1	7	1	6	2	3	5	1	7
N2			C4	11			11	0	11	0	11	0	9	2	9	2	6	5	5	6	1	10
N2			D4	8			8	0	7	1	7	1	7	1	5	3	4	4	3	5	2	6
N2	CynstaRDMp	25 µg/mL	B5	12			12	0	12	0	12	0	12	0	9	3	9	3	4	8	3	9
N2			C5	6			6	0	5	1	5	1	5	1	4	2	4	2	3	3	1	5
N2			D5	10			10	0	10	0	10	0	10	0	9	1	6	4	6	4	2	8
N2	AndpanHDMp	25 µg/mL	B6	8			8	0	8	0	8	0	5	3	5	3	3	5	3	5	1	7
N2			C6	11			11	0	10	1	10	1	8	3	8	3	6	5	4	7	1	10
N2			D6	5			5	0	4	1	4	1	4	1	3	2	2	3	2	3	1	4
N2	InoobIODM_479	25 µg/mL	B7	1			1	0	1	0	1	0	1	0	0	1	0	1	0	1	0	1
N2			C7	8			8	0	8	0	8	0	8	0	8	0	6	2	5	3	1	7
N2			D7	6			6	0	6	0	6	0	6	0	4	2	2	4	0	6	0	6
N2	LinrhiE_2018	25 µg/mL	B8	5			5	0	5	0	5	0	5	0	5	0	5	0	4	1	0	5
N2			C8	12			12	0	12	0	12	0	11	1	10	2	8	4	7	5	5	7
N2			D8	9			9	0	8	1	8	1	8	1	6	3	6	3	5	4	2	7
N2	AntcinE_2018	25 µg/mL	B9	10			10	0	8	2	7	3	7	3	6	4	6	4	3	7	2	8
N2			C9	13			13	0	13	0	13	0	13	0	10	3	5	8	3	10	2	11
N2			D9	8			8	0	7	1	7	1	6	2	6	2	3	5	1	7	0	8
N2	GloodoE0054_2018	25 µg/mL	B10	13			13	0	12	1	12	1	11	2	10	3	7	6	4	9	4	9
N2			C10	9			9	0	8	1	8	1	8	1	6	3	5	4	2	7	1	8
N2			D10	12			12	0	11	1	11	1	9	3	10	2	7	5	6	6	1	11
N2	Reserpine	30 µM	B11	6			6	0	6	0	5	1	3	3	2	4	2	4	2	4	2	4
N2			C11	7			7	0	7	0	6	1	4	3	4	3	3	4	3	4	1	6
N2			D11	10			10	0	9	1	7	3	8	2	8	2	7	3	5	5	1	9

Table 44 - Counting list of Experiment LS_E01_005 Plate B Part 2

Life Span Assay N2

Trial Number: LS_E01_005 **B** **Experimentator:** **Date:** 12.10.2018

							Date																	
N2	DMSO	1%	E2	15			15	0	15	0	15	0	15	0	15	0	6	9	7	8	1	14
N2			F2	16			16	0	16	0	14	2	13	3	13	3	5	11	7	9	5	11
N2			G2	10			10	0	10	0	8	2	9	1	7	3	3	7	4	6	3	7
N2	DryforRDMp	100 µg/mL	E3	19			19	0	19	0	17	2	15	4	15	4	6	13	6	13	6	13
N2			F3	9			9	0	9	0	9	0	9	0	9	0	5	4	5	4	1	8
N2			G3	8			8	0	7	1	7	1	7	1	5	3	3	5	3	5	0	8
N2	CynpanRDMp	100 µg/mL	E4	10			10	0	10	0	10	0	9	1	9	1	5	5	5	5	1	9
N2			F4	5			5	0	5	0	5	0	5	0	4	1	3	2	2	3	1	4
N2			G4	11			11	0	11	0	11	0	11	0	11	0	5	6	4	7	2	9
N2	CynstaRDMp	100 µg/mL	E5	9			9	0	9	0	9	0	9	0	8	1	5	4	5	4	4	5
N2			F5	11			11	0	11	0	8	3	8	3	7	4	4	7	4	7	1	10
N2			G5	13			13	0	13	0	13	0	12	1	10	3	8	5	8	5	1	12
N2	AndpanHDMp	100 µg/mL	E6	6			6	0	6	0	6	0	6	0	6	0	3	3	2	4	0	6
N2			F6	9			9	0	9	0	8	1	8	1	7	2	5	4	3	6	1	8
N2			G6	10			10	0	10	0	10	0	10	0	10	0	4	6	2	8	1	9
N2	InooblODM_479	100 µg/mL	E7	7			7	0	6	1	6	1	6	1	6	1	4	3	1	6	0	7
N2			F7	11			11	0	10	1	10	1	10	1	10	1	5	6	6	5	0	11
N2			G7	16			16	0	16	0	15	1	13	3	13	3	8	8	9	7	4	12
N2	LinrhiE_2018	100 µg/mL	E8	13			12	1	12	1	11	2	10	3	8	5	5	8	4	9	2	11
N2			F8	12			12	0	10	2	9	3	9	3	9	3	6	6	4	8	3	9
N2			G8	9			9	0	9	0	9	0	8	1	7	2	5	4	3	6	1	8
N2	AntcinE_2018	100 µg/mL	E9	7			7	0	7	0	7	0	7	0	7	0	5	2	5	2	2	5
N2			F9	12			12	0	10	2	10	2	9	3	9	3	6	6	4	8	1	11
N2			G9	10			10	0	10	0	10	0	10	0	9	1	9	1	6	4	1	9
N2	GloodoE0054_2018	100 µg/mL	E10	8			8	0	8	0	8	0	8	0	8	0	2	6	2	6	2	6
N2			F10	15			15	0	14	1	13	2	13	2	13	2	7	8	4	11	3	12
N2			G10	10			10	0	9	1	8	2	7	3	7	3	3	7	4	6	0	10
N2	DMSO	1%	E11	19			19	0	19	0	16	3	12	7	12	7	8	11	2	17	4	15
N2			F11	13			13	0	13	0	12	1	10	3	8	5	8	5	6	7	1	12
N2			G11	19			19	0	18	1	16	3	14	5	12	7	8	11	3	16	1	18

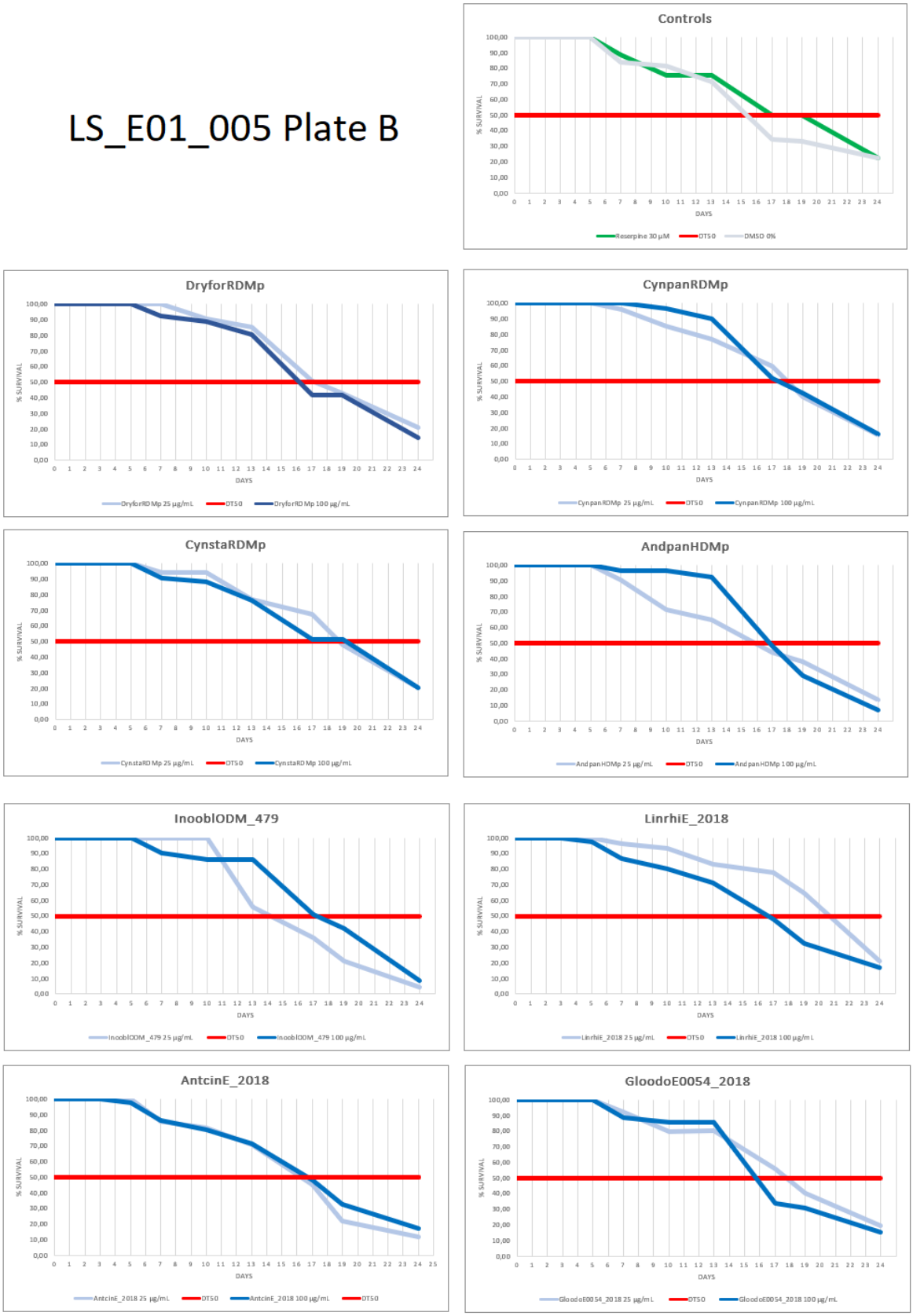


Figure 34 - Lifespan screening assay LS01_005 Plate B Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 45 - Counting list of Experiment LS_E01_005 Plate C Part 1

Life Span Assay N2

Trial Number: LS_E01_005 **C** **Experimentator:** **Date:** 12.10.2018

Date

Strain	Drug	Conc.	Well	X_0	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
					12.10.2018	0	15.10.2018	3	17.10.2018	5	19.10.2018	7	22.10.2018	10	25.10.2018	13	29.10.2018	17	31.10.2018	19	5.11.2018	24	8.11.2018	27
N2	Reserpine	30 µM	B2	6		0	6	0	6	0	6	0	6	0	5	1	4	2	4	2	1	5		
N2			C2	6		0	6	0	6	0	6	0	5	1	5	1	4	2	3	3	1	5		
N2			D2	7		0	7	0	7	0	7	0	7	0	6	1	4	3	4	3	1	6		
N2	DryforRDMp	25 µg/mL	B3	5		0	5	0	5	0	5	0	5	0	4	1	4	1	2	3	1	4		
N2			C3	11		0	11	0	11	0	11	0	10	1	8	3	8	3	6	5	3	8		
N2			D3	10		0	10	0	10	0	10	0	8	2	7	3	4	6	4	6	3	7		
N2	CynpanRDMp	25 µg/mL	B4	12		0	12	0	12	0	11	1	11	1	9	3	9	3	5	7	2	10		
N2			C4	7		0	7	0	6	1	6	1	6	1	6	1	4	3	2	5	0	7		
N2			D4	6		0	6	0	6	0	5	1	5	1	5	1	4	2	2	4	1	5		
N2	CynstaRDMp	25 µg/mL	B5	11		0	11	0	10	1	10	1	10	1	9	2	7	4	7	4	2	9		
N2			C5	14		0	14	0	14	0	14	0	14	0	12	2	9	5	8	6	2	12		
N2			D5	5		0	5	0	5	0	5	0	4	1	4	1	4	1	4	1	0	5		
N2	AndpanHDMp	25 µg/mL	B6	8		0	8	0	8	0	8	0	8	0	6	2	4	4	4	4	2	6		
N2			C6	7		0	7	0	7	0	7	0	7	0	5	2	3	4	3	4	1	6		
N2			D6	8		0	8	0	7	1	7	1	7	1	6	2	6	2	6	2	2	6		
N2	InooblODM_479	25 µg/mL	B7	10		0	10	0	10	0	10	0	9	1	6	4	6	4	4	6	1	9		
N2			C7	16		0	16	0	16	0	14	2	14	2	14	2	7	9	7	9	2	14		
N2			D7	10		0	10	0	10	0	9	1	9	1	8	2	5	5	6	4	1	9		
N2	LinrhiE_2018	25 µg/mL	B8	9		0	9	0	9	0	9	0	8	1	7	2	5	4	5	4	2	7		
N2			C8	10		0	10	0	10	0	10	0	8	2	8	2	5	5	4	6	2	8		
N2			D8	13		0	13	0	13	0	13	0	13	0	13	0	9	4	9	4	4	9		
N2	AntcinE_2018	25 µg/mL	B9	9		0	9	0	8	1	8	1	8	1	7	2	5	4	5	4	2	7		
N2			C9	12		0	12	0	12	0	12	0	11	1	9	3	7	5	7	5	1	11		
N2			D9	9		0	9	0	9	0	9	0	9	0	7	2	4	5	4	5	0	9		
N2	GloodoE0054_2018	25 µg/mL	B10	11		0	11	0	9	2	9	2	8	3	6	5	7	4	7	4	0	11		
N2			C10	21		0	21	0	21	0	19	2	16	5	15	6	7	14	7	14	2	19		
N2			D10	9		0	9	0	9	0	9	0	9	0	9	0	7	2	4	5	2	7		
N2	Reserpine	30 µM	B11	10		0	10	0	9	1	9	1	8	2	6	4	6	4	5	5	2	8		
N2			C11	9		0	9	0	9	0	9	0	6	3	6	3	6	3	5	4	4	5		
N2			D11	12		0	12	0	12	0	11	1	10	2	7	5	8	4	7	5	3	9		

Table 46 - Counting list of Experiment LS_E01_005 Plate C Part 2

Life Span Assay N2

Trial Number: LS_E01_005 **C** **Experimentator:** **Date:** 12.10.2018

						Date																		
N2	DMSO	1%	E2	10		0	10	0	10	0	10	0	10	0	9	1	6	4	5	5	3	7		
N2			F2	12		0	12	0	11	1	9	3	9	3	9	3	6	6	5	7	0	12		
N2			G2	9		0	9	0	9	0	8	1	6	3	5	4	5	4	3	6	1	8		
N2	DryforRDMp	100 µg/mL	E3	14		0	14	0	13	1	13	1	12	2	12	2	8	6	7	7	1	13		
N2			F3	10		1	9	1	8	2	8	2	8	2	8	2	7	3	6	4	2	8		
N2			G3	11		0	11	0	11	0	11	0	10	1	10	1	10	1	6	5	4	7		
N2	CynpanRDMp	100 µg/mL	E4	11		0	11	0	11	0	11	0	10	1	10	1	4	7	6	5	3	8		
N2			F4	12		0	12	0	12	0	12	0	10	2	10	2	5	7	3	9	1	11		
N2			G4	5		0	5	0	5	0	5	0	5	0	4	1	2	3	2	3	1	4		
N2	CynstaRDMp	100 µg/mL	E5	7		0	7	0	6	1	6	1	6	1	5	2	3	4	4	3	2	5		
N2			F5	12		0	12	0	12	0	11	1	9	3	9	3	6	6	7	5	4	8		
N2			G5	12		0	12	0	11	1	10	2	10	2	10	2	9	3	6	6	4	8		
N2	AndpanHDMp	100 µg/mL	E6	9		0	9	0	9	0	9	0	9	0	7	2	6	3	5	4	1	8		
N2			F6	6		0	6	0	5	1	5	1	5	1	4	2	4	2	4	2	1	5		
N2			G6	6		0	6	0	6	0	5	1	4	2	4	2	1	5	1	5	1	5		
N2	InooblODM_479	100 µg/mL	E7	11		0	11	0	11	0	11	0	10	1	10	1	6	5	4	7	1	10		
N2			F7	7		0	7	0	7	0	7	0	7	0	6	1	5	2	5	2	1	6		
N2			G7	11		0	11	0	11	0	11	0	10	1 2	8	3	6	5	8	3	3	8		
N2	LinrhiE_2018	100 µg/mL	E8	11		0	11	0	11	0	11	0	9	2	10	1	10	1	9	2	1	10		
N2			F8	8		0	8	0	8	0	8	0	8	0	7	1	7	1	4	4	1	7		
N2			G8	12		0	12	0	12	0	12	0	11	1	11	1	6	6	5	7	2	10		
N2	AntcinE_2018	100 µg/mL	E9	8		0	8	0	8	0	8	0	8	0	7	1	4	4	4	4	1	7		
N2			F9	4		0	4	0	4	0	4	0	4	0	4	0	1	3	2	2	1	3		
N2			G9	10		0	10	0	10	0	10	0	9	1	9	1	7	3	6	4	2	8		
N2	GloodoE0054_2018	100 µg/mL	E10	6		0	6	0	6	0	4	2	4	2	4	2	3	3	2	4	2	4		
N2			F10	7		0	7	0	7	0	7	0	7	0	5	2	2	5	2	5	1	6		
N2			G10	11		0	11	0	11	0	10	1	10	1	9	2	3	8	3	8	1	10		
N2	DMSO	1%	E11	9		0	9	0	9	0	9	0	8	1	7	2	6	3	6	3	1	8		
N2			F11	15		0	15	0	15	0	14	1	11	4	11	4	7	8	7	8	3	12		
N2			G11			0	0	0	0		0		0		0			0		0		0		

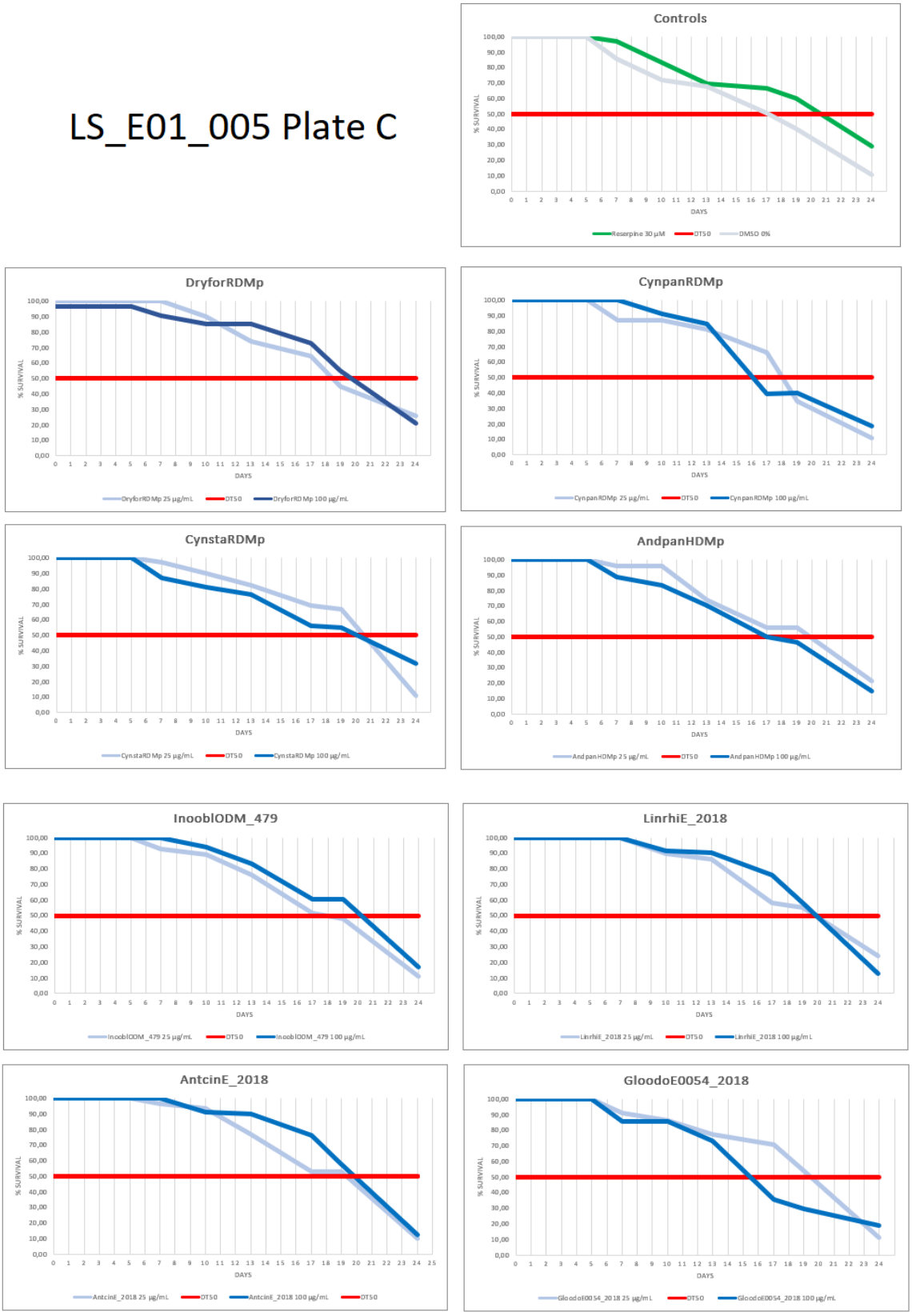


Figure 35 - Lifespan screening assay LS01_005 Plate C Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 47 - Counting list of Experiment LS_LR01 Plate A Part 1

Life Span Assay N2

Trial Number: LS_LR01 **A** **Experimentator:** KM **Date:** 21.2.2019

					Date																	
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	**Drug**	**Conc.**	**Well**	**X_0**	25.2.2019	4	27.2.2019	6	1.3.2019	8	4.3.2019	11	7.3.2019	14	11.3.2019	18	13.3.2019	20	15.3.2019	22	18.3.2019	25
N2			B2	12	12	0	12	0	9	3	8	4	7	5	7	5	6	6	6	6	3	9
N2	Reserpine	30 µM	C2	13	13	0	13	0	13	0	11	2	11	2	11	2	10	3	9	4	7	6
N2			D2	21	21	0	21	0	18	3		21		21		21		21		21		21
N2			B3	25	25	0	25	0	25			25		25		25		25		25		25
N2	LR01_01	25 µg/mL	C3	10	10	0	10	0	9	1	8	2	8	2	7	3	7	3	6	4	2	8
N2			D3	9	9	0	9	0	9	0	9	0	9	0	7	2	7	2	7	2	5	4
N2			B4	11	11	0	11	0	8	3	8	3	8	3	8	3	6	5	6	5	3	8
N2	LR01_02	25 µg/mL	C4	17	17	0	17	0	13	4	13	4	12	5	11	6	11	6	11	6	9	8
N2			D4	18	18	0	18	0	16	2	16	2	16	2	12	6	12	6	11	7	8	10
N2			B5	9	9	0	9	0	8	1	8	1	8	1	8	1	7	2	5	4	4	5
N2	LR01_03	25 µg/mL	C5	18	18	0	18	0	17	1	14	4	14	4	12	6	12	6	12	6	10	8
N2			D5	12	12	0	12	0	11	1	11	1	10	2	8	4	8	4	4	8	3	9
N2			B6	15	15	0	15	0	13	2	12	3	12	3	9	6	8	7	7	8	3	12
N2	LR01_06	25 µg/mL	C6	11	11	0	11	0	10	1	7	4	7	4	5	6	5	6	4	7	4	7
N2			D6	12	12	0	12	0	11	1	8	4	8	4	8	4	7	5	6	6	6	6
N2			B7	7	7	0	7	0	7	0	6	1	6	1	5	2	4	3	3	4	1	6
N2	LR01_07	25 µg/mL	C7	14	14	0	14	0	13	1	11	3	11	3	10	4	10	4	7	7	5	9
N2			D7	18	18	0	18	0	18	0	18	0	16	2	13	5	12	6	12	6	9	9
N2			B8	9	9	0	9	0	9	0	9	0	9	0	8	1	8	1	8	1	6	3
N2	LR01_08	25 µg/mL	C8	14	14	0	14	0	12	2	13	1	13	1	12	2	10	4	8	6	5	9
N2			D8	12	12	0	12	0	12	0	11	1	11	1	10	2	10	2	10	2	4	8
N2			B9	13	13	0	13	0	12	1	13	0	12	1	7	6	7	6	6	7	3	10
N2	LR01_09	25 µg/mL	C9	13	13	0	13	0	11	2	11	2	11	2	11	2	9	4	9	4	8	5
N2			D9	10	10	0	10	0	10	0	10	0	10	0	8	2	8	2	8	2	6	4
N2			B10	16	16	0	16	0	14	2	12	4	12	4	10	6	10	6	7	9	4	12
N2	LR01_10	25 µg/mL	C10	15	15	0	15	0	11	4	11	4	11	4	6	9	6	9	6	9	2	13
N2			D10	13	13	0	13	0	12	1	12	1	12	1	12	1	8	5	5	8	4	9
N2			B11	6	6	0	6	0	4	2	4	2	4	2	4	2	4	2	1	5	1	5
N2	Reserpine	30 µM	C11	16	16	0	16	0	14	2	12	4	12	4	12	4	11	5	10	6	10	6
N2			D11	20	20	0	20	0	16	4		20		20		20		20		20		20

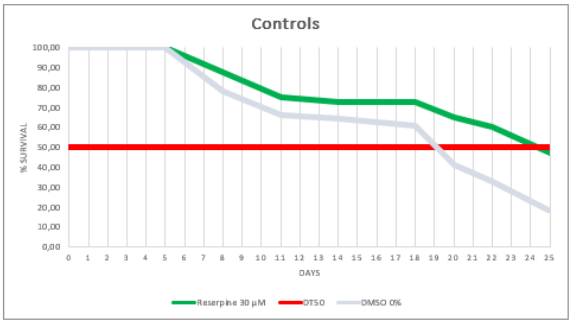
Table 48 - Counting list of Experiment LS_LR01 Plate A Part 2

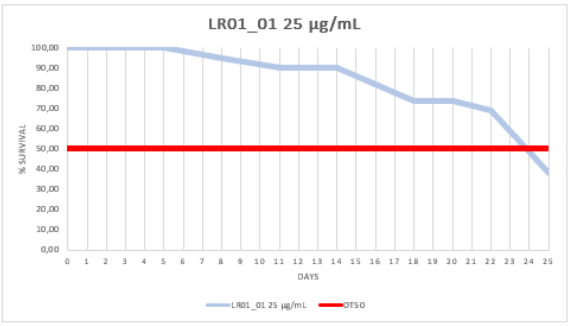
Life Span Assay N2

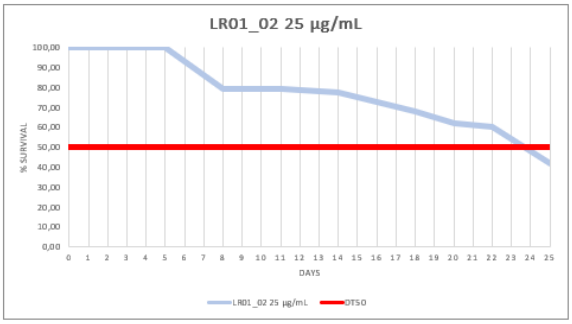
Trial Number: LS_LR01 **A** **Experimentator:** KM **Date:** 21.2.2019

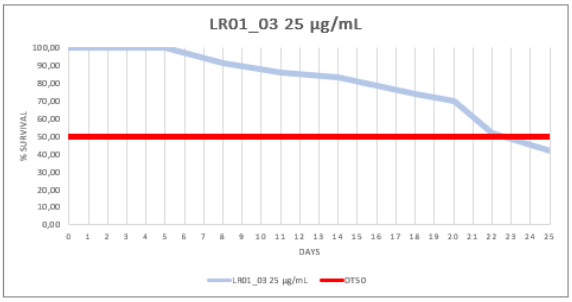
					Date																	
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	DMSO	1%	E2	10	10	0	10	0	9	1	8	2	8	2	9	1	7	3	7	3	3	7
N2			F2	7	7	0	7	0	5	2	3	4	4	3	4	3	2	5	2	5	1	6
N2			G2	12	12	0	12	0	12	0	10	2	10	2	7	5	7	5	6	6	3	9
N2	LR01_11	25 µg/mL	E3	12	12	0	12	0	11	1	10	2	10	2	10	2	7	5	7	5	2	10
N2			F3	12	12	0	12	0	9	3	8	4	8	4	7	5	5	7	4	8	3	9
N2			G3	10	10	0	10	0	8	2	7	3	7	3	7	3	6	4	6	4	2	8
N2	LR01_13	25 µg/mL	E4	11	11	0	11	0	8	3	7	4	7	4	7	4	7	4	6	5	3	8
N2			F4	12	12	0	12	0	11	1	10	2	10	2	9	3	9	3	9	3	5	7
N2			G4	17	17	0	17	0	11	6	10	7	10	7	8	9	7	10	6	11	5	12
N2	LR01_14	25 µg/mL	E5	14	14	0	14	0	13	1	11	3	10	4	10	4	9	5	9	5	6	8
N2			F5	11	11	0	11	0	9	2	6	5	6	5	5	6	5	6	4	7	3	8
N2			G5	13	13	0	13	0	9	4	9	4	8	5	6	7	5	8	4	9	2	11
N2	LigrhiScE	25 µg/mL	E6	13	13	0	13	0	11	2	9	4	9	4	8	5	8	5	5	8	4	9
N2			F6	9	9	0	9	0	8	1	7	2	7	2	5	4	5	4	4	5	1	8
N2			G6	15	15	0	15	0	12	3	13	2	13	2	12	3	10	5	10	5	7	8
N2	XanarmPDMp_I	25 µg/mL	E7	17	17	0	17	0	16	1	16	1	15	2	13	4	13	4	13	4	6	11
N2			E8	17	17	0	17	0	15	2	14	3	14	3	10	7	10	7	10	7	8	9
N2	XanarmPDMp_I	100 µg/mL	E9	12	12	0	12	0	9	3	9	3	8	4	8	4	7	5	7	5	4	8
N2			E10	16	16	0	16	0	15	1	13	3	13	3	12	4	12	4	12	4	9	7
N2	XanarmFDMp_I	25 µg/mL	F7	12	12	0	12	0	9	3	8	4	7	5	6	6	6	6	6	6	4	8
N2			F8	8	8	0	8	0	6	2	6	2	5	3	3	5	3	5	3	5	1	7
N2	XanarmFDMp_I	100 µg/mL	F9	15	15	0	15	0	12	3	12	3	10	5	9	6	9	6	5	10	5	10
N2			F10	12	12	0	12	0	10	2	10	2	10	2	9	3	6	6	6	6	4	8
N2	XanarmSDMp_I	25 µg/mL	G7	8	8	0	8	0	7	1	7	1	7	1	7	1	7	1	5	3	4	4
N2			G8	11	11	0	11	0	9	2	9	2	9	2	8	3	8	3	5	6	4	7
N2	XanarmSDMp_I	100 µg/mL	G9	15	15	0	15	0	12	3	10	5	9	6	9	6	7	8	6	9	3	12
N2			G10	11	11	0	11	0	10	1	10	1	10	1	8	3	8	3	7	4	3	8
N2	DMSO	1%	E11	10	10	0	10	0	9	1	8	2	8	2	7	3	6	4	3	7	3	7
N2			F11	12	12	0	12	0	9	3	8	4	7	5	7	5	6	6	3	9	2	10
N2			G11	9	9	0	9	0	8	1	8	1	7	2	6	3	4	5	4	5	2	7

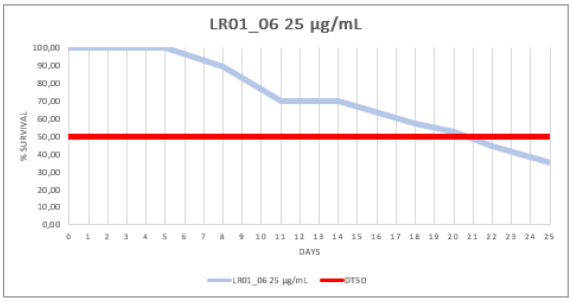
LR01_Plate A

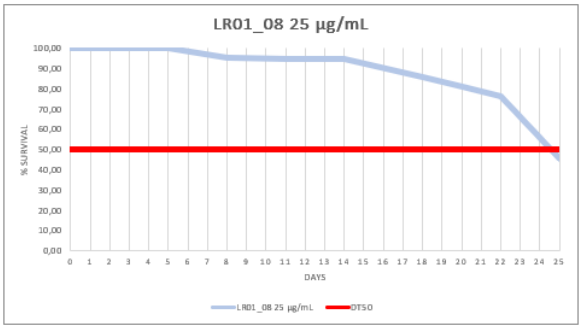

Controls
% SURVIVAL
DAYS
Reserpine 30 µM
DTSO
DMSO 0%

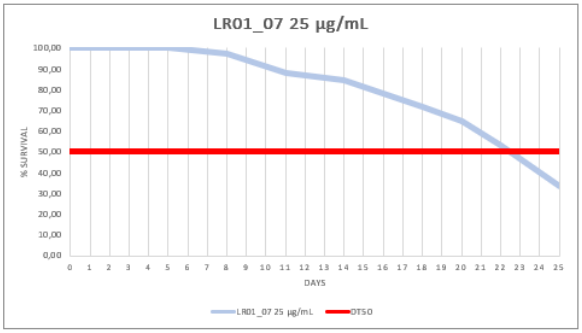

LR01_01 25 µg/mL
% SURVIVAL
DAYS
LR01_01 25 µg/mL
DTSO

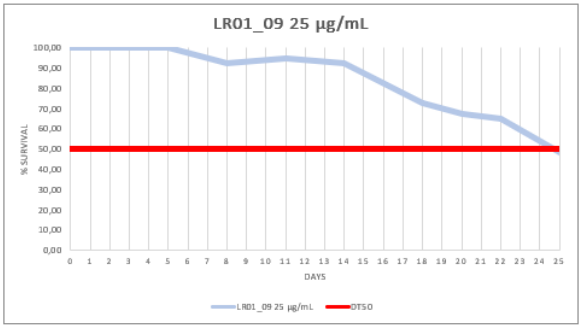

LR01_02 25 µg/mL
% SURVIVAL
DAYS
LR01_02 25 µg/mL
DTSO

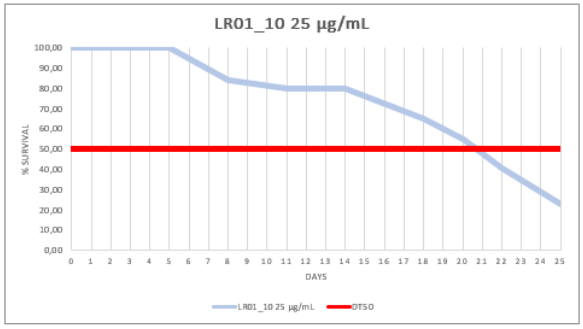

LR01_03 25 µg/mL
% SURVIVAL
DAYS
LR01_03 25 µg/mL
DTSO


LR01_06 25 µg/mL
% SURVIVAL
DAYS
LR01_06 25 µg/mL
DTSO


LR01_08 25 µg/mL
% SURVIVAL
DAYS
LR01_08 25 µg/mL
DTSO


LR01_07 25 µg/mL
% SURVIVAL
DAYS
LR01_07 25 µg/mL
DTSO


LR01_09 25 µg/mL
% SURVIVAL
DAYS
LR01_09 25 µg/mL
DTSO


LR01_10 25 µg/mL
% SURVIVAL
DAYS
LR01_10 25 µg/mL
DTSO

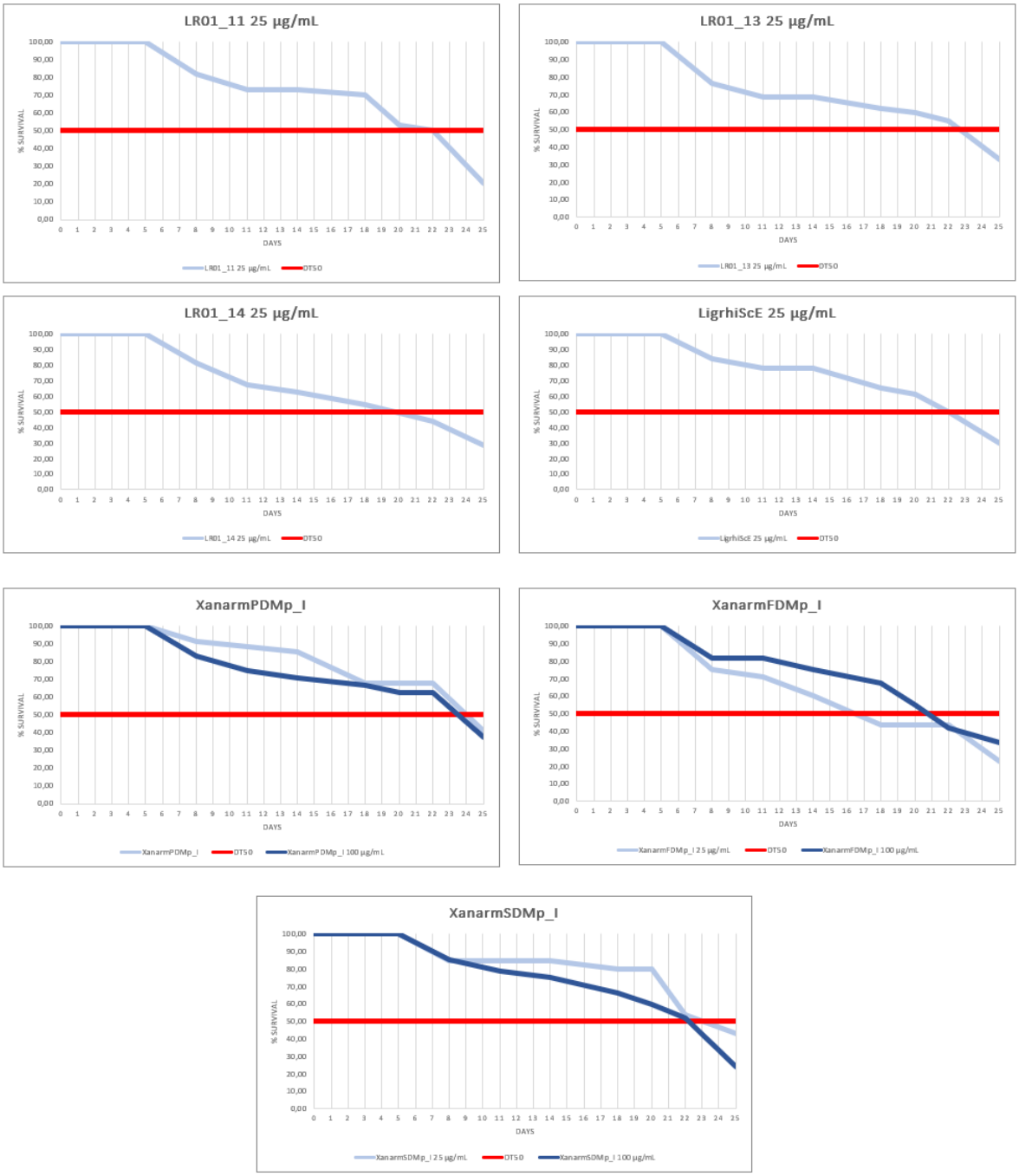


Figure 36 - Bioactivity Investigation assay LR01 Plate A Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 49 - Counting list of Experiment LS_LR01 Plate B Part 1

Life Span Assay N2

Trial Number: LS_LR01 **B** **Experimentator:** KM **Date:** 21.2.2019

					Date																	
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	Drug	Conc.	Well	X_0	25.2.2019	4	27.2.2019	6	1.3.2019	8	4.3.2019	11	7.3.2019	14	11.3.2019	18	13.3.2019	20	15.3.2019	22	18.3.2019	25
N2	Reserpine	30 µM	B2	17	17	0	17	0	13	4	12	5	12	5	12	5	10	7	9	8	7	10
N2			C2	9	9	0	9	0	7	2	6	3	5	4	5	4	5	4	3	6	3	6
N2			D2	12	12	0	12	0	10	2	7	5	7	5	5	7	5	7	5	7	3	9
N2	LR01_01	25 µg/mL	B3	13	13	0	13	0	11	2	11	2	9	4	9	4	7	6	4	9	4	9
N2			C3	10	10	0	10	0	7	3	6	4	6	4	6	4	6	4	4	6	2	8
N2			D3	15	15	0	15	0	15	0	14	1	14	1	11	4	10	5	10	5	5	10
N2	LR01_02	25 µg/mL	B4	7	7	0	7	0	6	1	6	1	6	1	4	3	3	4	3	4	2	5
N2			C4	9	9	0	9	0	8	1	7	2	7	2	7	2	7	2	4	5	4	5
N2			D4	10	10	0	10	0	9	1	7	3	6	4	5	5	5	5	4	6	2	8
N2	LR01_03	25 µg/mL	B5	18	18	0	18	0	14	4	13	5	13	5	11	7	11	7	9	9	7	11
N2			C5	12	12	0	12	0	11	1	10	2	10	2	7	5	7	5	7	5	6	6
N2			D5	17	17	0	17	0	13	4	12	5	12	5	10	7	10	7	7	10	3	14
N2	LR01_06	25 µg/mL	B6	21	21	0	21	0	18	3		21		21		21		21		21		21
N2			C6	12	12	0	12	0	11	1	10	2	10	2	7	5	7	5	5	7	4	8
N2			D6	11	11	0	11	0	10	1	10	1	10	1	8	3	7	4	6	5	3	8
N2	LR01_07	25 µg/mL	B7	11	11	0	11	0	10	1	9	2	9	2	8	3	8	3	8	3	4	7
N2			C7	17	17	0	17	0	16	1	12	5	12	5	10	7	10	7	9	8	4	13
N2			D7	10	10	0	10	0	9	1	9	1	9	1	6	4	6	4	5	5	3	7
N2	LR01_08	25 µg/mL	B8	14	14	0	14	0	11	3	11	3	10	4	9	5	9	5	7	7	6	8
N2			C8	9	9	0	9	0	8	1	8	1	6	3	5	4	4	5	4	5	2	7
N2			D8	6	6	0	6	0	5	1	4	2	3	3	3	3	3	3	2	4	1	5
N2	LR01_09	25 µg/mL	B9	12	12	0	12	0	9	3	7	5	7	5	6	6	4	8	0	12	0	12
N2			C9	6	6	0	6	0	5	1	5	1	5	1	5	1	5	1	5	1	2	4
N2			D9	18	18	0	18	0	15	3	14	4	12	6	10	8	10	8	9	9	8	10
N2	LR01_10	25 µg/mL	B10	11	11	0	11	0	10	1	10	1	10	1	8	3	8	3	7	4	5	6
N2			C10	12	12	0	12	0	8	4	7	5	7	5	7	5	7	5	5	7	1	11
N2			D10	17	17	0	17	0	13	4	11	6	11	6	10	7	8	9	5	12	3	14
N2	Reserpine	30 µM	B11	8	8	0	8	0	8	0	8	0	8	0	8	0	7	1	6	2	4	4
N2			C11	17	17	0	17	0	13	4	11	6	10	7	10	7	10	7	9	8	8	9
N2			D11	14	14	0	14	0	9	5	8	6	8	6	8	6	8	6	7	7	6	8

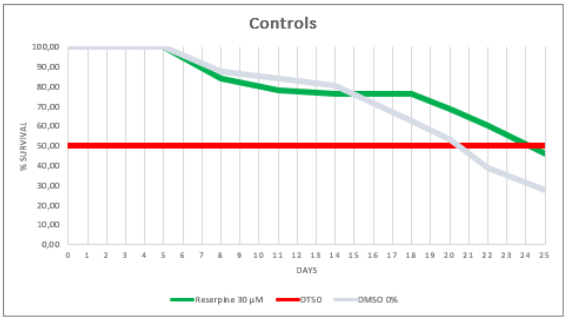
Table 50 - Counting list of Experiment LS_LR01 Plate B Part 2

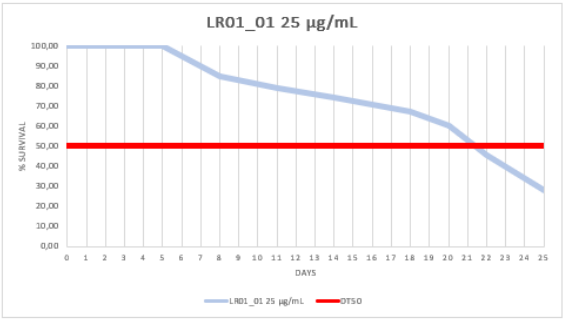
Life Span Assay N2

Trial Number: LS_LR01 **B** **Experimentator:** KM **Date:** 21.2.2019

					Date																	
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	DMSO	1%	E2	16	16	0	16	0	14	2	13	3	13	3	11	5	11	5	6	10	3	13
N2			F2	9	9	0	9	0	7	2	6	3	5	4	4	5	4	5	4	5	3	6
N2			G2	14	14	0	14	0	14	0	14	0	14	0	10	4	8	6	4	10	2	12
N2	LR01_11	25 µg/mL	E3	7	7	0	7	0	7	0	7	0	7	0	7	0	7	0	1	6	2	5
N2			F3	11	11	0	11	0	10	1	10	1	10	1	10	1	10	1	6	5	5	6
N2			G3	16	16	0	16	0	13	3	13	3	13	3	10	6	9	7	9	7	4	12
N2	LR01_13	25 µg/mL	E4	14	14	0	14	0	11	3	9	5	9	5	8	6	8	6	7	7	4	10
N2			F4	10	10	0	10	0	8	2	8	2	8	2	6	4	6	4	6	4	5	5
N2			G4	4	4	0	4	0	3	1		4		4		4		4		4		4
N2	LR01_14	25 µg/mL	E5	9	9	0	9	0	7	2	7	2	7	2	7	2	7	2	6	3	5	4
N2			F5	11	11	0	11	0	9	2	8	3	7	4	6	5	6	5	5	6	1	10
N2			G5	6	6	0	6	0	5	1	4	2	4	2	4	2	3	3	1	5	1	5
N2	LigrhiScE	25 µg/mL	E6	11	11	0	11	0	9	2	8	3	6	5	6	5	5	6	4	7	3	8
N2			F6	13	13	0	13	0	13	0	11	2	11	2	10	3	10	3	10	3	6	7
N2			G6	7	7	0	7	0	7	0	7	0	6	1	2	5	2	5	1	6	0	7
N2	XanarmPDMp_l	25 µg/mL	E7	10	10	0	10	0	9	1	6	4	6	4	6	4	6	4	6	4	3	7
N2			E8	9	9	0	9	0	9	0	9	0	9	0	9	0	6	3	6	3	2	7
N2	XanarmPDMp_l	100 µg/mL	E9	13	13	0	13	0	12	1	11	2	10	3	8	5	8	5	7	6	6	7
N2			E10	16	16	0	16	0	14	2	12	4	12	4	11	5	11	5	8	8	6	10
N2	XanarmFDMp_l	25 µg/mL	F7	9	9	0	9	0	7	2	6	3	6	3	6	3	6	3	3	6	2	7
N2			F8	11	11	0	11	0	8	3	9	2	7	4	4	7	4	7	4	7	4	7
N2	XanarmFDMp_l	100 µg/mL	F9	13	13	0	13	0	12	1	12	1	11	2	11	2	10	3	7	6	3	10
N2			F10	15	15	0	15	0	10	5	9	6	8	7	7	8	7	8	5	10	1	14
N2	XanarmSDMp_l	25 µg/mL	G7	6	6	0	6	0	5	1	5	1	5	1	2	4	2	4	2	4	2	4
N2			G8	9	9	0	9	0	8	1	7	2	7	2	7	2	7	2	6	3	5	4
N2	XanarmSDMp_l	100 µg/mL	G9	8	8	0	8	0	6	2	6	2	6	2	5	3	4	4	3	5	3	5
N2			G10	17	17	0	17	0	12	5	12	5	11	6	9	8	8	9	5	12	5	12
N2	DMSO	1%	E11	18	18	0	18	0	15	3	14	4	14	4	12	6	11	7	8	10	5	13
N2			F11	8	8	0	8	0	7	1	7	1	6	2	6	2	6	2	4	4	2	6
N2			G11	14	14	0	14	0	12	2	12	2	12	2	10	4	8	6	6	8	5	9

LR01_Plate B


Controls
% SURVIVAL
DAYS
Reserpine 30 µM
DTSO
DMSO 0%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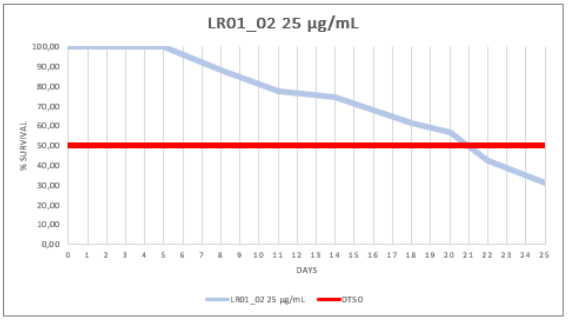
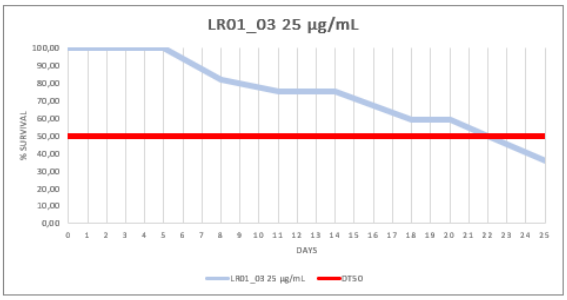
% SURVIVAL
DAYS
LR01_01 25 µg/mL
DTSO


LR01_02 25 µg/mL
% SURVIVAL
DAYS
LR01_02 25 µg/mL
DTSO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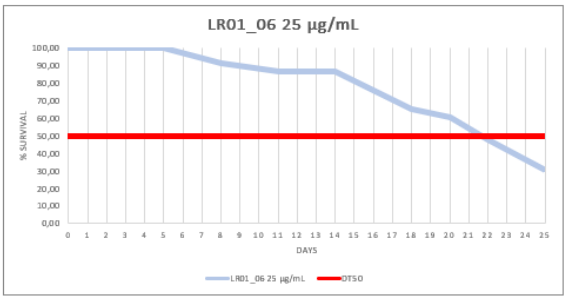
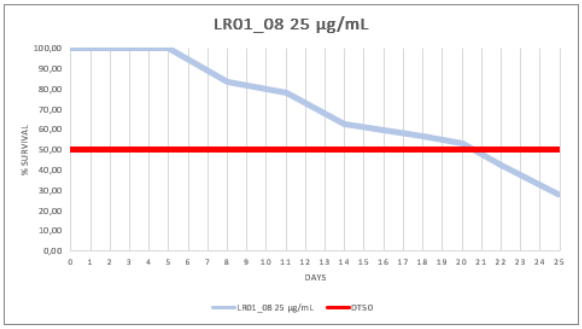
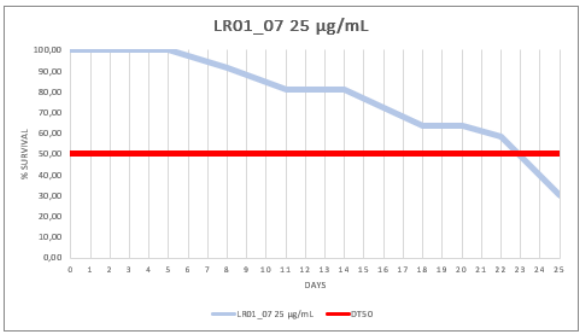
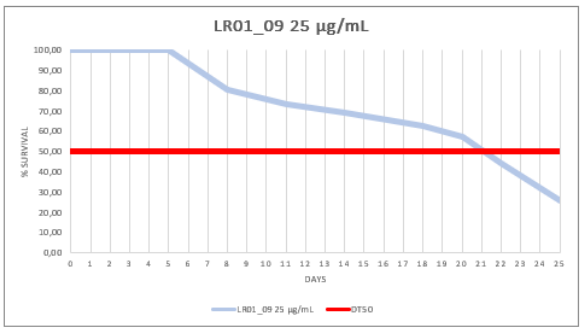
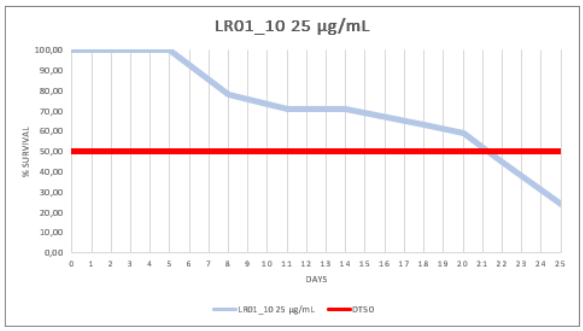
% SURVIVAL
DAYS
LR01_03 25 µg/mL
DTSO


LR01_06 25 µg/mL
% SURVIVAL
DAYS
LR01_06 25 µg/mL
DTSO


LR01_08 25 µg/mL
% SURVIVAL
DAYS
LR01_08 25 µg/mL
DTSO


LR01_07 25 µg/mL
% SURVIVAL
DAYS
LR01_07 25 µg/mL
DTSO


LR01_09 25 µg/mL
% SURVIVAL
DAYS
LR01_09 25 µg/mL
DTSO


LR01_10 25 µg/mL
% SURVIVAL
DAYS
LR01_10 25 µg/mL
DTSO

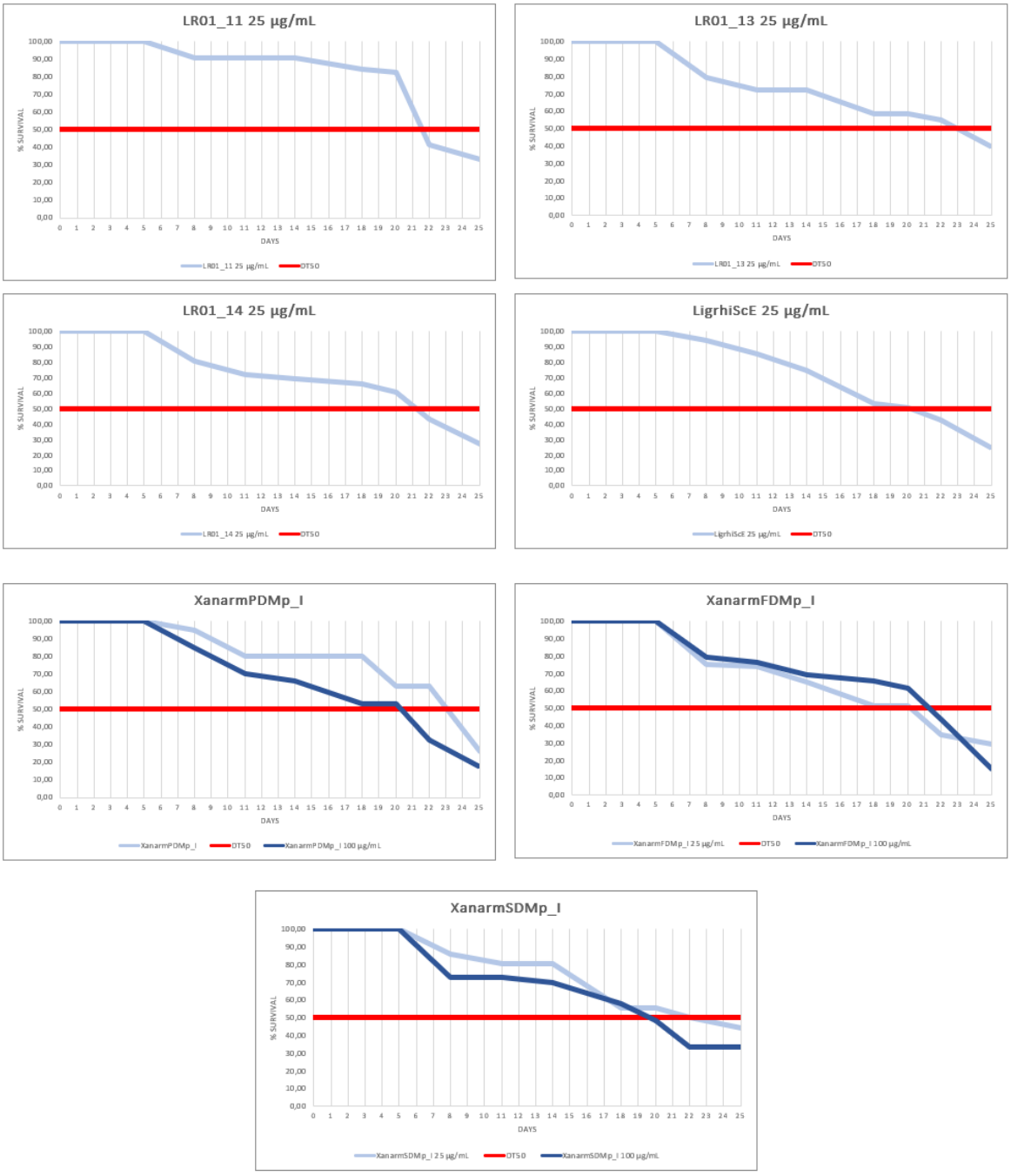


Figure 37 - Bioactivity Investigation assay LR01 Plate B Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.

Table 51 - Counting list of Experiment LS_LR01 Plate C Part 1

Life Span Assay N2

Trial Number: LS_LR01 C **Experimentator:** KM **Date:** 21.2.2019

					Date																	
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
Strain	Drug	Conc.	Well	X_0	25.2.2019	4	27.2.2019	6	1.3.2019	8	4.3.2019	11	7.3.2019	14	11.3.2019	18	13.3.2019	20	15.3.2019	22	18.3.2019	25
N2	Reserpine	30 µM	B2	13	13	0	13	0	11	2	11	2	8	5	6	7	6	7	6	7	6	7
N2			C2	9	9	0	9	0	8	1	7	2	7	2	5	4	5	4	5	4	4	5
N2			D2	12	12	0	12	0	11	1	9	3	9	3	8	4	7	5	6	6	6	6
N2	LR01_01	25 µg/mL	B3	9	9	0	9	0	7	2	7	2	4	5	3	6	2	7	2	7	1	8
N2			C3	6	6	0	6	0	6	0	6	0	6	0	5	1	5	1	4	2	3	3
N2			D3	15	15	0	15	0	13	2	11	4	11	4	7	8	7	8	6	9	5	10
N2	LR01_02	25 µg/mL	B4	14	14	0	14	0	14	0	14	0	14	0	9	5	9	5	7	7	2	12
N2			C4	9	9	0	9	0	8	1	8	1	7	2	6	3	6	3	5	4	4	5
N2			D4	16	16	0	16	0	16	0	12	4	10	6	5	11	5	11	3	13	1	15
N2	LR01_03	25 µg/mL	B5	16	16	0	16	0	14	2	14	2	14	2	12	4	12	4	10	6	8	8
N2			C5	14	14	0	14	0	12	2	13	1	13	1	9	5	9	5	7	7	7	7
N2			D5	16	16	0	16	0	15	1	13	3	13	3	11	5	9	7	8	8	4	12
N2	LR01_06	25 µg/mL	B6	14	14	0	14	0	13	1	11	3	11	3	9	5	9	5	6	8	2	12
N2			C6	11	11	0	11	0	9	2	8	3	8	3	5	6	5	6	3	8	1	10
N2			D6	7	7	0	7	0	6	1	5	2	5	2	5	2	5	2	5	2	5	2
N2	LR01_07	25 µg/mL	B7	19	19	0	19	0	14	5	13	6	13	6	12	7	11	8	11	8	9	10
N2			C7	23	23	0	23	0	19	4		23		23		23		23		23		23
N2			D7	19	19	0	19	0	17	2	17	2	16	3	16	3	15	4	12	7	10	9
N2	LR01_08	25 µg/mL	B8	11	11	0	11	0	9	2	9	2	8	3	8	3	6	5	4	7	4	7
N2			C8	14	14	0	14	0	11	3	11	3	10	4	10	4	10	4	9	5	5	9
N2			D8	10	10	0	10	0	7	3	6	4	6	4	6	4	6	4	5	5	3	7
N2	LR01_09	25 µg/mL	B9	16	16	0	16	0	15	1	13	3	10	6	8	8	8	8	8	8	4	12
N2			C9	15	15	0	15	0	12	3	12	3	10	5	10	5	10	5	8	7	4	11
N2			D9	12	12	0	12	0	9	3	9	3	8	4	6	6	5	7	5	7	2	10
N2	LR01_10	25 µg/mL	B10	16	16	0	16	0	14	2	13	3	12	4	10	6	9	7	9	7	6	10
N2			C10	10	10	0	10	0	7	3	7	3	6	4	6	4	6	4	5	5	4	6
N2			D10	9	9	0	9	0	8	1	7	2	7	2	7	2	7	2	4	5	2	7
N2	Reserpine	30 µM	B11	14	14	0	14	0	10	4	10	4	10	4	8	6	8	6	8	6	6	8
N2			C11	17	17	0	17	0	10	7	11	6	11	6	10	7	9	8	7	10	7	10
N2			D11	14	14	0	14	0	9	5	8	6	8	6	8	6	8	6	8	6	4	10

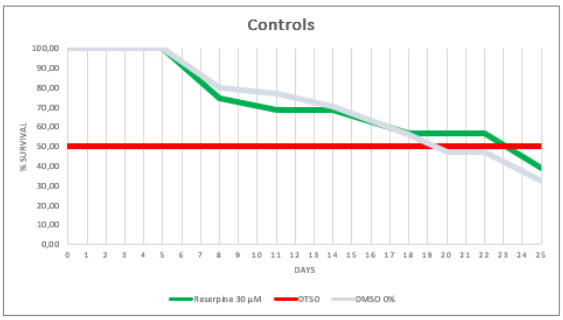
Table 52 - Counting list of Experiment LS_LR01 Plate C Part 2

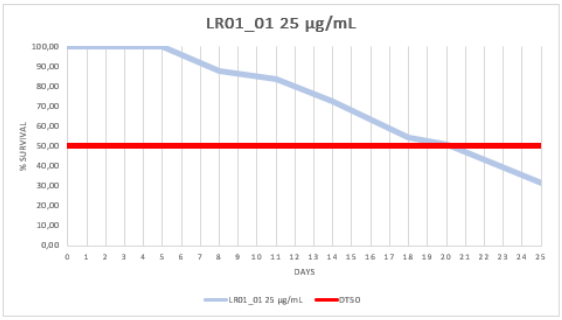
Life Span Assay N2

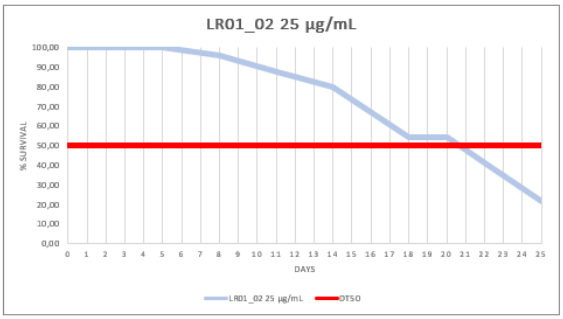
Trial Number: LS_LR01 C **Experimentator:** KM **Date:** 21.2.2019

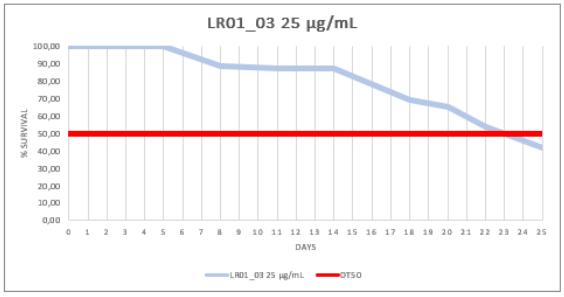
					Date																	
					live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead	live	dead
N2	DMSO	1%	E2	10	10	0	10	0	10	0	7	3	7	3	6	4	6	4	6	4	3	7
N2			F2	11	11	0	11	0	10	1	9	2	7	4	6	5	5	6	5	6	3	8
N2			G2	11	11	0	11	0	9	2	9	2	9	2	7	4	5	6	5	6	4	7
N2	LR01_11	25 µg/mL	E3	8	8	0	8	0	8	0	8	0	7	1	4	4	3	5	3	5	1	7
N2			F3	15	15	0	15	0	13	2	13	2	13	2	13	2	11	4	6	9	3	12
N2			G3	15	15	0	15	0	12	3	12	3	12	3	11	4	10	5	10	5	2	13
N2	LR01_13	25 µg/mL	E4	10	10	0	10	0	9	1	9	1	8	2	7	3	7	3	5	5	3	7
N2			F4	12	12	0	12	0	11	1	10	2	9	3	8	4	7	5	5	7	3	9
N2			G4	5	5	0	5	0	4	1	4	1	4	1	4	1	4	1	3	2	3	2
N2	LR01_14	25 µg/mL	E5	15	15	0	15	0	13	2	13	2	12	3	9	6	9	6	8	7	7	8
N2			F5	10	10	0	10	0	8	2	8	2	8	2	6	4	6	4	6	4	4	6
N2			G5	12	12	0	12	0	9	3	8	4	8	4	7	5	6	6	6	6	2	10
N2	LigrhiScE	25 µg/mL	E6	20	20	0	20	0	16	4	16	4	16	4	11	9	11	9	8	12	6	14
N2			F6	11	11	0	11	0	6	5	7	4	7	4	6	5	6	5	5	6	4	7
N2			G6	12	12	0	12	0	8	4	6	6	5	7	4	8	3	9	3	9	2	10
N2	XanarmPDMp_I	25 µg/mL	E7		0	0	0	0	0			0		0		0		0		0		0
N2			E8	14	14	0	14	0	12	2	12	2	11	3	10	4	10	4	10	4	5	9
N2	XanarmPDMp_I	100 µg/mL	E9	7	7	0	7	0	6	1	6	1	6	1	5	2	5	2	5	2	3	4
N2			E10	12	12	0	12	0	11	1	11	1	9	3	8	4	8	4	6	6	3	9
N2	XanarmFDMp_I	25 µg/mL	F7	13	13	0	13	0	11	2	11	2	10	3	10	3	10	3	8	5	3	10
N2			F8	9	9	0	9	0	8	1	8	1	8	1	8	1	7	2	6	3	5	4
N2	XanarmFDMp_I	100 µg/mL	F9	12	12	0	12	0	11	1	11	1	11	1	8	4	7	5	5	7	4	8
N2			F10	10	10	0	10	0	9	1	9	1	9	1	8	2	7	3	5	5	5	5
N2	XanarmSDMp_I	25 µg/mL	G7	15	15	0	15	0	9	6	9	6	9	6	8	7	8	7	8	7	6	9
N2			G8	19	19	0	19	0	19	0	18	1	16	3	13	6	13	6	13	6	9	10
N2	XanarmSDMp_I	100 µg/mL	G9	11	11	0	11	0	11	0	11	0	8	3	8	3	8	3	6	5	1	10
N2			G10	12	12	0	12	0	8	4	8	4	7	5	7	5	7	5	4	8	3	9
N2	DMSO	1%	E11	6	6	0	6	0	4	2	4	2	4	2	3	3	3	3	3	3	2	4
N2			F11	9	9	0	9	0	9	0	8	1	8	1	8	1	6	3	4	5	2	7
N2			G11	9	9	0	9	0	8	1	6	3	6	3	6	3	6	3	4	5	2	7

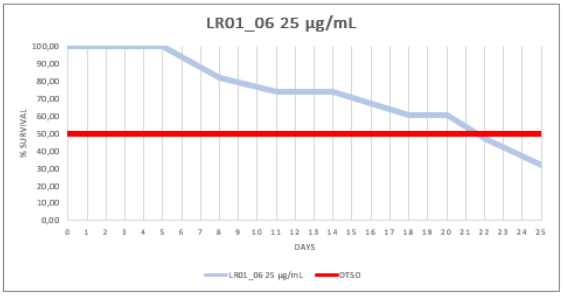
LR01_Plate C

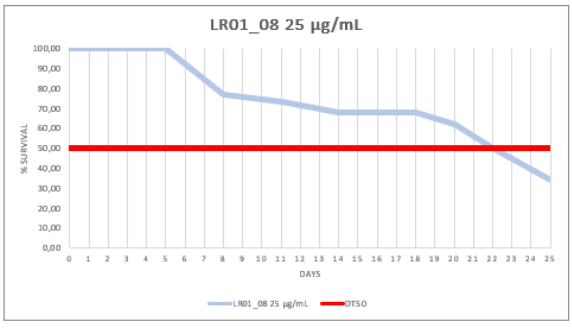

Controls
% SURVIVAL
DAYS
Reserpine 30 µM
DTSO
DMSO 0%

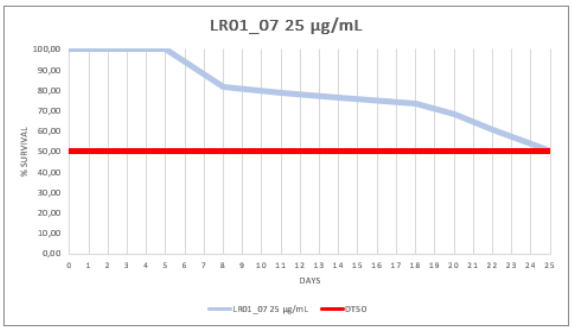

LR01_01 25 µg/mL
% SURVIVAL
DAYS
LR01_01 25 µg/mL
DTSO

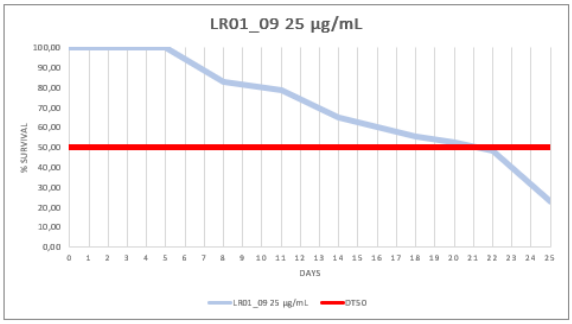

LR01_02 25 µg/mL
% SURVIVAL
DAYS
LR01_02 25 µg/mL
DTSO

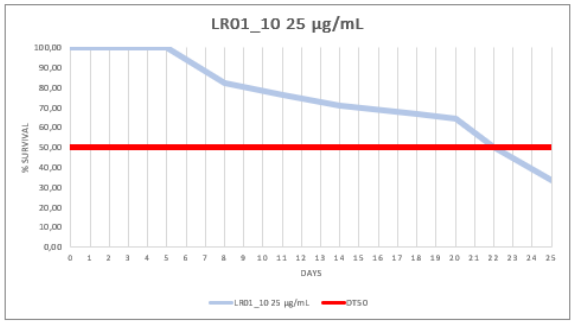

LR01_03 25 µg/mL
% SURVIVAL
DAYS
LR01_03 25 µg/mL
DTSO


LR01_06 25 µg/mL
% SURVIVAL
DAYS
LR01_06 25 µg/mL
DTSO


LR01_08 25 µg/mL
% SURVIVAL
DAYS
LR01_08 25 µg/mL
DTSO


LR01_07 25 µg/mL
% SURVIVAL
DAYS
LR01_07 25 µg/mL
DTSO


LR01_09 25 µg/mL
% SURVIVAL
DAYS
LR01_09 25 µg/mL
DTSO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% SURVIVAL
DAYS
LR01_10 25 µg/mL
DTSO

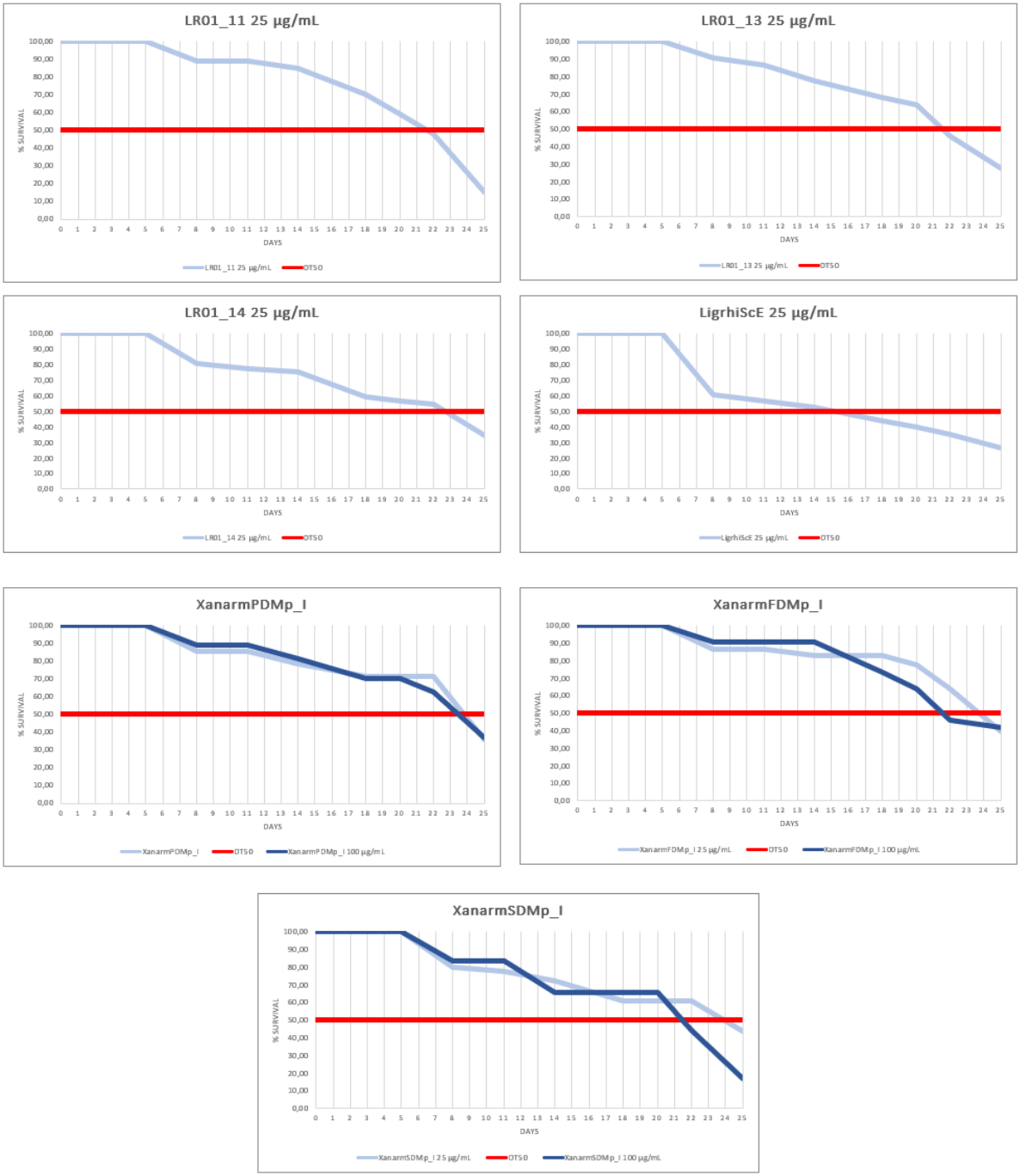

Figure 38 - Bioactivity Investigation assay LR01 Plate C Charts of the tested extracts at a concentration of 25 (light blue) and 100 µg/ml (dark blue), respectively and controls – reserpine (green) and DMSO (gray) at a concentration of 30µM and 1%, respectively.