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„Identifying health promoting effects of herbal extracts and natural compounds in *Caenorhabditis elegans* based health span assay“

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List of abbreviation

AD	Alzheimer's Disease
B.C.	Before Christ
CBD	Cannabidiol
CBG	Cannabigerol
C. elegans	*Caenorhabditis elegans*
ddH2O	double-distilled water
D. melanogaster	*Drosophila melanogaster*
DMSO	Dimethyl sulfoxide
DT_{50}	Death time 50 %
E. coli	*Escherichia coli*
f.c.	Final concentration
FuDR	5-Fluorodeoxyuridine
HTS	High throughput screening
IGF	insulin-like growth factor
IIS - pathway	insulin/insulin-like growth factor (IGF) signaling (IIS) pathway
IR	Infrared
LC_{50}	lethal concentration 50%
LSA	Life span assay
NCs	Natural compounds
NGM	Nematode Growth Medium
NPs	Natural products
PD	Parkinson's Disease
PDD	Phenotypic drug discovery

RNAi	RNA-interference
ROS	Reactive oxygen species
RT	Room temperature
S. cerevisiae	*Saccharomyces cerevisiae*
SD	Standard deviation
SPA	Stress Protection Assay
TCM	Traditional Chinese medicine
TDD	Target-based drug discovery
WHO	World health organization
YFP	Yellow fluorescent protein

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1 Abstract

Aiming at identifying natural products with health-promoting properties, in this master thesis 19 different herbal and fungal extracts, together with seven natural compounds and two CBD formulations were tested in a survival and motility assay using the model organism *Caenorhabditis elegans* (*C. elegans*). Those samples which led to a significantly increased survival rate of the worms, were further tested in a lifespan assay. Additionally, a protocol for a stress protection assay was established and optimized by the integration of a semi-automated IR-based worm tracker ("WMicroTracker").

In the course of a protocol optimization assessing the nematodes' stress resistance, we could determine a worm density of 100 worms per well as most suitable for the worm tracker and show the superiority of flat bottom 96-well plates over round bottom plates. Furthermore, two different stressors have been evaluated, H_2O_2 and heat shock. We determined heat shock treatment with 31.5°C ± 1.5 °C as suitable stressor and consider it as less prone to interference compared to H_2O_2.

The initial screening showed that five plant extracts, three fungal extracts and four natural compounds significantly increased the survival rates of *C. elegans*. Four plant extracts and three fungal extracts were examined in more detail in a lifespan assay, in which the selected four plant extracts and two fungal extracts revealed a significant prolongation of the nematode's lifespan. These hit extracts will be promising starting materials for further chemical and biological profiling and the discovery of bioactive constituents dedicated to healthy ageing.

1.1 Zusammenfassung

Ziel dieser Masterarbeit war es Naturprodukte zu identifizieren, die gesundheitsfördernde Eigenschaften haben. Dafür wurden 19 verschiedene Pflanzen- und Pilzextrakte sowie sieben Naturstoffe und zwei CBD-Formulierungen auf ihre Auswirkungen auf die Überlebensrate und die Motilität von *Caenorhabditis elegans* (*C. elegans*) getestet. Jene Proben, die zu einer signifikant erhöhten Überlebensrate der Würmer führten, wurden dann in einem sogenannten ‚*Lifespan Assays*' genauer untersucht.

Im zweiten Teil dieser Arbeit wurde an einem Protokoll für einen ‚*Stress-Protection Assay*' gearbeitet, um zusätzliche Informationen zur Resilienz der getesteten Würmer zu erhalten. Für eine Optimierung des Protokolls wurde der halbautomatische IR-basierte Wurm-Tracker ("WMicroTracker") integriert. Dabei konnte die passende Wurmdichte von 100 Würmern pro well ermittelt werden und gezeigt werden, dass 96-well Platten mit Flachboden jenen mit abgerundetem Boden zu robusteren Messergebnissen führten. Weiters wurden H_2O_2 und Hitze als Stressoren getestet und verglichen. Unsere Versuche identifizierten eine Hitzebehandlung von 31.5°C ± 1.5 °C als überlegenen Stressor gegenüber H_2O_2, da dieser von weniger äußeren Faktoren abhängig zu sein scheint.

Das erste Screening von Naturstoffmaterialien zeigte, dass fünf Pflanzenextrakte, drei Pilzextrakte und vier Reinstoffe die Überlebensrate von *C. elegans* signifikant erhöhten. Vier Pflanzenextrakte und drei Pilzextrakte wurden daraufhin einem ‚*Lifespan Assay*' unterzogen, in dem die vier ausgewählten Pflanzenextrakte und zwei der Pilzextrakte im Wurmmodel zu einer signifikanten Verlängerung der Lebensspanne führten. Diese "Hit"-Extrakte sind vielversprechende Ausgangsmaterialien für weitere chemische und biologische Untersuchungen zur Identifizierung von Naturstoffen, die zu einem gesunden Altern beitragen können.

2 Aim of the work

The Aim of this thesis was to identify extracts and natural compounds that extend the lifespan of *C. elegans* while helping to maintain or improve health status during this time.

3 Introduction

3.1 Ageing

According to the world health organization (WHO), the average life expectancy is increasing constantly. Countries all over the world are encountering an increasing proportion of the older population. This effect is largely owed to the improvement of the living and sanitary conditions, health care and food supply. It is estimated, that in 2050 the number of people over 60 years old will be over 2 billion [1]. However, ageing comes along with several physiological changes like impairment of hearing and visual ability, moving function and changes in the hormonal status. Furthermore, it is a major risk factor for the development of chronic diseases, such as neurodegenerative disorders, cancer, osteoporosis, metabolic disorders, and cardiovascular disease [2, 3]. All these conditions can negatively impact the quality of life. In order to increase the quality of life in older age, it is necessary to find new therapeutic approaches for the prevention, alleviation and the treatment of the ageing related conditions [4].

On a biological level, the additional years are accompanied by the accumulation of cellular and molecular damage, resulting in various biological alterations, such as metabolic dysfunction, disturbed protein homeostasis and modified cellular signaling [3]. An accumulation of these changes inevitably leads to cellular changes and ageing processes such as, apoptosis, or unrestrained cell growth, which subsequently affect human health [5].

Although the ageing process as well as the related development of diseases are not fully uncovered to date, on a cellular base *Carlos López-Otín et al., 2013* defined nine hallmarks of ageing playing a crucial role in senescence: Genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication. These hallmarks have fulfilled the following criteria (i)

manifestation during the normal ageing process (ii) accumulation of this marker causes accelerated aging (iii) and for some it has been shown that experimental decreasing of the markers results in a delayed ageing process. These nine hallmarks often display interrelations, meaning a change in one marker often leads to a change in one or several others, while each of them influences the process of ageing in its own complex way. Genomic instability, epigenetic alterations, telomere attrition, and loss of proteostasis are defined as primary hallmarks that cause damage. In response to the damage, antagonistic hallmarks react, including mitochondrial dysfunction, deregulated nutrient-sensing, and cellular senescence. Stem cell exhaustion and senescent intercellular communication are defined as the integrative hallmarks, which are the „culprits of the phenotype". They represent the consequences of the other two groups of hallmarks and are responsible for the functional decline in ageing [2].

Defining and understanding the hallmarks and their effects on ageing, provides a basis for exploring new molecular mechanisms of ageing. In addition, the hallmarks may open the door to new approaches for developing interventions in aging to improve healthspan [2].

3.1.1 Healthy Ageing

Delaying ageing and the associated conditions is complex and difficult since ageing not only depends on cellular mechanisms but also on social and environmental factors. Due to the lack of knowledge about the ageing process and required conditions for healthy ageing, there is still no generally valid definition of what healthy ageing really is. The WHO defines healthy ageing "*as the process of developing and maintaining the functional ability that enables wellbeing in older age"* [3]. Healthy ageing already starts before birth with one's genetic heritage, where gene expression can be affected by experiences in the womb and several other ambient influences [3]. The health status depends on the intrinsic capability, and functional ability of an individual, which is defined as the sum of health-associated attributes that enable people to cope with everyday tasks, depending on the environmental conditions including social interactions, relationships, social policies, health care, work environment and the support system. Intrinsic capability include the individual mental and physical capacities as well as the interactions between these two and the individual [3]. On a scientific base, (healthy) ageing is investigated by means of mammalian model

organisms, such as rats (*Rattus norvegicus domestica*), mice (*Mus musculus*) and domestic dogs (*Canis lupus*) and non-human primates. However, besides financial reasons, studies on mammals are time-consuming and raise ethical concerns. Simpler model organisms, such as *Caenorhabditis elegans (C. elegans)* and *Drosophila melanogaster* have several advantages, such as short life span, easy maintenance, less legal requirements and constitute excellent alternatives to mammalian animal models [6, 7].

C. elegans played a key role in the research of physiological processes like ageing and led to the discovery of insulin/insulin-like growth factor (IGF) signaling (IIS) pathway in lifespan regulation. Due to the different methods of investigating and influencing ageing processes in *C. elegans*, it is an important model organism in the field of ageing research [8].

3.2 *C. elegans* as a model organism

3.2.1 Overview

C. elegans is a non-parasitic, 1 mm long roundworm with a life span of about 2-3 weeks and generation time of about 2-3 days at 20°C [9]. The worm consists of less than 1000 cells but still has different organs, cell types and tissues. Its transparent cuticle enables an uncomplicated observation of structures inside the body through a dissecting microscope. Historically, detailed research on *C. elegans* began in the 1960, when Sydney Brenner aimed to develop the worm as a genetic model for examining developmental biology and neurobiology [10]. For his work on organ development and genetics of cell death, he received the Nobel Prize in physiology or medicine in 2002 [11].

The maintenance of the worm is simple and inexpensive. *C.* elegans convinces with a short generation time, a large brood size of about 300 progenies and its well understood and simple anatomy [12]. The genome of the worm with more than 19.000 genes has been completely sequenced [13]. Resources like a genome-wide RNA-interference (RNAi) library and a map of protein interactions constitute another advantage of the organism [14]. RNAi makes it possible to quickly and specifically determine a loss of function phenotype of genes, therefore is a highly valuable tool in screening of genomes of *C. elegans* [15].

At the first sight, it seems like *C. elegans* is very different compared to humans. However, on the genetic level 50-80% of the worm genes have homologs in humans [12]. Moreover, worm and human share processes like apoptosis, autophagy, mitochondrial regulation, fat-storage, as well as lifespan regulating pathways, such as the IIS pathway. In *C. elegans* the IIS pathway serves as a linker between nutrient levels and metabolism, longevity, growth and behavior, influencing life span, development and reproduction [8, 16].

Due to the biologically conservation of the IIS pathway among various species, results from *C. elegans* studies could shed a light on its function in higher organisms. Because of the large number of orthologs, results from *C. elegans* studies are important factors in early drug discovery. Moreover, the conclusion can be drawn that results with a positive outcome on a disease phenotype in *C. elegans* are able to serve as an important measure in the prediction of human diseases [17].

Natural habitat and isolation of *C. elegans*

C. elegans can be found worldwide in semi-rotten parts of plants, fruits and compost. Their natural diet includes bacteria and yeast, but also small eukaryotes [18, 19]. The isolation of *C. elegans* for laboratory reasons is simple. In a first step, a sample of rotten vegetal substrate is placed on a petri dish with an *E.coli* lawn. Attracted by the food source, worms crawl out of the substrate, towards the bacteria. *C. elegans* can then be identified, isolated, and cultivated on a new agar plate [9]. In the laboratory, worms can be cultured on nematode growth medium (NGM) plates, seeded with OP50, at 16 °C. OP50 is an uracil-requiring *E. coli* strain, which functions as a food source in the laboratory. Uracil shortness in the medium prevents OP50 overgrowth and the formation of a thick bacterial layer, obscuring the worms [20].

3.2.2 Sexual forms & life cycle

Sexual forms

There are two sexual forms of the *C. elegans* wild type, they can either appear as self-fertilizing hermaphrodite or as male. In a normal population, 99.8-99.9% of the offspring develop into hermaphrodites and only 0.1-0.2% develop into males. When hermaphrodites reach the L4 stage, they produce amoeboid sperm, which is stored in

the spermatheca. Once the adult stage has been reached, the worms start with the production of eggs. Hermaphrodites fertilize their own eggs, while one individual can produce up to 300 progenies, limited by the amount of sperm it possesses. For further reproduction, sperm-depleted hermaphrodites can mate with one of the rarely occurring males. Even though hermaphrodites and males both have diploid chromosomes, they differ in the number of X chromosomes. Males have a genotype of X0 while hermaphrodites show a XX genotype, which explains the predomination of this sex [10].

Life cycle

The life cycle of the worm starts with the embryogenesis, lasting 16 hours. After hatching from the eggs, the worms are called L1 larva. The L1 stadium lasts 16 hours and is followed by three further larval stages called L2, L3 and L4, which are reached one after another at time intervals of 8-18 hours (Figure 1). Each larval stage is followed by a sleep-like period, called “lethargus”. This period of inactivity is mandatory for the animals to shed and to build a new cuticle [10].

Food shortage leads to the activation of an alternative life cycle in a L1 larvae. Starved larvae switch into a special L2 stage, called “*dauer*” stage (L2d). (“*dauer*” is the german word for “enduring”) [21]. *Dauers* have a thicker cuticle making them more resistant to chemicals, harmful substances and protected against stress from various environmental influences. *Dauer* larvae are able to survive for several months in this stage. When the food supply increases, *dauers* continue their development to a L4 larvae [12].

Characterization of different stages

Larvae in the four stages of development differ primarily in the size. L1 larvae reach a length of approximately 250 µm, L2 worms grow up to a length of 360-380 µm. L3 larvae are 490-510 µm long and L4 reach a length of 620-650 µm. The adult worm has a length of about 1000 µm [22]. In contrast to adult worms, L4 larvae have a closed vulva, visible by a ventral white spot, while adults show gonads and an open vulva. The whole cycle from egg to a completely developed adult lasts about three days at 25°C [23]. *Dauers* are approximately 400 µm long and significantly thinner than worms,

that undergo the normal development cycle. They are further distinguished by a thick cuticle that surrounds the entire worm including the pharynx, which is thereby closed.

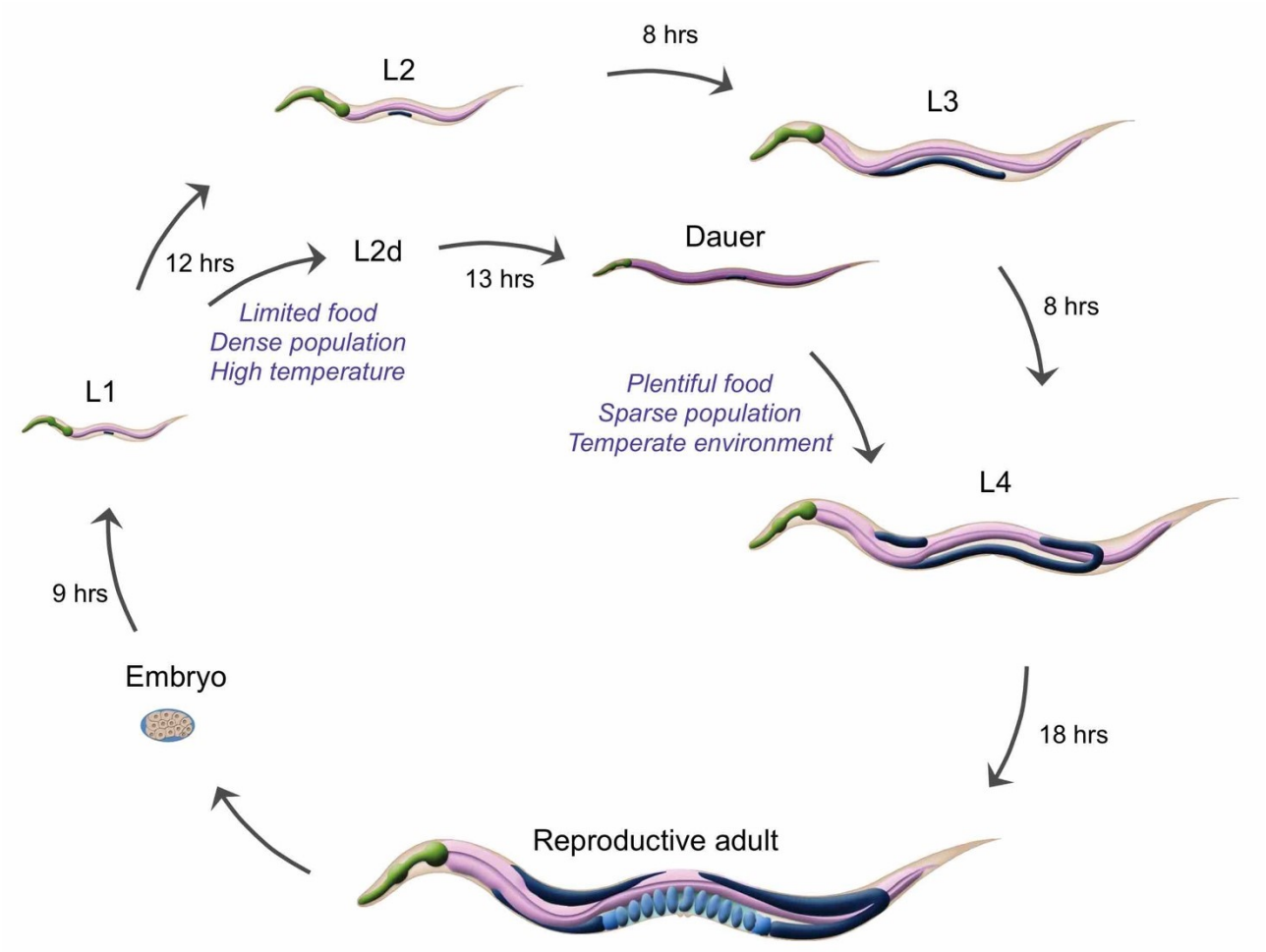

Figure 1: Lifecycle of C. elegans the figure shows the life cycle of *C. elegans* with the individual stages - from the embryonic stage to the reproductive adult worm. Under certain conditions such as food shortage, high temperatures or a large population density, the worm can change into a "dauer" stage. In this stage it is possible for the worm to survive for several months - when conditions improve again the worm can develop normally to L4 and then to an adult worm. Image from Z. F. Altun and D. H. Hall, “Introduction.,” WormAtlas, 2009, doi: doi:10.3908/wormatlas.1.1 [23].

3.2.3 Anatomy of *C. elegans*

C. elegans has a simple anatomy (Figure 2). Like other nematodes, it has a cylindrical, unsegmented tubular body shape. The body consists of an inner and an outer tube, separated by a fluid-filled tube, called pseudocoelom, which functions as a hydro skeleton. The hydrostatic pressure of the pseudocoelom plays an important role in the excretory system and tissue functions. The outer tube comprises of collagenous cuticle, hypodermis, muscles, neurons, and excretory system, whereas the inner tube consists of the alimentary system and in the adult worm the reproductive system [23].

In the young adult stage, the cuticle has a thickness of about 0.5 µm and consists of the four main layers epicuticle, cortical, medial and basal layers. The epicuticle functions as the outer cuticle and consists of lipids covered with a thin glycoprotein-rich coat. The cortical layer is located directly below the epicuticle and consists of collagens and cuticulin. Adjacent to this layer lies a fluid-filled zone called the medial zone, which contains collagen struts that connect the medial zone to the basal and cortical zones [24].

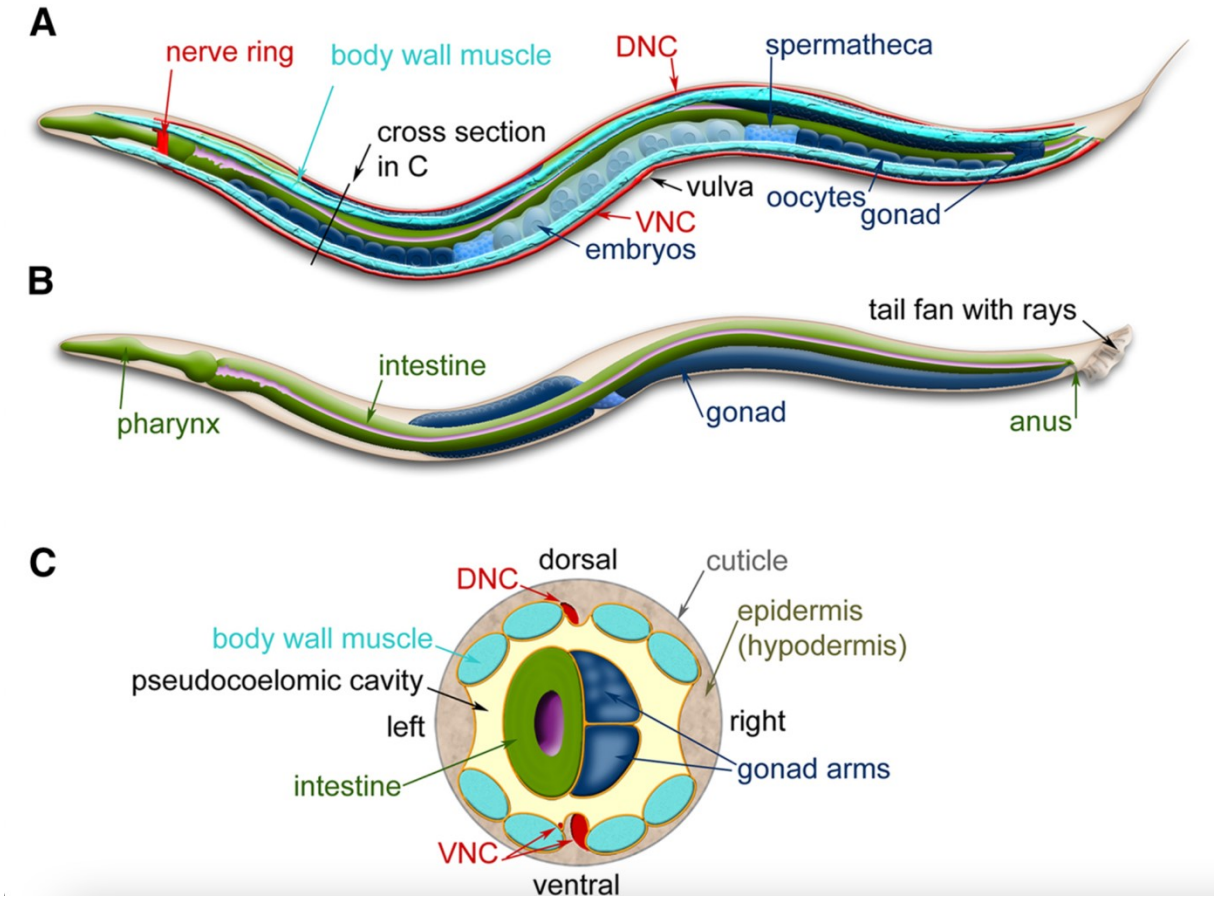

Figure 2: Anatomy of C. elegans. Figure shows the anatomical characteristic of a hermaphrodite (A) and male (B). (C) shows a Cross-section of a hermaphrodite anterior region. Image from A. K. Corsi, "A Transparent window into biology: A primer on Caenorhabditis elegans," WormBook, pp. 1–31, Jun. 2015, doi: 10.1895/wormbook.1.177.1. [10].

Alimentary system

The alimentary system consists of 127 cells and can be divided into pharynx, intestine and rectum. The pharynx, stomodeum (primary mouth region of the annelid larva) and the buccal cavity form the foregut, the intestine form the midgut and the hindgut is

formed through the rectum and the anus [25]. The mouth of the worm consists of six symmetrical lips, forming a circular hollow of about 1-3 µm, which forwards the food to a tube-like muscular pump, called pharynx. The pharynx contains 42 cells and 20 neurons. The cells are divided in nine epithelial cells, nine marginal cells, 20 muscle cells and four gland cells. Moreover, the different cells of the pharynx enable peristaltic movements and pumping to transport food into the intestine of the worm. The pharynx consists of the isthmus, the corpus, and the terminal bulb (Figure 3). In the anterior isthmus, bacteria are concentrated and squeezed for further digestion. Through the isthmus peristalsis, the bacteria are forwarded to the grinder in the terminal bulb, where they are crushed and subsequently being transported to the intestinal lumen [25].

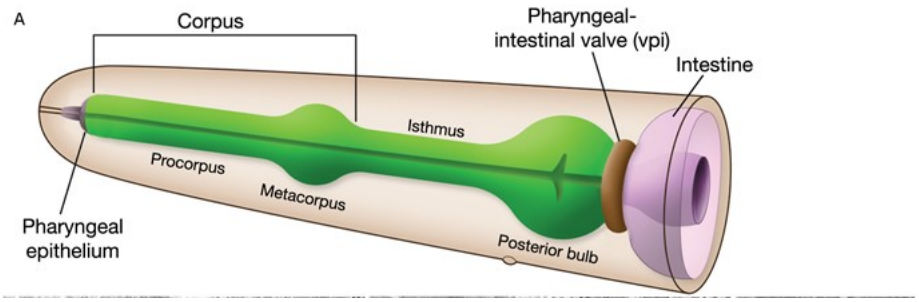

Figure 3: schematic presentation of the pharynx. Figure shows the pharynx with its different functional parts: Corpus consisting of procorpus and metacorpus, isthmus and terminal bulb (posterior bulb). Anterior the buccal cavity is connecting to the pharynx, at the posterior end the pharyngeal-intstinal valve is connected to the pharynx. The pharyngeal-intestinal valve is then connected to the intestine. Image from Z. F. Altun and D. H. Hall, "Alimentary system, overview," 2009, doi: doi:10.3908/wormatlas.1.2. [25]

The intestine in *C. elegans* is not only responsible for digestion and absorption of nutrients, but also plays a role in the synthesis and storing of macromolecules, initiation of the innate immune response in the event of pathogen infestation, and in the production of yolk for germ cells [26]. The intestine consists of 20 large epithelial cells, arranged as bilateral symmetric pairs that form a ring-shaped formation. Those pairs are building a long tube with a lumen, which is composed of eight intestinal rings, containing four cells each [24]. At the beginning of the worm development, the intestine fills the entire body cavity; as the nematode develops, the intestine becomes more curved, in order to make room and enable the gonadal growth. Due to this specific growing process, the intestine is connected to the body wall only at the anterior and posterior ends, whereas the rest of the intestine is free to move [26].

The hindgut consists of the last intestinal segment and the excretory organ, which is only a passageway connecting the intestine and the exterior. The passage consists of the rectum, the rectal gland and a valve between the rectum and the intestine. The rectum opens into the anus, which is the actual passage to the exterior. The entire excretory passage consists only eleven cells of three different cell types [27].

Reproductive system

Males and hermaphrodites differ in the reproductive system. As outlined in *WormAtlas,* 2009, “Reproductive system, overview” R. Lints and D. H. Hall, the reproduction is mainly facilitated by the hermaphrodites, which provide the anatomical prerequisites for self-fertilization and egg deposition, while males only provide sperm for additional fertilization of sperm depleted hermaphrodites. The reproductive system can be divided into the germ line, the somatic gonads and the egg laying apparatus. The uterus, part of the egg laying apparatus, can be found in the middle of the body. It is leading into two symmetrical U-shaped tubes, consisting of the somatic gonad and the germ line. In contrast, the reproductive system in males only contain a germ line and somatic gonads [28].

Muscle

Two main types of muscles in the worm can be distinguished. The non-straited muscles and the obliquely straited muscles/sarcomeres, with the latter being known as *somatic muscles*. The *somatic muscles* form the body wall, containing 95 body wall muscles cells, which are organized in four bundles. Non-straited muscles are called *single sarcomeres* and are found mainly in the anal sphincter, pharynx and uterus [29].

Nervous system

Adult worms have 302 neurons, belonging to two independent nervous systems: The somatic nervous system, containing 282 neurons and the pharyngeal system comprising only 20 neurons. A single pair of RIP interneurons enables the intercommunication between the systems. The somatic nervous system is located between the hypodermis and the body wall muscles. Hypodermis and neurons share a basal laminar that isolates the neurons from the muscles. The small pharyngeal nervous system is located directly among the muscles of the pharynx [30].

3.2.4 Preclinical methods

The increasing health hazard and the declining quality of life through the years, described in chapter 3.1, make ageing an urgent field for the discovery of therapeutic approaches. Thus, it is essential to find novel drugs to maintain and prolong the well-being in older age [31]. Generally, two different approaches, used for screening in drug discovery can be distinguished: the phenotype directed and the target directed screening.

Target-based drug discovery (TDD)

Receptors, enzymes, ion channels or other macromolecules constitute possible targets, whose activity can be modified by various compounds. When a ligand, for instance a drug molecule, binds to a target, it modulates the target's function and thereby causes a pharmacological effect, such as the negative inotropic effect of acebutolol when bound to the β1-adrenoreceptor [32]. In TDD the focus lies on finding compounds that can modulate a known disease-specific target. Modulation of the target results in an alleviating or healing effect on the symptoms. Therefore, library screening is used to discover target-modulators, which can then be further optimized for their selectivity, pharmacokinetic properties, potency and cellular activity [33]. TDD is predominantly used in the pharmaceutical industry and in the drug development [34].

Phenotypic drug discovery (PDD)

In PDD drug candidates are tested in a complex biological system, such as model organisms and cell cultures with functional or biochemical markers, to observe physiological effects. PDD aims to find drugs (small molecules) that positively affect a diseases phenotype [35]. This method of drug development focuses on the phenotypic effects of the tested samples without any prior knowledge about their molecular target(s) and molecular pathways [36]. In further studies, the target is identified and validated by means of phenotypic cellular screening. The identification and characterization of the target enables the design of further small molecules that bind to the same target [33].

TDD versus PDD

For many years, TDD was the dominant approach in drug discovery, but in recent years PDD has gained more and more attention [36]. PDD has the advantage that the target can remain unknown, making this approach particularly suitable for finding the so-

called "first in class" molecules. Furthermore, molecules acting via undiscovered mechanisms of action can be identified. However, the path from the discovery of a potential hit to the effective drug is long and can easily go wrong. In comparison, TDD is mainly used to discover molecules when the target is already known and validated as a druggable target. The approach is highly efficient to refine molecules, binding to the specific target. In this way, drugs can be optimized in their structure and efficacy. In most cases, structures found by PDD show a higher transferability from *in vitro* to *in vivo* and a better clinical efficacy than those identified by TDD [36]. Both approaches have their advantages and disadvantages and are important for drug development [37]. Compared to each other, PDD seems to be superior to TDD in the development of first in class drugs, while TDD is more suitable for the further development of drugs based on the first in class molecules [37]. To successfully develop new drugs, a combination of both approaches is suitable to exploit the potential of both assays [33].

3.2.5 Organisms for preclinical research for ageing related disease

Primitive organisms, such as yeast (*Saccharomyces cerevisiae*), fruit flies (*Drosophila melanogaster*), the roundworm *C. elegans,* but also rodents and non-human primates constitute important model organisms for ageing research. Some genes have shown to affect the lifespan, either by influencing the ageing process as such or impacting the development and the progress of ageing-related disorders [38].

Unicellular species have long been considered to be immortal. However, this assumption has been refuted by detailed research, which revealed the ageing process of the single cell fungus *Saccharomyces cerevisiae* [39]. On a genetic level, mammals and yeast share genes, making *S. cerevisiae* a useful organism for early preclinical research [38]. Ageing studies in yeast focus on two different approaches: The replicative life span and the chronological lifespan. The replicative lifespan is defined as the number of buds (outgrowth of the parent body) produced before the death, while the chronological lifespan is defined as the survival time of the cell, when it is in a non-dividing state. In the replicative lifespan approach, it is possible to analyse how many daughter cells (buds) the mother cell of the yeast can produce. Thus, statements can be made about the reproductive capacity of the yeast. Briefly, yeast models, particularly those for chronological aging, are primarily used for investigation of effects on dividable cells such as neurons [39].

Drosophila melanogaster (*D. melanogaster)* has proven to be a good model organism for research on age-related processes [40, 41]. The fruit flies have a life span of approximately 60-80 days and a generation time of about 10 days. *D. melanogaster* is cultivated in bottles, in which the flies undergo four different stages of their life cycle. The stages can be examined in different regions in the bottle. Another benefit of using the fruit fly in research is the uncomplicated and inexpensive handling [42]. About 60% of the genes of *D. melanogaster* have a homolog in humans. Furthermore 75% of genes that have been identified to be responsible for human diseases have a homolog in flies [43]. Moreover, the flies enable researchers to identify and characterize single gene mutations that result in an altered lifespan. Therefore, it is possible to test drugs that affect lifespan and to identify the pathways responsible for them using mutants [40, 41].

Rodent model organisms, such as rats and mice are mammals and in contrast to the previously mentioned model organisms, they are more closely related to human than yeast or fruit flies. Therefore, results obtained in rodent studies can more likely be related to humans. Especially mice are commonly used in research as model organisms, also in the field of ageing research. In mice, many genes that play a role in ageing have been identified. Mutated genes appear to extend lifespan by up to 50%. In various experiments, it has been proven that food abstinence leads to a prolonged lifespan in rats and mice [38]. A major disadvantage of lifespan testing in mice compared to, e.g. worms is their long life expectancy. Mice have a lifespan of up to four years, *C. elegans* in contrast has a lifespan of 2-3 weeks [12, 44]. Rodents could prove to be particularly important in the treatment of age-related diseases. There are mouse lines that display Werner's syndrome, Alzheimer's disease, diabetes and other age-related diseases [38].

C. elegans is a well suited as a model organism for ageing-related diseases because of the high similarity in genes, responsible for many disorders, with humans. Furthermore, *C. elegans* exhibits aging-related characteristics that can be seen both anatomically and behaviourally. Due to the simplicity of the genetic mutation of the worm, it is possible to study the effects of potential drugs on different pathways through various mutants [45].

3.2.6 *C. elegans* as a model organism for ageing related disease

When it comes to the discovery of anti-ageing compounds, focusing solely on lifespan prolonging effects is considered to be insufficient to provide evidence of delayed ageing [46]. In this context, the Geroscience Network pointed out the importance of considering healthspan in lifespan studies [46]. *C. elegans* enables the observation and analysis of ageing-related changes, playing a key role for a better understanding of age-related changes, affecting the nervous, the muscular and the reproductive system. Furthermore, some of the ageing symptoms in *C. elegans* can be compared to humans and can be monitored under the dissecting microscope, e.g. pharyngeal pumping rate, body movement, brood size and body size [47].

As the worm ages, the structure of the reproductive system changes which leads to a decreasing reproduction rate [48]. Moreover, a decline in motility, comparable to human frailty, caused by a loss of muscular integrity and sarcomere density can be examined over the time [45]. Furthermore, sinusoidal coordinated movements become increasingly uncoordinated. These muscular changes result in an abnormal, rigid, rectilinearly oriented body shape of the worm. In young adults, the pharyngeal pumping rate reaches a maximum of 300 pumps per minute. Due to older age and progressive loss of motility function the pumping rate declines continuously leading to a full loss of function at approximately day twelve of adulthood. Beside the previously outlined effect, other morphological and biochemical changes can be detected with different methods of measurement, for example the accumulation of the yolk protein can be tested using electron microcopy [49]. Different assays can be combined to investigate healthspan prolonging effects like antioxidant capabilities [50].

One hypothesis outlines the role of reactive oxygen species (ROS) in ageing. Oxidative stress can be caused by an imbalance between the production of ROS and their depletion [51]. *C. elegans* has been frequently used to study the effects of ROS *in vivo*: Accumulation of ROS leads to a decreased healthspan, increases susceptibility to toxins, and decreases the lifespan. Meanwhile, increased stress resistance leads to an increased healthspan, better resistance to toxins, and an increased lifespan. In *C. elegans*, stress resistance or the amount of ROS can be measured in different ways, such as measuring antioxidant enzymes and transcription factors. Antioxidant enzymes that can be determined are glutathione peroxidase, superoxide dismutase, peroxiredoxins, catalase, glutaredoxin. As many of the pathways involved in stress

resistance also have orthologs in humans, *C. elegans* suits as a model organism in the research of antioxidant properties. The antioxidant properties of the extracts shown in *C. elegans* could indicate a possible similar effect in humans [52].

For the investigation of samples in disease specific assay, the *C. elegans* mutant strains provide an indispensable tool [53]. To name some examples, the mutant strain "SS104" with the genotype glp-4(bn2) I, accumulates more fat than N2 wildtype worms and is therefore used as a model of metabolic syndrome [54]. Moreover, other mutant *C. elegans* strains can serve as model organisms for neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) [53]. For example, due to constitutive β-amyloid plaque aggregation in muscles, which is regulated by the unc-54 promoter, CL2006 is ideal for AD research. Growing β-amyloid accumulation causes aging-related progressive paralysis. When paralysis occurs, the worm is only able to move its head, but not its body. Natural product research has made considerable use of CL2006, which enables phenotypic evaluation of β-amyloid aggregation by observing the paralysis state of the worms [55, 56]. The mutant strain NL5901, expressing YFP marked α-synuclein is used as model for Parkinson diseases. The transparency of the worm enables the observation of α-synuclein levels under the fluorescence microscope. The amount of α-synuclein can be quantified by using software like ImageJ, whereby the fluorescence intensity in the body wall muscles correlates with the levels of α-synuclein [57].

3.3 Natural compounds, mushroom and plant extract tested in *C. elegans* lifespan assay

The plant kingdom constitutes an excellent source of therapeutic agents. The first records of the use of medicinal plants date back to 2600 BC, but until the 19th century, their use was purely empirical. In the 19th century plants were studied scientifically for the first time. Friedrich Sertürner, a German pharmaceutical assistant, was the first who successfully isolated the sleep-inducing and analgesic agent from *Papaver somniferum*, named *morphium* (referring to the Greek god Morpheus). This evoked great interest in phytochemistry leading to isolation of further compounds [58].

Between 1981 and 2010, 1073 new chemical drugs have been developed, belonging to the group of small molecules. Over 50% of them were either derivates of natural compounds (NCs) or inspired by nature and only 36% were fully synthetic [59].

The importance of drugs, deriving or inspired by nature can be shown by examples like antineoplastic substances as paclitaxel, vinblastine and vincristine. Further areas of application of NCs are for example AD, where galantamine isolated from *Galanthus nivalis* L., a cholinesterase inhibitor is used [60, 61].

Herbal and fungal preparations have been used in traditional medicine for centuries. Well-documented and detailed ethnopharmacological reports on plant material and their preparations provide information on possible pharmacological effects and therapeutic areas and form a good basis for further pharmacological and phytochemical studies by means of bioactivity-guided isolation. Most of these preparations are multicomponent mixtures, usually affecting several targets. A special characteristic of these multicomponent mixtures is that the single constituents can influence each other [58].

The interactions of NCs have an impact on the bioavailability of the single compounds and can improve their bioactivity in a synergistical, additive or antagonistic way. Most NCs have a complex stereochemistry and 3D structure, making chemical synthesis challenging or often impossible [62]. To avoid complex laborious synthesis, these compounds can be isolated from natural sources and either be used directly or as a lead structure for further modifications [58].

However, working with natural sources brings some shortcomings. One major problem is the availability and the amount of starting material, needed for the isolation. Further problems can appear in terms of recollection, as plant habitats vanish through anthropic pressure. Changes in the chemical composition, such as those that can occur during the seasons, can further diminish the timeframe for the recollection. Moreover, local circumstances, such as natural disasters and wars, can further limit the availability of plants, growing only in rare places [58].

Lonicera japonica Thunb.

Lonicera japonica Thunb., commonly known as Japanese honeysuckle, is a twinging vine which belongs to the family of Caprifoliaceae. It has a long-standing history in

traditional Chinese medicine (TCM), where it is used to treat dysentery and swelling, reduce hot flashes, prolong life, and protect the body against infections. Furthermore, it finds its application as a healthy beverage providing several positive effects, such as preventing diseases, strengthening the body, and increasing moisture of the skin. Apart from China, *Lonicera japonica* have been cultivated and used in more distant regions like Brazil, Argentina, Mexico, New Zealand, Australia, and United States. So far, nearly 140 chemical compounds have been isolated from the plant [63]. In TCM aerial parts of *Lonicera japonica* (flower, flower bud, leaf, and caulis) are used and can be consumed as tee or tincture [64]. However, organs differ in their chemical composition, which causes variations in the pharmacological activities [65]. Antibacterial, antiviral, anti-inflammatory, antipyretic and blood fat reducing activities could already been attributed to natural compounds like Hederagenin and chlorogenic acid in different studies which have been summarized in the review *"Lonicera japonica Thunb.: Ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine*" written by *X. Shang, H. Pan, M. Li, X. Miao, and H. Ding* [63].

***Angelica sinensis* (Oliv.) Diels**

Angelica sinensis (Oliv.) Diels is a traditional medicinal plant and belongs to the family of Apiaceae. *Angelica sinensis* is also known as *dong quai* in TCM, where the dried roots are traditionally used to treat amenorrhea and irregular menstruation, release pain, tonify and to increase the ability of the cardiovascular system. It´s pharmacological effects are displayed in the improvement of the immune function and provides anti-atherosclerotic effects, preventing myocardial infarction events and anti-arrhythmic activities. The phytochemical composition of *Angelica sinensis* root has already been examined in detail, The root mainly consists of organic acids, amino acids, vitamins, polysaccharides and phthalides [66].

***Scutellaria barbata* D. Don**

Scutellaria barbata D. Don belongs to the family of Lamiaceae and is widely used in traditional Korean and Chinese medicine. Traditionally, *Scutellaria barbata* is used for purification and detoxification of the body and relief of heat ailments. Ethanolic extracts of *Scutellaria barbata* are considered to have anti-microbial and anti-inflammatory effects. So far, more than 203 components have been isolated from the plant, where

flavonoids and neo-clerodane diterpenes seem to be the predominant structure classes [67].

Ganoderma lucidum (CURTIS) P. KARST.

Ganoderma lucidum (CURTIS) P. KARST. is a Basidiomycota that belongs to the family of Ganodermataceae. The fruiting body is console-shaped and is characterized by a bright lacquer-like shining red-orange color [68]. The mushroom has a longstanding tradition in wide parts of Asia, where it is called "mushroom of life", with *Ling-Zhi* being the Chinese designation and *Reishi* mushroom as the Japanese term [69, 70]. The effects of *Reishi* range from anti-atherosclerotic, anti-inflammatory, antibacterial, anti-diabetic, chemo-preventive, antitumor, sleep promoting, anti-inflammatory, chemo and radio protective, antioxidant and radical-scavenging, to hypolipidemic, immunomodulation, anti-fibrotic, analgesic, hepatoprotective, anti-androgenic, anti-angiogenic, antiviral, anti-aging, hypoglycemic, anti-ulcer, estrogenic activity and anti-herpetic properties [70]. *Ganoderma lucidum* contains a wide spectrum of constituents, including protein, steroids, polysaccharides, vitamins, triterpenoids, enzymes, nucleotides, sterols, fatty acids and minerals. Polysaccharides and triterpenes are considered to be responsible for the pharmacological effects like anti-inflammatory, anti-atherosclerotic, antiaging and antitumor. Besides triterpenes and polysaccharides, the active components include alkaloids, steroids, nucleotides, vitamins, fatty acids, lectins, adenosines, and essential minerals [69].

Lignosus rhinocerus (Cooke) Ryvarden

Lignosus rhinocerus (Cooke) Ryvarden, also called Tiger milk mushroom is a fungus and belongs to the family of Polyporaceae [71]. Extracts isolated from the sclerotium of the mushroom displaying anti-inflammatory, antioxidant, antimicrobial and anti-asthmatic activity as well as immunostimulatory effects in human. Recent studies have proven a high absorption rate and bioavailability after oral intake [72].

Genista tinctoria L.

Genista tinctoria L. a small plant with yellow flowers belongs to the family of Fabaceae and is located in Europe. *Genista tinctoria* is known as dyer’s greenweed, deriving from its use in the past to dye textile in Europe. The flavonoids apigenin and luteolin, some glycosides and the isoflavone genistein are responsible for the coloring properties [73]. The high content of isoflavones and flavonoids is characteristically for *Genista tinctoria*.

Flowers, herb and seeds are traditionally used for medicinal purposes as diuretics, emetics and cathartic in the folk medicine of Europe. *Genista tinctoria* differs from other Genista species mainly by its high isoflavone content, genistein, which has phytoestrogen properties, but also affects the thyroid gland. Moreover, researchers attributed an antitumoral effect to genistein [74].

Macrocybe titans (H.E Bigelow & Kimbr.) Pegler, Lodge & Nakasone

Macrocybe titans (H.E Bigelow & Kimbr.) Pegler, Lodge & Nakasone is part of the Tricholomataceae family and can be found in South America. The cone shaped mushroom can reach a fresh weight of up to 30 kg and a size of 75 cm [75]. Distinctive characteristic of the fungus are the squamulose striped surface and the various refractive pseudocystidia, which can be observed under the dissecting microscope [76]. *Macrocybe titans* has only been little mycochemically and pharmacologically investigated yet. The few known components that have been identified to date include the polysaccharide fucogalactan some β-Glucans and a triglyceride named macrocybin [77, 78]. Polysaccharides isolated from the family of Tricholomataceae showed anti-inflammatory, immunomodulatory, anti-sepsis, anti-oxidative and antitumor activities [77].

Resveratrol

Resveratrol is one of the best examined natural polyphenols. It was originally isolated from *Veratrum grandiflorum* O.Loes, but can also be found in vine, grapes, peanuts, berries, and soy [79–81]. The pharmacological benefits of resveratrol reach from antioxidant, anti-inflammatory, antidiabetic, antiaging to anticancer, neuroprotective and cardioprotective activities [79, 80]. Resveratrol is used in the prevention and in treatment of chronic diseases such as obesity, diabetes, neurodegeneration, cardiovascular disease, cancer and in the process of aging [82, 83].

Pterostilbene

Trans-3,5-dimethoxy-4′-hydroxystilbene (Pterostilbene) is a natural polyphenol and as such is an analog of resveratrol. Compared to resveratrol, pterostilbene seems to have a higher bioavailability due to additional two methoxy groups (Figure 4). Pterostilbene can be found for example in blueberries, almonds and grapes. Pterostilbene is considered to have antioxidant and anti-inflammatory properties [84].

Figure 4: Structural formula of A: Resveratrol and B: Pterostilbene

***Lycium barbarum* Mill.**

Lycium barbarum Mill., belonging to the Solanaceae family, has been used in TCM for more than 2000 years. The herb is rich in betaine, phenols, 2-O-β-d-glucopyranosyl-l-ascorbic acid, polysaccharides, carotenoids, flavonoids, cerebrosides and vitamins. Especially the lipopolysaccharides comprised in the herb exhibit antiaging effects, antioxidant and antiapoptotic immunoregulative activities and DNA damage reducing qualities [85].

***Panax ginseng* C.A.Mey**

Panax ginseng C.A.Mey, also known as Korean ginseng, belongs to the family of Araliaceae and has been used in TCM for more than 5000 years. Its most important medicinal constituents include ginsenosides and glycosylated triterpenes. The various triterpenes have demonstrated anti-inflammatory, antiproliferative and antiapoptotic activities in mice and human cells [86].

***Punica granatum* L.**

Punica granatum L., known as pomegranate, is a member of the plant family Lythraceae. The seeds and fruit of the pomegranate are consumed worldwide for hundreds of years. Due to its abundance in bioactive substances, the flowers, leaves, fruit and seeds are additionally of great interest in scientific circles. *In vitro* and *in vivo*

studies indicated that compounds derived from the pomegranate reduce platelet aggregation and oxidative stress, effect endothelial cell function in a positive way and contribute to blood pressure regulation [87].

***Panax notoginseng* (Burkill) F.H.Chen**

Panax notoginseng (Burkill) F.H.Chen belongs to the family of Araliaceae. The plant is also known as *Sanchi* or *Sanqi* in China and has been applied as a treatment of cerebrovascular diseases for centuries worldwide. Panax notoginseng contains many bioactive components, most importantly including ginsenosides, saponins, polyacetylenes and volatile oils [88].

***Astragalus mongholicus* Bunge *& Astragalus membranaceus* Fisch ex. Bunge**

Astragalus membranaceus Bunge also called *Huangqi* and *Astragalus membranaceus* Fisch ex. Bunge belongs to the Fabaceae family and have been utilized in TCM for more than 200 years. The main components include flavonoids, saponins and polysaccharides. Various studies, including studies on rodents, have indicated antioxidant, immune regulatory, anti-inflammatory, and anticancer qualities [89, 90].

***Withania somnifera* L. Dunal.**

Withania somnifera L. Dunal. generally known as *Ashwagandha* belongs to the Solanaceae family. *Ashwagandha* played an important role in the Indian medicine, where it was mainly used as an antistress agent and potent adaptogen. The leaves, root and stem comprise various bioactive compounds, including alkaloids, tannins, flavonoids, glycosides and steroid lactones. Steroid lactones include withnaolides, the main secondary metabolites of the plant and the main ingredients responsible for the effects attributed to the plant [91].

***Centella asiatica* (L.) Urb.**

Centella asiatica (L.) Urb. also known by the name of *Bua-bok, Tiger grass, Gotu Kola* or *Indian Pennywort*, belongs to the Apiaceae family. Traditionally, the plant is predominantly used in Southeast Asia as a medicinal plant because of its wound healing properties. Further qualities of *Centella asiatica* include antioxidant, antimicrobial, neuroprotective and anti-inflammatory activity. The main bioactive constituents are the certain triterpenes, the so-called scentelloids [92].

***Convolvulus prostratus* Forssk**.

Convolvulus prostratus Forssk., also known as *Shankhpushpi*, is a traditional medicinal plant in Ayurveda. The plant belongs to the family of Convolvulaceae and is believed to have anxiolytic, antioxidant, neuroprotective, immunomodulatory, analgesic, antidiabetic, cardioprotective and antimicrobial properties, owed to the bioactive compounds of *Convolvulus prostratus* include phenols, flavonoids, and alkaloids [93].

***Passiflora incarnata* L.**

Passiflora incarnata L., belonging to the Passifloraceae family, has been extensively phytochemically and pharmacologically investigated. The flowers, fruits, and remaining aerial parts of the plant are used for medical purposes, whereby anxiolytic, spasmolytic, and antihelmintic abilities are attributed to the plant. Phenols, alkaloids, cyanogenic glycosides and flavonoids comprise the bioactive substances of the plant [94].

***Salvia officinalis* L.**

Salvia officinalis L. originates from the Mediterranean and the Middle East and belongs to the Lamiaceae family. Owing to its properties, *Salvia officinalis* is used in the treatment of ulcers, seizures, tremors, paralysis, hyperglycemia, inflammation, and diarrhea. Due to its long established use and broad application range, most of the bioactive compounds of *Salvia officinalis* have been studied extensively, including fatty acids, glycosidic derivatives, carbohydrates, alkaloids, terpenes/terpenoids, poly acetylenes, phenolic compounds, steroids and waxes. Bioactive constituents show antimicrobial, antidementia, antioxidant, antimutagenic, anticancer, antinociceptive properties [95].

Cynara scolymus L.

Cynara scolymus L., also known as cardoon, belongs to the plant family Asteraceae. The leaves of the plant mainly contain phenolic components, responsible for the antiproliferative, antidiabetic and antimicrobial properties of cardoon [96].

***Gynostemma pentaphyllum* (Thunb.) Makino**

Gynostemma pentaphyllum (Thunb.) Makino, also known as *Jiaogulan* in TCM meaning herb of immortality, belongs to the Cucurbitaceae family. The plant includes a wide spectrum of bioactive compounds such as flavonoids, sterols, lignans, vitamins,

polysaccharides, amino acids, gypenosides. Its various constituents exhibit pharmacological properties including antidementia, anticancer, hepatoprotective, antiatherogenic, lipid regulating and hypoglycemic properties. Despite its high biological activity *Gynostemma pentaphyllum* displays very low pharmacological toxicity, causing an enhanced interest in the plant in recent years [97].

***Cistus creticus* L.**

Cistus creticus L. also known as Pink Rock Rose, belongs to the Cistaceae family. The bush is native to Europe, Western Asia and Mediterranean Areas and its main bioactive components include polyphenols, thus accounting for the plants antioxidant and antimicrobial properties [98].

***Ranunculus ternatus* Thunb.**

The *Ranunculus ternatus* Thunb. plant belongs to the Ranunculaceae family and is used in TCM for the treatment of tuberculosis, faucitis, breast cancer and neck scrofula. Polysaccharides isolated from the root of the plant are attributed with antioxidant, antitumor and immunomodulatory properties [99].

***Polygala tenuifolia* Willd./sibirica**

The root of the *Polygala tenuifolia* Willd./sibirica is commonly referred to as *yuanzhi* or *onji* in eastern Asia. The plant belongs to the Polygalaceae family and is well documented in the Chinese, Korean, Japanese and European Pharmacopoeias. In oriental medicine it is used to treat inflammatory diseases, anxiety and memory disorders. Bioactive compounds include phenolic compounds such as xanthones oligosaccharide esters and triterpenoids [100].

***Fomitopsis betulina* (Bull.) B. K. Cui, M. L. Han & Y. C. Dai**

Fomitopsis betulina (Bull.) B. K. Cui, M. L. Han & Y. C. Dai is a higher Basidiomycota and belongs to the family Fomitopsidaceae. In the northern hemisphere the fungus is growing on trees. In folk medicine, the mushroom is known for its antimicrobial, anticancer and anti-inflammatory activity. The main bioactive components of *Fomitopsis betulina* are triterpenes [101].

Cannabidiol (CBD) and Cannabigerol (CBG)

CBD and CBG are isolated from the plant *Cannabis sativa* L. . The phytocannabinoids have been used to treat different conditions, such as stress, anxiety, sleeping disorders

and pain for more than 6000 years. Cannabinoids act on the endocannabinoid system, which consists of two different types of cannabinoid receptors. These receptors play critical roles in the inflammatory response, giving cannabinoids an anti-inflammatory effect [102].

Spermidine

Spermidine is a naturally occurring polyamine. Due to its positive charge it can interact with negatively charged molecules such as RNA, DNA and proteins. Spermidine therefore can be involved in physiological as well as pathological processes, such as cell differentiation, cell growth, tissue regeneration and growth. Spermidine has antioxidant and anti-inflammatory properties. Furthermore, it improves protease and leads to enhanced mitochondrial function, which can result in higher autophagy. The natural concentration of spermidine declines with age and the externally supplied supplementation is associated with a prolonged lifespan [103].

Salicin and salicylic acid

Salicin and salicylic acid are part of the group of salicylates and are compounds which can be isolated from various *Salix* species and have been used in folk medicine for centuries. They are used to treat headaches, rheumatic diseases, toothache, backache and menstruation. Salicin is the prodrug for salicylic acid, which attacks cyclooxygenases and thus interferes with prostaglandin synthesis and thus with inflammatory and pain processes [104].

Chlorogenic acid

The phenolic compound chlorogenic acid, also known as 5-O-caffeoylquinic acid, belongs to the family of hydroxycinnamic acids. The acid is attributed with antioxidant, antimicrobial, antidiabetic, antilipidemic, antihypertensive and anti-inflammatory properties. Chlorogenic acid is found in many foods and plants such as artichokes, apples, coffee beans, carrots, turmeric, tomatoes, potatoes, pears and tea [105].

Ascorbic acid

Ascorbic acid, also known as vitamin C, is an essential micronutrient for humans that cannot be synthesized by the body. Vitamin C is a powerful antioxidant, has wound-healing effects, and contributes to immune defense at various cellular levels. Severe vitamin C deficiency results in the potentially fatal disease called scurvy [106].

4 Materials and Methods

4.1 Instruments and Lab Ware

Instrument	Supplier
Microscope shaker	IKA MS 3 digital
Analytical balance	BP210D, Sartorius
Balance	Sartorius LC4801P
Centrifuge	Zentrifuge Heraeus Megafuge 1.0R
Dissecting scope	Zeiss
Lamina airflow	Steril Werkbank B10
Microscope	Nikon Labophot-2; Reichert-Jung Neovar 2
Rotavapor	BUCHI Switzerland
Shaker	IKA MS3 digital
Vortex	Stuart variable speed Mini Vortex Mixer
Worm Tracker	WMicrotracker one, Phylum tech
Electronic stirrer	Variomag; Electronicrührer MULTIPOINT HP
Incubator - 16°C	Memmert GTR0214
Incubator – 25°C	INCU-Line Tower, VWR
Florescence microscope	Axio Observer. Z1 Zeiss
Ultrasonic bath	Transsonic T 460, Elma
96-well Plate Reader	Tecan Genios

Lab Ware	Supplier
Erlenmeyer Flask	SCHOTT DURAN
Falcons – 15 ml	CELLSTAR TUBES; Greiner bio-one

Falcons 50 ml	Sarstedt, 50 ml Tube
Multi-channel pipette	Eppendorf Research (30-300 µL); StarLab (100-1200 µL)
Parafilm	Bemis Parafilm “M”
Pasteur Pipette	Sarstedt: Round petri plates (92X16mm), sterile, with vents
Petri plates	Gosselin; Round Petri plate PS na 3 ventsH14
Pipette boy	Integra Pipetboy 2
Pipette Tips	Eppendorf; Sarstedt; StarLab
Reagent Reservoir	VWR North American Cat. No. 89094-680
Serological Pipettes	Sarstedt, Serological Pipette
Vial	6 ml & 10 ml - Sempercare
Cell Culture Plate	Sarstedt TC plate, 96 well, Standard, F. flat bottom, transparent non-pyrogenic, non-cytotoxic; DNase/RNase/DNA-free; sterile
Eppendorf Tube	500 Eppendorf Tubes; Safe-Lock Tubes

4.2 Chemicals and Reagents

Reagent	Supplier/Charge
Agar	BioScience; Art.-Nr. 6494.3
Calcium chloride anhydrous	Sigma-Aldrich; C1016-100G
Calciumchlorid	Sigma; C1016-100G
Cholesterol	Sigma-Aldrich; C8503-25G
Citric acid monohydrate	Sigma; C1909-25G
Copper (II) sulfate pentahydrate	Sigma; C8027-500G
Danchlor	Klorin 2250FR122Q
ddH_2O	purified by ion exchanger

DMSO	Emsure; Merck 1.02952.1000
EtOH	Sigma-Aldrich; 24194-2.5L-R
$FeSO_4 7H_2O$	Sigma; 215422-250G
disodium salt dihydrate	E1644-250G
K_2HPO4	Sigma; P3786-100G-D
KH_2PO4	Sigma; P5655-500G
$MgSO_4$	Sigma; 63535-50G-F
$MnCl_2 4H_2O$	Sigma 63535-50G
Na_2HPO_4	Sigma; 57907-100G
NaOH	Merck 1.064621000
Peptone	Sigma-Aldrich; 91249-500G
$ZnSO_4 7H_2O$	Sigma; Z4750-100G
Potassiium citrate tribasic	Sigma b0153-500G-F
Monohydrate	Sigma; 60153-500G-F
Potassium phosphate dibasic	Sigma-Aldrich; P3786-500G
Potassium phosphate monobasic	Sigma-Aldrich; P5655-500G
Sodium chloride	Sigma-Aldrich; S7653-1KG
Sodium phosphate dibasic	Sigma-Aldrich; S7907-100G
Tris	Roth 284216280
Methanol	VWR – 83638.320
Reserpine	Sigma-Aldrich; 83580-1G

4.3 Composition of Reagents and Media

NGM Agar	
NaCl	3 g
Agar	17 g
Peptone	2,5 g
ddH_2O	975 mL

All compounds were mixed in an Erlenmeyer flask. Afterwards, the flask was transferred to a water bath and heated for a few minutes, followed by autoclavation (121°C, 2 bar). Then, the flask was reheated on the water bath and the following components were added under the lamina airflow:

1M $CaCl_2$	0,5 mL
5mg/ml Cholesterol in EtOH	1 mL
1M $MgSO_4$	1 mL
1M KPO_4	25 mL

After swirling with the magnetic stirrer, 10 ml of the hot NGM agar were spread onto each 10 cm petri dish. The agar was left to cool and solidify for one hour before the dishes were packed into sterile bags and stored upside down at 4 °C in the refrigerator.

S Basal	
NaCl	5.85 g
K_2HPO_4	1 g
KH_2O_4	6 g
ddH_2O	ad 1000mL
5 mg/ml cholesterol in EtOH	1 ml

Inorganic reagents were mixed and autoclaved. Continuing that, 1 ml of a 5 mg/ml ethanolic cholesterol solution was added to the mixture under the lamina airflow.

S Medium / S- Complete	
S Basal	1L
1M KCitrate Buffer (pH=6)	10 mL
Trace Metal Solution	10 mL
1M $CaCl_2$	3 mL
1M $MgSO_4$	3 mL

All compounds were dissolved and the mixture was autoclaved.

S Buffer	
0,05M K_2HPO_4	129 mL
0,05M KH_2PO_4	871 mL
NaCl	5.85 g
ddH_2O	ad 1000 ml

All substances were dissolved and subsequently autoclaved.

M9 Buffer	
K_2HPO_4	3 g
KH_2PO_4	6 g
NaCl	5 g
1M $MgSO_4$	1 ml
ddH_2O	ad 1000 ml

All substances were dissolved and subsequently autoclaved.

Trace metal Solution	
Na_2EDTA	1.86 g
$FeSO_47H_2O$	0.96 g
$MnCl_24H_2O$	0.2 g
$ZnSO_47H_2O$	0.29 g
$CuSO_45H_2O$	0.025 g
ddH_2O	ad 1000 ml

All substances were dissolved and subsequently autoclaved.

KPO_2 Buffer with pH6	
K_2HPO_4	108.3 g
KH_2PO_4	35.6 g
ddH_2O	ad 1000 ml

All substances were dissolved and subsequently autoclaved. Afterwards the pH value was checked.

1M KCitrate Buffer pH=6	
Citric acid H_2O	20 g
K_3Citrate H_2O	293.5 g
ddH_2O	ad 1000 ml

All substances were dissolved and subsequently autoclaved. At the end the pH value was checked.

X Reagent (for 3 Plates)	
Danchlor (5% NaOCl)	1 ml
5M NaOH	0,5 ml

Both solutions were mixed directly before use.

LB Medium	
Bacto-tryptophone	10 g
Bacto-yeast	5 g
NaCL	5 g
ddH2O	970 ml

The pH value was adjusted to 7 by means of 1M NaOH, then the solution was autoclaved.

OP-50 *E.coli* stock	
LB Medium	250 ml
ddH2O	30 ml
S Medium	To adjust the concentration
E. coli cells (cell bank stock stored at -70°C)	500 µl

250 ml LB Medium were inoculated with 500 µl of standardized *E. coli* OP50 working cell bank stock (stored at -70°C) and stored on a shaker for 24 hours at room temperature (20°C ± 1). Afterwards, the solution was centrifuged at 3000 g for 10 minutes. The supernatant was decanted to isolate OP50. Afterwards, the Pellet was washed two times with ddH_2O and the remaining water was removed. At the end, the pellet was air-dried, weighted and dissolved in S Medium to a concentration 100mg/ml.

4.4 Plant and mushroom material

Plant and fungal material, as well as Natural Products (NPs) were selected by Mag. pharm. Martina Redl, who performed a broad literature search based on ethnopharmacological data during her PhD project.

Table 1 – Plant and Mushroom material for extract preparation.

Extract ID	Species	Plant Family	Used Organ	Extraction Method	Source of extract and plant material	Charge number
SPr 41	*Lycium barbarum* Mill.	Solanaceae	Cortex	70 Vol % EtOH	Plantasia GmbH	460799
Spr 42	*Lonicera japonica Thumb.*	Caprifoliaceae	Caulis	70 Vol % EtOH	Plantasia GmbH	84 0850
SPr 43	*Panax Ginseng C.A.Mey*	Araliaceae	Radix	70 Vol % EtOH	Dr. Kottas	P21301630
SPr 45	*Punicae granatum* L.	Lythraceae	Semen	70 Vol % EtOH	Alfred Galke GmbH	38770
SPr 46	*Panax notoginseng* (Burkill) F.H.Chen	Araliaceae	Radix	70 Vol % EtOH	Plantasia GmbH	751115
SPr 47	*Astragalus mongholicus* (Bunge) *and Astragalus membranaceus* Fisch ex. Bunge	Fabaceae	Radix	70 Vol % EtOH	Plantasia GmbH	640033
SPr 48	*Angelica sinensis (*Oliv.) Diels	Apiaceae	Radix	70 Vol % EtOH	Plantasia GmbH	78 0023
SPr 49	*Withania somnifera* (L.) Dunal	Solanaceae	Radix	70 Vol % EtOH	Alfred Galke GmbH	44618
SPr 51	*Centella asiatica* (L.) Urb.	Apiaceae	Herba	70 Vol % EtOH	Alfred Galke GmbH	41639
SPr 52	*Convolvulus prostratus* Forssk.	Convolvulaceae	Herba	70 Vol % EtOH	Alfred Galke GmbH	124741

Extract ID	Species	Plant Family	Used Organ	Extraction Method	Source of extract and plant material	Charge number
Spr 53	*Passiflora incarnata* L.	Passifloraceae	Herba	70 Vol % EtOH	Dr. Kottas	unknown
SPr 54	*Salvia officinalis* L.	Lamiaceae	Folium	70 Vol % EtOH	Dr. Kottas	unknown
SPr 55	*Cynara cardunculus* L.	Asteraceae	Folium	70 Vol % EtOH	Dr. Kottas	W21201631
SPr 56	*Scutellaria barbata* D. Don	Lamiaceae	Herba	70 Vol % EtOH	Plantasia GmbH	76 0577
SPr 57	*Gynostemma phentaphyllum* (Thunb.) Makino	Cucurbitaceae	Herba	70 Vol % EtOH	Alfred Galke GmbH	44300
SPr 58	*Cistus creticus* L.	Cistaceae	Herba	70 Vol % EtOH	Alfred Galke GmbH	Creti : 37886 – incan. 41034
SPr 59	*Ranunculus ternatus.* Thunb.	Ranunculaceae	Radix	70 Vol % EtOH	Plantasia GmbH	1108515
SPr 60	Polygala tenuifolia Willd. /sibirica	Polygalaceae	Radix	70 Vol % EtOH	Plantasia GmbH	93 0101
SPr 61	*Fomitopsis betulina* (Bull.) B. K. Cui, M. L. Han & Y. C. Dai	Fomitopsidaceae	Karposoma	70 Vol % EtOH	Wild harvest	unknown
SPr 62	*Ganoderrma lucidum* (CURTIS) P. KARST.	Ganodermataceae	Karposoma	70 Vol % EtOH	Plantaisa	JR-20080605-A1
Spr 63	*Lignosus rhiocerus* (Cooke) Ryvarden	Polyporaceae	Sklerotium	70 Vol % EtOH	Tyroler Glückspilze	AN310

Extract ID	Species	Plant Family	Used Organ	Extraction Method	Source of extract and plant material	Charge number
SPr 69	*Genista tinctoria* L.	Fabaceae	Herba	70 Vol % EtOH	Dr. Kottas	W22201222
SP 70	*Macrocybe titans* (H.E Bigelow & Kimbr.) Pegler, Lodge & Nakasone	Tricholomataceae	Karposoma	70 Vol % EtOH	Tyroler Glückspilze	AN399

Table 2: Natural Compounds and pure substances

Extract Number	Compound name	Source	Charge number
SPr 64	Spermidine	SIGMA - ALDRICH	85558
SPr 65	CBD/CBG oil (12.5 % CBD, 2.5 % CBG)	Fa. Smart Greenery	unknown
SPr 66	CBD extract University (10%)	University of Vienna	unknown
SPr 67	Salicylic acid	GATT - KOLLER	21J01961
SPr 68	Salicin	University of Vienna	788A
SPr 71	Chlorogenic acid	ROTH	6385
SPr 72	Pterostilbene	University of Vienna	unknown
SPr 73	Levamisole	SIGMA - ALDRICH	L9756
SPr 74	Ascorbic acid	APOfit Handels GmbH	0463

4.5 Herbal/Fungal extracts and natural compounds

4.5.1 Extraction of herbal and fungal drug substances

For each extract, the plant and fungal material was powdered in a coffee grinder or a mortar. Around 300 mg of the pulverized mushroom/plant material were mixed with 9 ml of freshly prepared 70% EtOH (v/v). The mixture was covered with aluminum foil and sonicated for 20 minutes at RT. Afterwards the solution was centrifuged at 3500 g and the supernatant was filtered through cotton wool into a round bottom flask. The remaining pellet was washed with 5 ml of 70% EtOH and placed into the ultrasonic bath for 5 minutes. Thereafter, the solution was centrifuged at 3500 g and the supernatant was filtered through cotton wool. The filtrates were combined and evaporated. Subsequently, the dried extract was diluted in MeOH, transferred into a pre-weighted vial and the solvent was subsequently evaporated. For the bioactivity screening, about 2 mg of the extract were transferred to a pre-weighted Eppendorf tube and diluted in 70% DMSO to reach a concentration of 20 mg/ml at first and a final concentration of 200 µg/ml.

4.6 Maintenance of *C. elegans*

The N_2 Bristol wildtype worms were cultured on NGM agar plates (100 mm x 15mm round petri dish, inoculated with 20mg OP50 each) at 16°C. In order to prevent the nematodes from starving and from switching into dauer stage, little squares were cut from the agar and transferred to new NGM agar plates, seeded with an OP50 lawn, every week.

4.6.1 Synchronization of *C. elegans* cultures

In order to obtain an age-synchronous worm population, nematodes were washed off from six agar plates, using 4 ml of sterile water per plate. Subsequently, the worms were transferred to two 15 ml falcons and cleaned from agar and bacteria via centrifugation at 1600 g. The cleansed worms were suspended in 7 ml ddH_2O and treated with 1.5 ml freshly prepared x-reagent, a bleaching solution, which leads to the lysis of the worms and thereby enables the isolation of the eggs. The worm lysis was observed through the light microscope. In order to prevent the eggs from being harmed by excessive exposure to x-reagent the reaction was stopped by adding 7ml M9 buffer, as soon as approximately 70% of the worms have been lysed. Thereafter, the

remaining egg pellet was washed two times with 10 ml of M9 buffer and once with 10 ml S medium as illustrated in Figure 5. After every centrifugation step, the supernatant was decanted, and the pellet was dissolved in fresh solvent. After the last washing process, the worm solution was transferred to an Erlenmeyer flask and put on a shaker for 48h until L1 hatched from the eggs.

Washing Procedure:

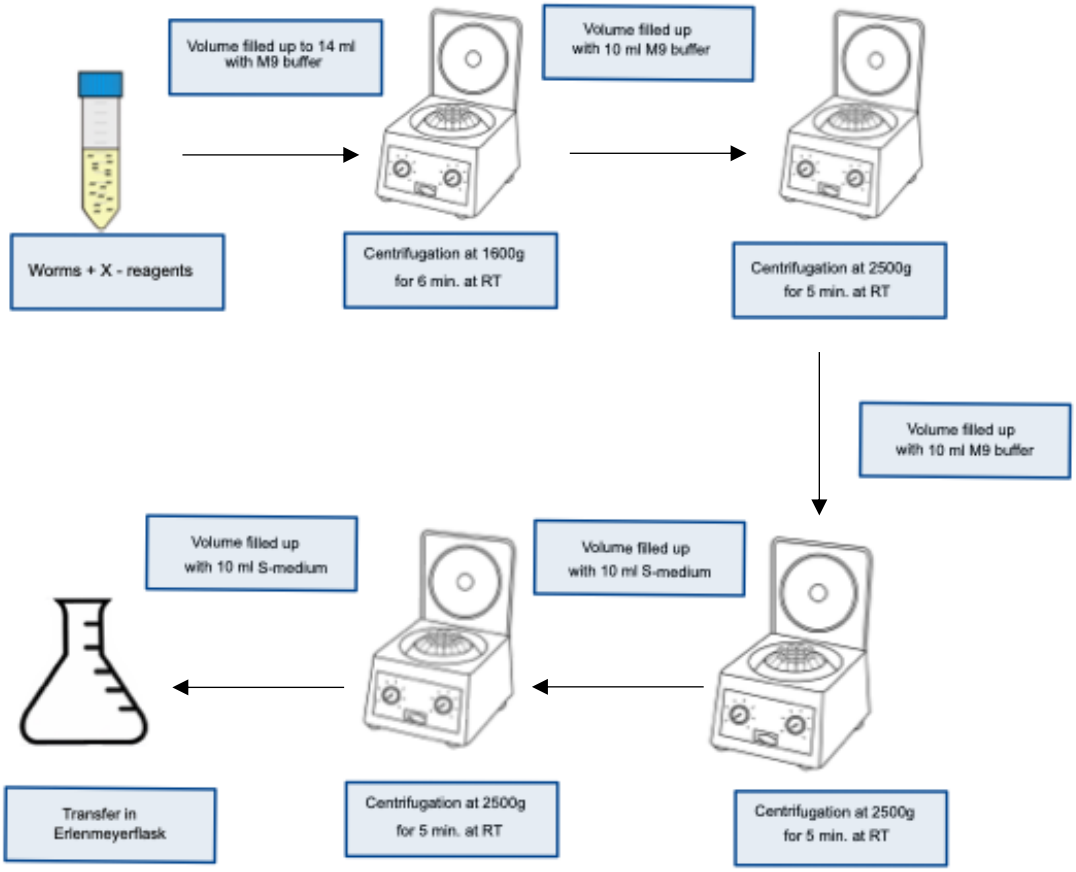

Figure 5: Washing procedure. Schematic presentation of the washing procedure. When about 70% of the worms have been lysed, lysis is stopped by adding M9 buffer up to 14 ml. Afterwards, the falcons are centrifuged at 1600 g for 6 min. After centrifugation, the supernatant is removed, the pellet is dissolved in 10 ml of M9 buffer. The falcons were centrifuged at 2500g for 5 min. This step was repeated twice. After the last centrifugation step, the supernatant is decanted and 10 ml of S-medium is added, followed by centrifugation at 2500g for 5 minutes. Then the supernatant is decanted again and the remaining pellet is dissolved in 10 ml of S-medium, the worm solution is transferred to an Erlenmeyer flask and shaken on the shaker for 48h until L1 hatch from the eggs.

4.6.2 Reseeding

After the L1 larvae had hatched, L1 were transferred into three 96-well plates. First, the outer wells of each plate were filled with 90 µl ddH_2O as a diffusion barrier. Second, the worm solution was added to the wells and the worm density in the solution was adjusted to 10 worms/100 µl to achieve a worm density of 5-20 worms per well in the case of survival assay and lifespan assay. For the stress protection assay the worm density was adjusted to about 100 worms per 100 µl. 1,2 ml 100 mg/ml OP50 stock was added to 20 ml of the adjusted solution to achieve a final concentration of 6mg/ml OP50. After 90 µl of the solution were added to the inner wells of the plate, the plates were stored at 25°C, until the worms evolved to L3.

4.6.3 Sterilization

In order to keep the worm population age-synchronous, the worms had to be sterilized, which was done by adding 22.5 µl of a 0.6 mM 5-Fluorodeoxyuridine (FuDR) solution in each well. The plates were sealed using parafilm and gently shook for one minute on a microtiter plate shaker. In a last step, the plates were stored at 25°C in the incubator.

4.7 Extract screening – survival assay

Prior the experiment, the worms were chunked, synchronized, and reseeded into 96 well plates, labeled with date, strain and operator, as described above in 4.6.2. In order to keep the worm population age-synchronous, 22.5 µl 0.6 mM FuDR was added. Prior to the experiment, the worms were observed under the light microscope to ensure that they have reached the young adult stadium. To facilitate the subsequent sample application and to reach the respective final concentrations (f.c), the stock solutions of the samples were prediluted in a dilution series, as illustrated in Figure 6.

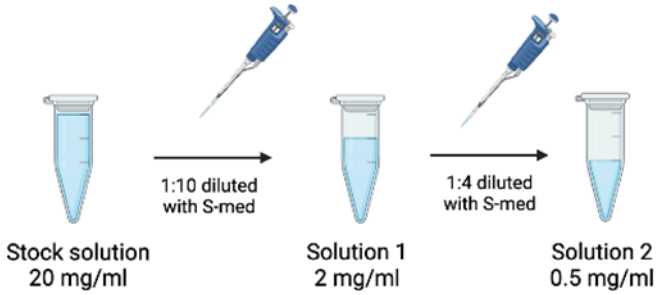

Figure 6: Schematic illustration of dilution series. The stock solution (SSo) had concentration of 20 mg/ml, solution extract dissolved in 70% DMSO. After diluting 1:10 with S-medium S1 had a concentration of 2 mg/ml extract in 7% DMSO. A further dilution step, by diluting S1 1:4 with 7% DMSO lead to the solution 2 (S2), having a concentration of 0.5 mg/ml in 7% DMSO.

12.5 µl of the prediluted samples were added to the wells, while the positive control quercetin (f.c. 200 µM) and the vehicle control DMSO (f.c. 0.7% DMSO) were added to six wells each. Extracts and NCs were added to three wells per condition. The extracts were tested in f.c. of 50 µg/ml and 200 µg/ml and the NCs were tested in f.c. between 10 and 200 µM. Afterwards, treated worms were incubated at 25°C for three days. To protect the worms from starving, 3.8 µl of 100 mg/mL OP50 solution was added to each well on the third day of adulthood. To secure sufficient oxygen supply, the plates were ventilated every second day. On the 11th day of adulthood, the survival rate was assessed by counting the number of live and dead worms under the light microscope. Worms were considered as dead if they did not show any movement, pharyngeal pumping or a reaction to a strong light stimulus. The raw data was tracked and processed in Microsoft Excel 2019, where the mean percentage of living nematodes compared to the total number of worms per condition was calculated for each plate. The mean survival rate values from each plate and condition were subsequently transferred to GraphPad PRISM 4.0 and presented in bar charts ± standard deviation (SD). The statistical significance was calculated using One Way ANOVA and Dunnett's post-test.

4.8 Motility assay

The worm motility was assessed by means of a semiautomatic IR based worm tracker "WMicrotracker one" by the company Phylumtech. Prior to the experiment, the worms were synchronized, reseeded into three 96 well plates and sterilized with 0.6 mM FuDR (f.c), as described above. On the first day of adulthood, worms were shaken on the microplate shaker and subsequently, the basal activity of the worms was measured in the worm tracker, "WMicroTracker". Following that, the samples in the respective

concentrations, positive control (quercetin f.c 200 µM) and vehicle control (f.c 0.7% DMSO) were added to the wells as described in 4.7. On third day of the experiment, 3.8 µl OP50 was fed to the worms. On the seventh day of adulthood, the motility was measured again.

The raw data was tracked and processed in Microsoft Excel 2019, where the mean worm motility of each treatment was calculated. The mean basal motility was set to 100% and the motility values from day seven were normalized to the basal activity value of the respective condition. The normalized data was graphically illustrated in bar charts ± SD using GraphPad PRISM 4.0. As a last step, the data was visually interpreted.

4.9 Lifespan assay

Prior to the assay, the worms were synchronized, reseeded into three 96 well plates and sterilized using 0.6 mM FuDR, as described above. Before the extracts were added, the worms were monitored under the light microscope to ensure their development to young adults. Thereafter, the total number of worms was assessed under the microscope. Dead worms in each well were documented and excluded in later calculations. Afterwards, samples in the respective concentrations, positive control (quercetin f.c 200 µM) and vehicle control (f.c 0.7% DMSO) were added to the wells as described in 4.7.. To protect the worms from starving, 3.8 µl of 100 mg/mL OP50 solution was added to each well on the third day of adulthood. To ensure sufficient oxygen supply, the plates were ventilated every second day. Over the course of the experiment, the number of live worms was counted three times a week.

The survival rate of the worms was recorded for each condition until only 30% of the worms were alive. For each point in time, raw survival data of each condition was recorded and processed with Microsoft Excel 2019: The mean survival rate of the worms from each treatment was computed separately for each plate and mean values were transferred to GraphPad PRISM 4.0. Survival curves for each plate were created by plotting the percentage of living worms per condition against the time. When only 50 % of the nematodes are alive, called death time 50% (DT_{50}) was calculated based on the survival curves. Mean DT_{50} were later determined using the survival curves of the three parallel experiments. The mean DT_{50} of the vehicle control was set to day 20 and DT_{50} values of each treatment were normalized to day

20. The deviation of lifespan was expressed as percentage of increase/decrease compared to the vehicle control. Statistical significance was calculated using One Way ANOVA and Dunnett's post-test.

4.10 Stress protection assay

4.10.1 H_2O_2 - concentration – finding

Prior to the implementation of the stress protection assay, a dose finding experiment was performed to find the best H_2O_2 concentration for stress induction. In preparation for the assay, the worms were chunked, synchronized, seeded into two 96 well plates and sterilized by adding 0.6mM FuDR as described in 4.6.3. On the first day of adulthood, the worms were treated with antioxidant ascorbic acid (f.c. 50 µM and 200 µM) and anthelmintic levamisole (f.c.10 µM) as a negative control or vehicle control DMSO 0.7% f.c. the worms were incubated alongside the samples for three days at 25°C. On the fourth day, the basal motility was recorded by means of the semiautomatic infrared-based worm tracker "WMicroTracker". Afterwards, H_2O_2 in final concentrations between 50 µM and 800 µM, was added. Figure 7 provides an overview of the sample distribution on the plate at the particular H_2O_2 concentrations. After H_2O_2 was added, using the WMicroTracker, the plates were alternately measured for 30 minutes once a day over the course of five days.

The obtained raw data was tracked and processed in Microsoft Excel 2019, where the mean worm motility of each treatment was calculated. In addition, mean basal activity was set to 100% and motility values from every measurement were normalized to the basal activity value of the respective condition. The normalized data, using GraphPad PRISM 4.0., was later graphically illustrated in bar charts ± SD and visually interpreted.

	A-B	1	2	3	4	5	6	7	8	9	10	11	12
H2O2 f.c	A												
800µM	B		Vit C 200 µM	Vit C 200 µM	Vit C 200 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Leva-misol 10 µM	Ctrl 0,7% DMSO	Blank (worms + dmso	
400 µM	C		Vit C 200 µM	Vit C 200 µM	Vit C 200 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Leva-misol 10 µM	Ctrl 0,7% DMSO	Blank (worms + dmso	
200 µM	D		Vit C 200 µM	Vit C 200 µM	Vit C 200 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Leva-misol 10 µM	Ctrl 0,7% DMSO	Blank (worms + dmso	
100 µM	E		Vit C 200 µM	Vit C 200 µM	Vit C 200 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Leva-misol 10 µM	Ctrl 0,7% DMSO	Blank (worms + dmso	
50 µM	F		Vit C 200 µM	Vit C 200 µM	Vit C 200 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Leva-misol 10 µM	Ctrl 0,7% DMSO	Blank (worms + dmso	
0 µM (Smed!!)	G		Vit C 200 µM	Vit C 200 µM	Vit C 200 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Vit C 50 µM	Leva-misol 10 µM	Ctrl 0,7% DMSO	Blank (worms + dmso	
	H												

Figure 7: Overview on the sample distribution. Schematic presentation of the distribution of samples and H_2O_2 in the given concentrations on a 96-well plate: Wells of each row were treated with different concentration of H_2O_2 for stress induction. Worms were incubated with ascorbic acid, levamisole in the given concentrations, 0,7% DMSO vehicle control and S-med (Blank).

4.10.2 Worm density & plate – type

Worm density

In a first step the worms were synchronized as described above. Age-synchronous L1 larvae were seeded into four 96 well plates with different densities between 25 - 200 worms per well. 10 wells were filled 25 and 200 worms and 20 wells were filled with 50 and 100 worms for each plate. The worms were then sterilized with 0.6 mM FuDR on the following day. After having developed to adult worms, the nematodes were treated with samples (vitamin C 200 µM), the negative control levamisole (f.c 20 µM) and the vehicle control DMSO (f.c 0.7%). Subsequently, the worms were incubated with the samples for three days at 25 °C. On the third day of adulthood the worm's basal motility was measured by means of the WMicroTracker. On day four H_2O_2 was added to the

worms at a final concentration of 2mM. Afterwards the motility of the worms was measured every two hours until the motility was close to zero.

The raw data was tracked and processed in Microsoft Excel 2019, where the mean worm motility of each treatment was calculated. In addition, the mean motility of the basal activity was set to 100% and motility values from every measurement were normalized to the basal activity value of the respective condition. The normalized data was transferred to GraphPad PRISM 4., graphically illustrated in bar charts ± SD and visually interpreted.

Plate – type

To find 96 well plates, which are most suitable for the worm tracker, two parallel experiments were performed using flat bottom and round bottom 96 well plates. For the experiment, the worms were synchronized and reseeded to two flat bottom and two round bottom 96 well plates in different worm densities between 25 – 200 worms per well. On each plate 10 wells were filled with 25 and 200 worms per well and 20 wells were filled with 50 and 100 worms per well. The worms were sterilized by adding 0.6 mM FuDR f.c. The following day, the young adult worms were treated with the samples (vitamin C 200 µM), the negative control (levamisole 20 µM) and the vehicle control 0.7% DMSO. This day is defined as day zero. The treated worms were then incubated at 25°C for three days. On the third day, basal motility was measured in the "WMicroTracker". On day four, H_2O_2 was added to the worms at a final concentration of 2 mM. Afterwards, the motility was measured every two hours until the motility was close to zero.

The raw data was tracked and processed in Microsoft Excel 2019, where the mean worm motility of each treatment was calculated. The mean basal motility was set to 100% and the motility values from every measurement were normalized to the basal activity value of the respective condition. The normalized data was later graphically illustrated in bar charts ± SD using GraphPad PRISM 4.0. Thereafter, the data was visually interpreted.

4.10.3 Heat shock Assay

The worms were chunked, synchronized, and reseeded into three flat bottom 96 well plates at a worm density of 100 worms/well. The next day, the worms were sterilized

by adding 0.6 mM FuDR and incubated for further 24 hours until the animals have grown into adults. Adult worms were treated with vitamin C in the following final concentrations: 50 µM, 100µM, 200 µM and 400 µM and the vehicle control. 0.7% DMSO. The worms were incubated at 25°C for three days, whereby on the third day of adulthood, the basal motility of the worms was measured in the worm tracker, “WMicroTracker”. Then, the two plates were stored in the incubator at 31.5°C ± 1.5 °C for 16 hours. The third plate was stored at 25°C as untreated control. After incubation, the motility of the worms in each plate was measured for 30 minutes, every two hours until motility was close to zero.

The raw data was tracked and processed in Microsoft Excel 2019: The mean worm motility of each treatment was computed and the mean motility of the basal activity was set to 100%. Motility values from each measurement were normalized to the basal activity value of the respective condition. Normalized data was graphically illustrated in motility curves by using GraphPad PRISM 4.0. The data was visually interpreted.

5 Results and Discussion

The aim of this study was to discover, whether different extracts, NCs and CBD formulations have a health promoting effect on *C. elegans* healthspan. Figure 8 provides an overview of the project workflow.

First, hydroethanolic extracts, NCs and CBD formulations, known for their ethnopharmacological use, were tested in the *C. elegans* based survival assay and motility assay. The survival assay provides first insights into the effects of the tested samples on the lifespan of the worms. However, it is less time consuming to perform compared to a lifespan assay and therefore allows a higher screening throughput. Nevertheless, since the goal was not only to find extracts that extend lifespan but also promote health, motility was observed as an additional health span parameter. The results of motility tracking were of importance in the selection of hit extracts. Hits from the survival assay were further tested in a *C. elegans* based lifespan assay to obtain more detailed information on the lifespan promoting effect. Furthermore, hits should be tested in a stress protection assay, to gain information on the antioxidant, stress-resisting properties of the samples. Prior to this assay of hit extracts, NCs and CBD formulations, optimizations of the protocol for the stress protection assay were conducted. Commonly used stress protection assays are based on time-consuming manual records of all death events under the dissecting microscope, which were assessed after treatment with either a thermal or a chemical stressor. Thereby, the workflow was meant to be facilitated and accelerated by the integration of a semi-automated, IR-based worm tracker. The integration of the “WMicroTracker” would also minimize the influence of the individual mode of operation. In order to set the best testing conditions for a smooth operation of the stress protection assay, parameters such as the ideally stressor, the best 96-well plate type, the right worm density had to be selected.

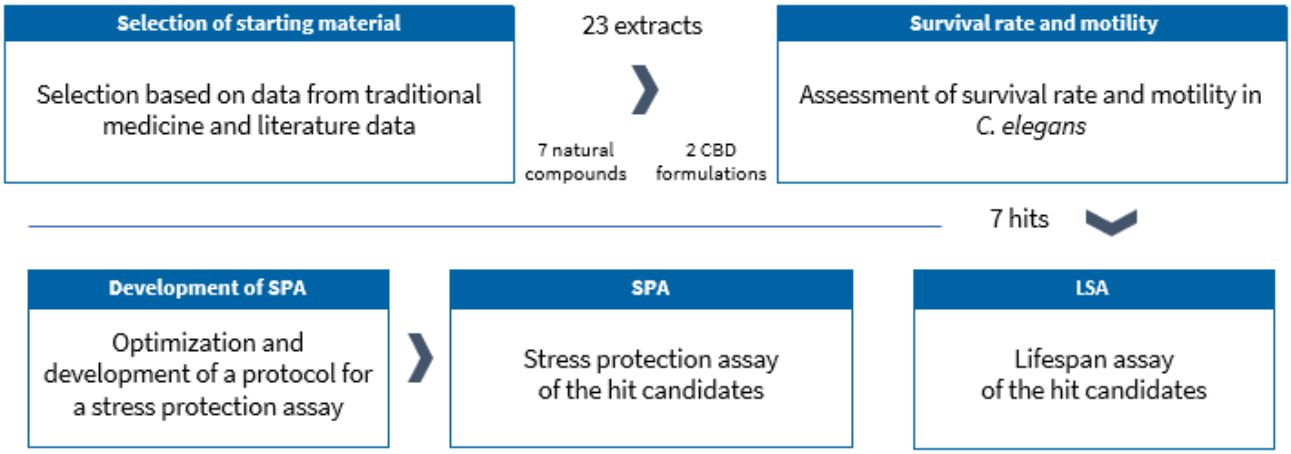

Figure 8: Workflow of the study. The study is building up on previous work of Mag.pharm. Martina Redl. 23 Extracts, seven NCs and two CBD formulations were tested in survival and motility assays. Seven extracts displayed a significant increase in the survival rate and showed promising health parameters, and where therefore selected as hit candidates. For further investigation hit candidates were tested for their effect on lifespan and their resilience in a stress protection assay (SPA), which required optimization and development of a protocol.

5.1 Development of stress protection Assay

ROS serve as signal molecules to regulate physiological and biological processes [107]. ROS are generated by various exogenous and endogenous processes, whereby an imbalance between antioxidant defense mechanisms and reactive oxygen species leads to the development of oxidative stress. The accumulation of cellular and molecular damage, caused by oxidative stress leads to a functional loss in old age. Therefore, oxidative stress plays a crucial role in the development of ageing associated diseases, such as cardiovascular and neurodegenerative diseases [108, 109].

C. elegans allows to investigate stress responses at the cellular and organismal level. Oxidative stress can be induced through a physical (thermal) or a chemical stressor [110]. Chemical stressors are prooxidative substances such as H_2O_2, juglone and paraquat while thermal stress can be induced by exposing worms to high temperatures [111–113]. In this study, the protective effects of the samples on stress exposed worms, were evaluated by measuring the motility of the worms in a semi-automatic IR-based worm tracker, “WMicroTracker”. For this reason, the methodology of the stress protection assay had to be adapted and testing parameters had to be determined in preliminary experiments.

1. Experiment

   In preparation for the assay, the worms were chunked, synchronized, seeded into two 96 well plates and sterilized by adding 0.6mM FuDR as described in 4.6. In the first experiment, oxidative stress should be induced by a chemical stressor H_2O_2. A literature search revealed different H_2O_2 concentrations from 0.6 mM to 5 mM for the induction of stress in liquid media [111–113]. Therefore, H_2O_2 was tested in final concentrations between 50 µM and 800 µM on 96 well plates, containing approximately 30 worms per well [114, 115]. However, H_2O_2 did not show any perceptible effects on the motility or the survival rate of the worms within five days in any concentration compared to the untreated worms. Thus, we assumed that the H_2O_2 concentration needs to be raised, to archive the desired effect and a final concentration of 2 mM H_2O_2 was determined as stressor for the next experiment.

2. Experiment

Besides finding the most suitable H_2O_2 concentration for chemical stress induction, it was necessary to determine at which worm density the worm tracker provides the most stable motility values, leading to the results that display the lowest standard deviation. Therefore, the worms were chunked, synchronized and reseeded into four 96-well plates at worm densities from 25 to 200 worms per well and sterilized by adding 0.6mM FuDR as described in4.6. Day after the worms were treated with either 200µM vitamin C (ascorbic acid) or 0.7% DMSO as vehicle control. After the worms had developed into adults, they were treated with 2 mM H_2O_2.

In order to determine, whether the shape of the bottom of the 96-well plates has an influence on results measured, the experiments were performed using two round bottom and two flat bottom 96 well plates.

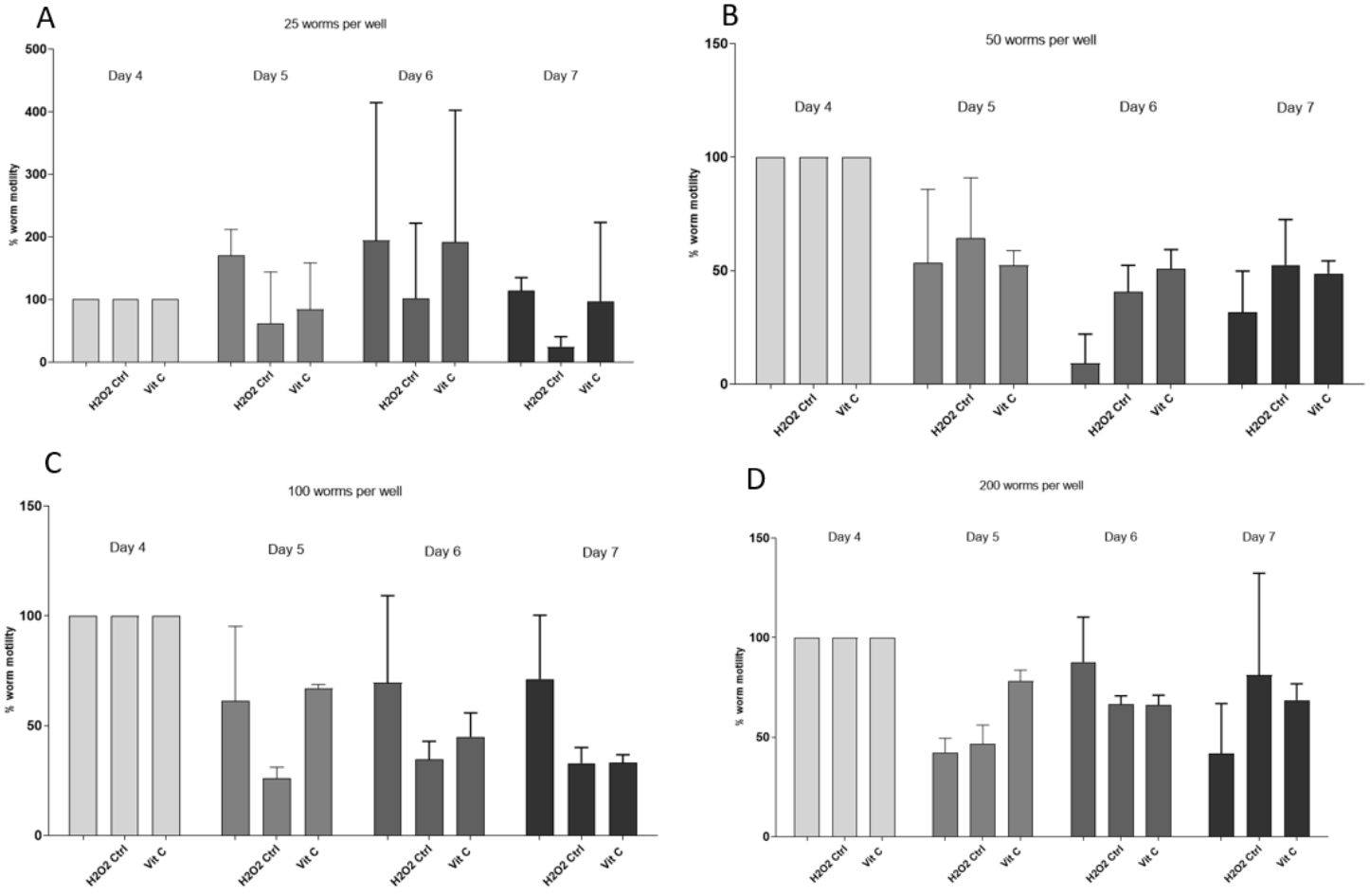

Figure 9 shows the mean motility of worms (vehicle containing 0.7% DMSO) with and without stress inducing H_2O_2 (2mM) and with and without pretreatment with vitamin C (200 µM f.c) on 96 well plates using a worm density of ~25 (A), ~50(B), ~100(C) and ~200(D) worms per well. Experiment was carried out in flat bottom 96 well plates. Bar charts represent the mean motility on day 5, 6 and 7 (age of worms after first day of adulty, corresponding to day 0, 1, 2, 3 after H_2O_2 treatment), of two parallel experiments, expressed as mean % of motility ± standard deviation (SD).

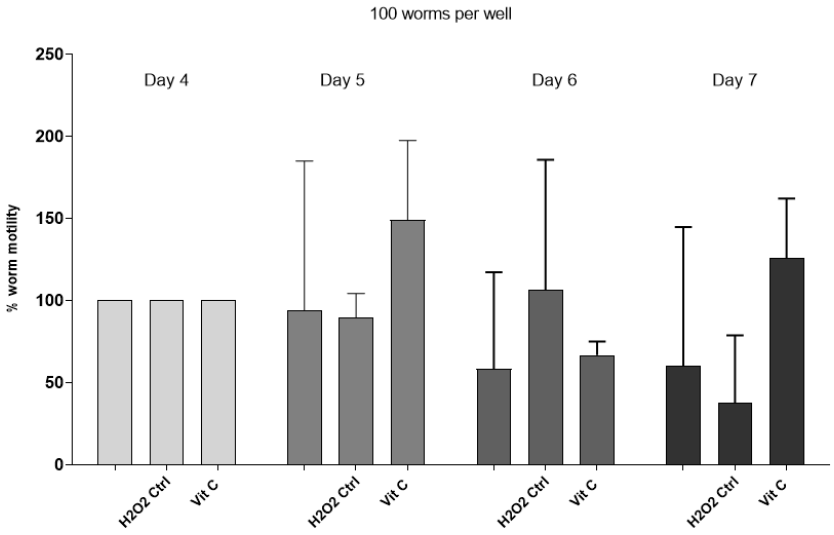

Figure 10 shows the mean motility of worms (vehicle containing 0.7% DMSO) with and without stress inducing H_2O_2 (2mM) and with and without pretreatment with vitamin C (200 µM f.c) on 96 well plates using a worm density of ~100 worms per well. Experiment was carried out in round bottom 96 well plates. Bar charts represent the mean motility on day 5, 6 and 7 (age of worms after first day of adulty, corresponding to day 0, 1, 2, 3 after H_2O_2 treatment), of two parallel experiments, expressed as mean % of motility ± standard deviation (SD).

The conducted evaluation indicated that motility values of worms treated in flat bottom plates (Figure 9) displayed a lower standard deviation compared to motility values of worms treated in round bottom plates (Figure 10). As for the worm density, a density of about 100 worms per well (Figure 9C) lead to the lowest standard deviation in the treated group. Based on these results a worm density of about 100 worms per well was selected for further stress protection experiments.

In the experiments in the flat bottom plates, treatment with H_2O_2 resulted in decreased motility in the worms, but not in the efficacy that would be necessary to obtain clear results. Results achieved in the round bottom plate experiments cannot be used for evaluation due to too high standard deviations. A possible reason for the delayed onset of the effect among all conditions might be that the concentration of H_2O_2 is still too low for the adult worms. Some experiments conducted by other researchers, showed however that after 24 hours significant effects were obtained using concentrations of 3 - 5mM [116]. Others however describe an LC_{50} of 50mM [111].

Ascorbic acid is known for its antioxidant effect, it was used as a treatment to determine the parameters that could influence the measurement with the worm tracker [117]. Vitamin C has proven to alleviate the effect of H_2O_2 on the worm motility on the first and second day (day 5 and day 6 of experiment) of the treatment, in comparison with

the H_2O_2 treated DMSO control group. However, the direct adding of H_2O_2 into the medium raises concerns on whether H_2O_2 was already captured in the medium by antioxidant test substances without affecting the worms. Since *E. coli* itself displays a catalase activity, it could affect the H_2O_2 as well. In order to circumvent this point of uncertainty, additional washing steps to remove samples and bacteria from the worms before adding the chemical stressor would have to be included in the workflow. Yet, further washing steps could complicate the assay and would require the establishment of a new protocol, as the worms could not be treated in 96 well plates but in Eppendorf tubes. Another way to avoid the aforementioned problem, would be to use a stressor, not affected by the presence of potential antioxidants. Hereby, thermal stress is induced by storing the worms in an elevated temperature over a prespecified timeframe. This method is independent from the reuptake of a stressor.

Our preliminary tests with heat shocks at 31.5 °C ± 1.5 °C over 16h lead to a phenotypic reduction in the motility of the worms. It will be important in further studies to test this stressor for robustness and validate its use. In further studies it must be verified whether the accumulation of ROS leads to the death of the worms or whether this is triggered by other factors. The formation of ROS and their associated damage are necessary to test the antioxidant capabilities of the extracts, NCs and CBD formulations. The use of ascorbic acid as a positive control must also be validated in further experiments, or if vitamin C proves to be unsuitable, another positive control must be found.

5.2 Survival and motility screening of natural products

19 Different plant extracts, four fungal extracts, seven natural compounds and two CBD formulations were tested in the *C. elegans* based survival assay and in the motility assay. Throughout the examination, the live worms were counted on the 11th day of adulthood whereas the motility was measured on the 7th day. The survival and the motility rates of the worms treated with the different samples were compared to the 0.7% DMSO vehicle control. The results were graphically illustrated using GraphPad PRISM 4.0. The statistical significance was calculated using One Way ANOVA and Dunnett's post-test. The following section contains a detailed description and graphical representation of the results from the survival screening. Additionally, motility graphs are shown for the samples that resulted in a significant increase in survival rate. Table 3 provides a tabular summary of the results of the survival and the motility assay.

Lycium barbarum Mill.

The hydroethanolic extract of *Lycium barbarum* cortex (SPr41) was tested in final concentrations of 50 µg/ml and 200 µg/ml in the *C. elegans* based survival assay. Worms were treated with 0.7% DMSO as vehicle control displayed a survival rate of 22.45% ± 10.58. Worms treated with SPr41 in both concentrations showed an increase in survival rate on day eleven, in comparison with the vehicle control. 200 µg/ml of SPr41 depicted a worm survival rate of 33.15% ± 7.89. In addition, extract concentrations of 50 µg/ml led to a survival rate of the worms of 32.02% ± 23.09. However, these effects were not statistically significant (Figure 11).

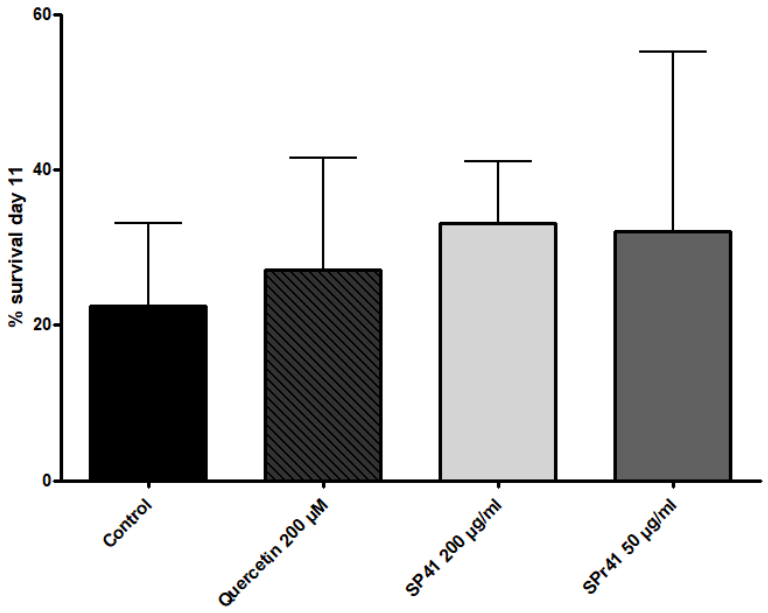

Figure 11: Effects of *Lycium barbarum* (SPr41) on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Lonicera japonica* Thunb.**

The effects of a hydroethanolic *Lonicera japonica* caulis (SPr42) extract on the survival rate of C. elegans were tested in final concentrations of 50 µg/ml and 200 µg/ml. In both concentrations the extract led to a significant increase in the survival rate of the worms compared with the DMSO 0.7% control ($p<0.01$), (Figure 12). Worms treated with the vehicle control exhibited a survival rate of 12.04% ± 0.59. The survival rate of worms which were treated with 200 µg/ml amounted to 23.90% ± 4.80 and the survival rate of the worms tested with 50 µg/ml SPr42 was 23.50% ± 2.79. On the 7th day of the experiment, the motility of the worms was measured using the worm tracker. Results presented in Figure 13 show an increased motility of the worms treated with SPr42 in both concentrations.

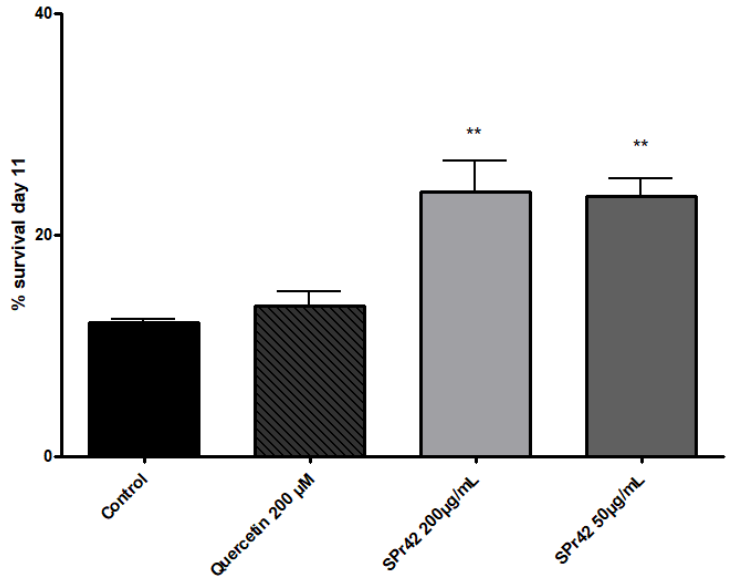

Figure 12: Effects of *Lonicera japonica* (SPr42) on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

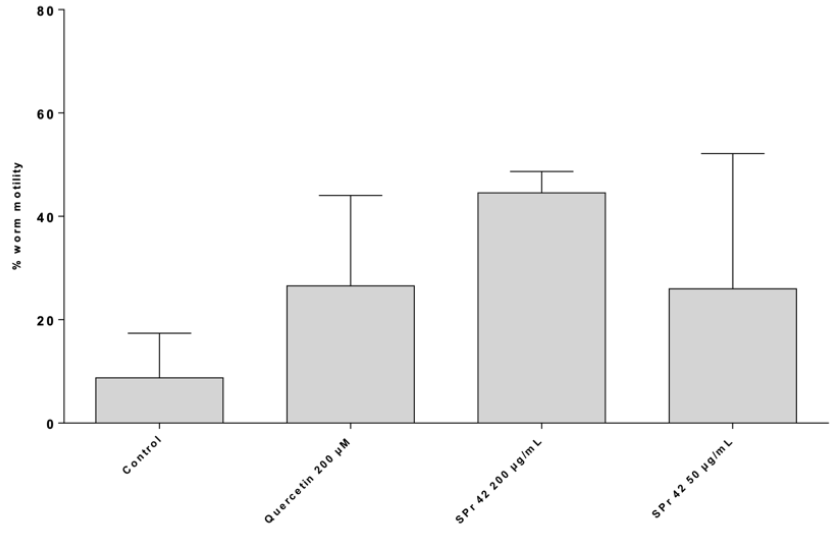

Figure 13: Motility of worms treated with *Lonicera japonica* extract. The bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Panax ginseng C.A.MEY.

The hydroethanolic extract of *Panax ginseng* radix (SPr43) was tested in final concentrations of 50 µg/ml and 200 µg/ml. Hereby, worms treated with 0.7% DMSO vehicle control exhibited a survival rate of 24.62% ± 5.64. Treatment of the worms with SP43, compared to the vehicle control, led to an increased survival rate in a dose dependent manner, on day 11 in both concentrations: concentrations of 200 µg/ml

SP43 led to a worm survival rate of 41.41% ± 10.93 and concentrations of 50 µg/ml led to a survival rate of the worms of 29.86% ± 15.56. Calculations with One Way ANOVA and Dunnett's post-tes thowever did not reveale statisitcal significance of the effect (Figure 14).

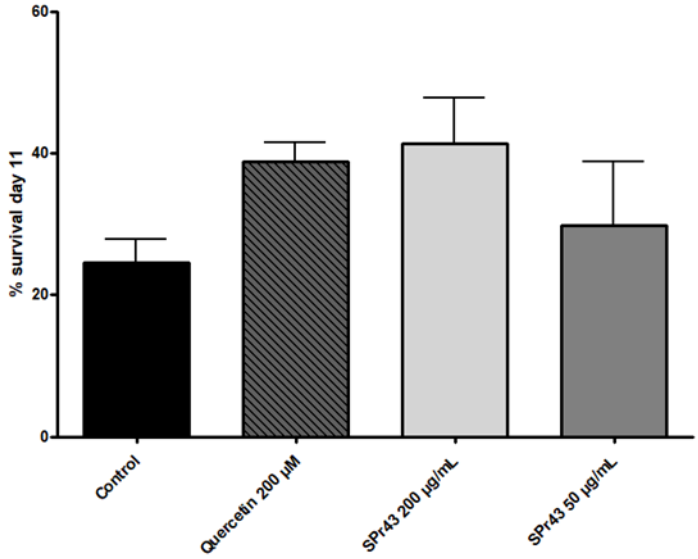

Figure 14: Effects of *Panax ginseng* (SPr43) extract on the survival rate of adult N2 wildtype worms in the given concentrations. The bar charts represent the mean survival rate on day 11 of adulthood, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Punica granatum* L.**

The effects of a treatment of *C. elegans* with an hydroethanolic *Punica granatum* semen (SPr45) extract on the survival rate in final concentrations of 50 and 200 µg/ml were evaluated. SP45 did result in an increased survival rate of the worms in both tested concentrations compared with the vehicle group, which displayed a survival rate of 24.62% ± 5.64. The effect of SPr45 on *C. elegans* resulted to be dose dependent. Thus, 200 µg/ml of the extract exhibited a survival rate of the worms of 41.14% ± 16.34, while 50 µg/ml led to a survival rate of the worms of 31.57% ± 16.42. These effects again were not statistically significant (Figure 15).

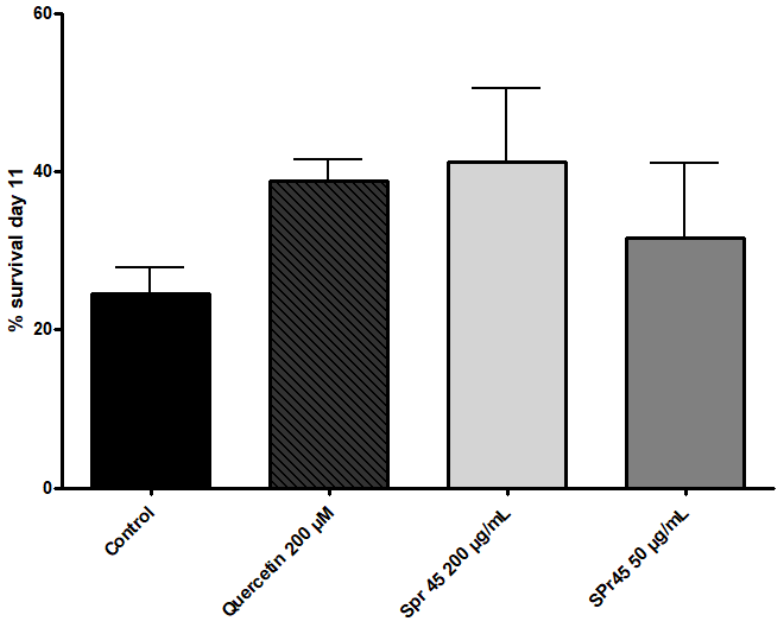

Figure 15: Effects of *Punica granatum* (SPr45) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Panax notoginseng* (Burkill) F.H.Chen ex C.Y.Wu & K.M.Feng**

The hydroethanolic extract of *Panax notoginseng* radix (SPr46) was tested in final concentrations of 50 µg/ml and 200 µg/ml. 200 µg/ml SPr46 led to an enhanced survival rate of the worms (37.01% ± 20.64), compared to the vehicle control (22.45% ± 10.58). Concentrations of 50 µg/ml however exhibited a survival rate of the worms of 18.67% ± 11.58, which is slightly below the survival rate of the vehicle group. Although we could see a dose dependency effect on the survival rate of the worms nevertheless, these effects were not statistically significant (Figure 17).

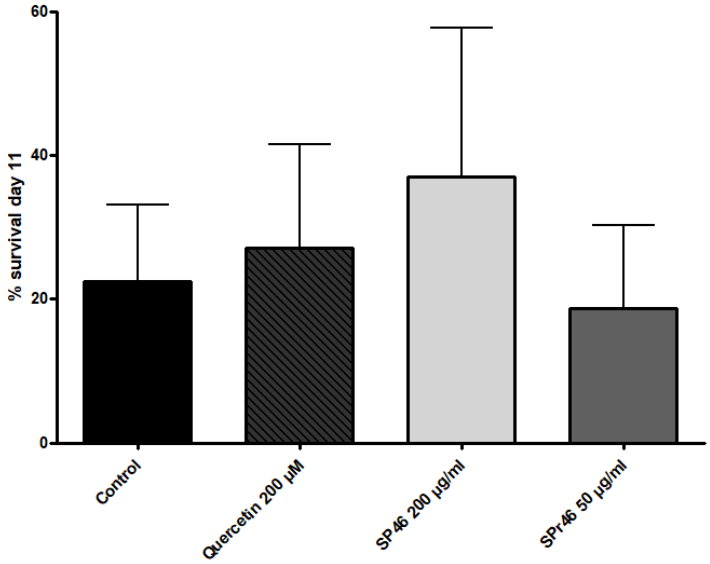

Figure 17: Effects of *Panax notoginseng* (SPr46) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

Astragalus mongholicus **(Bunge) and** ***Astragalus membranaceus*** **Fisch. Ex Bunge**

The hydroethanolic extract of *Astragalus mongholicus* and *Astragalus membranaceus* radix (SPr47) was tested in final concentrations of 50 and 200 µg/ml. Treatment with SPr47 in both concentrations led to an enhanced survival rate of the worms in comparison with the vehicle group (22.45% ± 10.58). 200 µg/ml of the extract displayed a worm survival rate of 50.14% ± 10.29 and 50 µg/ml led to a survival rate of the worms of 37.40% ± 4.07. The aforementioned effects were dose related, however not statistically significant (Figure 18).

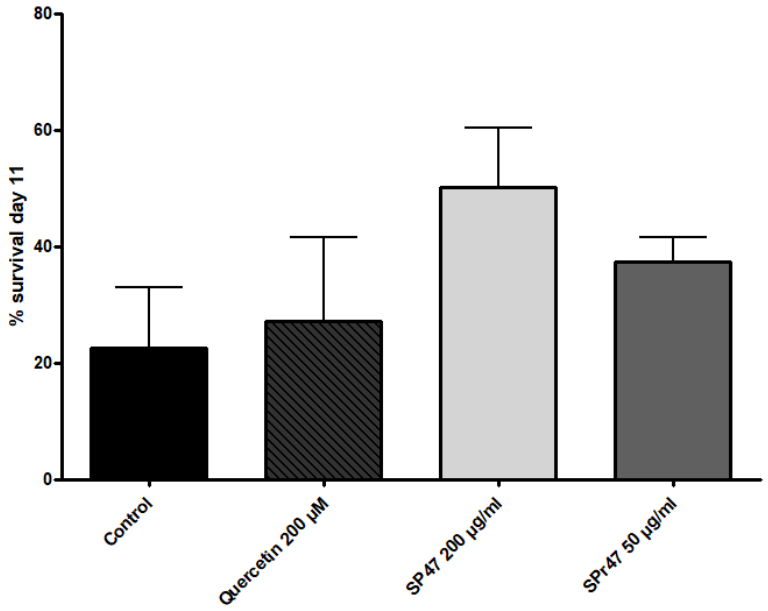

Figure 18: Effects of Astragalus mongholicus and Astragalus membranaceus (SPr47) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

*Angelica sinensis (*Oliv.) Diels

The survival rate of the worms treated with a hydroethanolic extract of *Angelica sinensis* radix (SPr48) was tested in 50 µg/ml and 200 µg/ml final concentrations. 200 µg/ml of the extract led to a significant increase in survival rate of the worms, compared to the DMSO 0.7% control (12.04% ± 0.59) ($p<0.01$) (Figure 19). The extract of *Angelica sinensis* shows a potent dose dependent effect. A final concentration of 200 µg/ml SPr48 caused a survival rate of the worms of 35.62% ± 10.36 while 50 µg/ml SPr48 led to a survival rate of 18.77% ± 6.25, which was elevated in comparison with the vehicle control group, however not statistically significant. In accordance with the survival data, motility measurement on the 7th day of the experiment revealed an increased motility of the worms which were treated with 200 µg/ml of SPr48. Figure 20 illustrates the results of the motility assay.

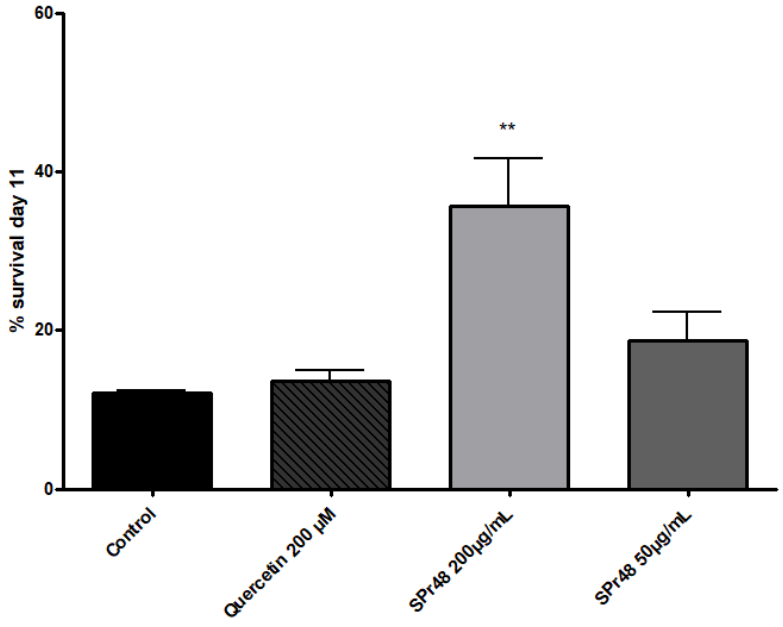

Figure 19: Effects of *Angelica sinensis* (SPr48) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

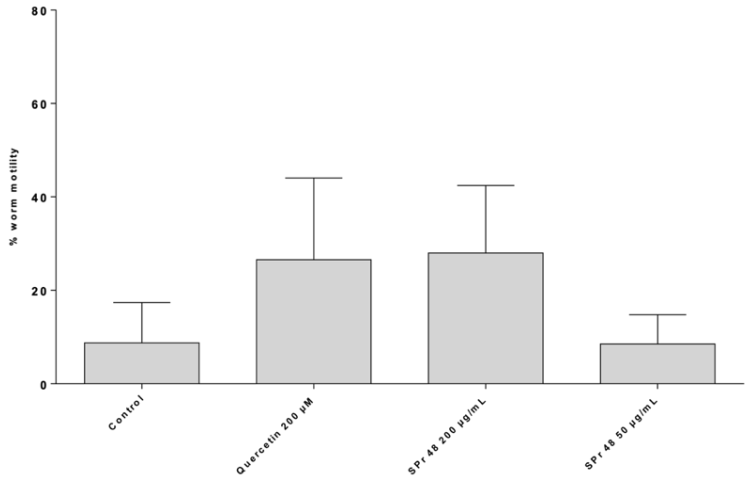

Figure 20: Motility of worms treated with Angelica *sinensis* extract. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Withania somnifera (L.) Dunal

The hydroethanolic extract of *Withania somnifera* radix (SPr49) was tested in the final concentrations of 50 µg/ml and 200 µg/ml (Figure 21). Worms treated with the vehicle control exhibited a survival rate of 24.62% ± 5.63. Treatment with SPr49 in both concentrations enhanced the survival rate of the worms in a dose-dependent manner, compared to the vehicle control. 200 µg/ml resulted in a worm survival rate of 33.38% ± 9.93. Within the lower concentration on the other hand a survival rate of the worms of 25.15% ± 9.01 could be observed. However, calculations with One Way ANOVA and Dunnett's post-test revealed no statisitcal significance of the effect.

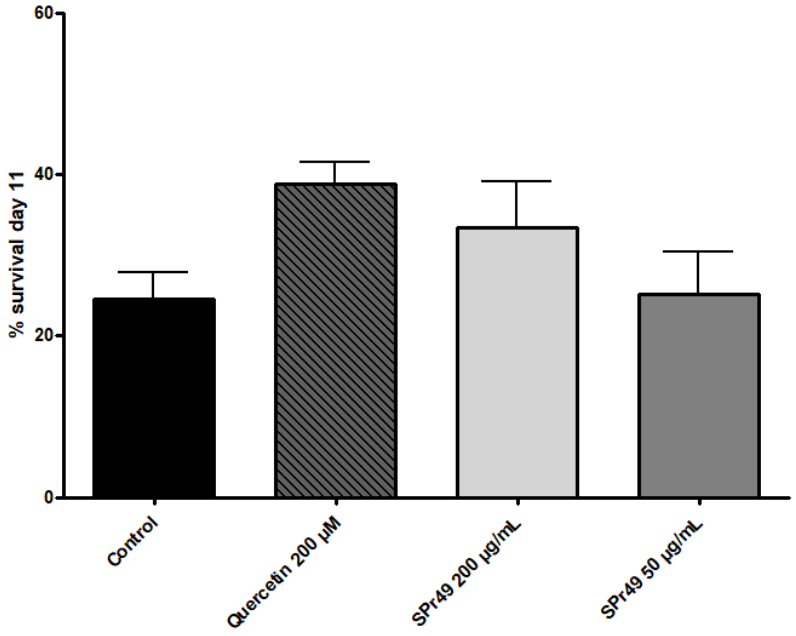

Figure 21. Effects of *Withania somnifera* (SPr49) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

Centella asiatica (L.) Urb.

The hydroethanolic extract of *Centella asiatica* herba (SPr51) was tested in final concentrations of 50 µg/ml and 200 µg/ml (Figure 22). SPr51 in 200 µg/ml concentration led to an enhanced survival rate of the worms of 33.37% ± 13.75,

compared to a survival rate of the worms of 24.62% ± 5.64 in the vehicle group. In the final concentration of 50 µg/ml the survival rate of the worms decreased compared to the vehicle control, reaching a value of 12.75% ± 10.03. The effect of SPr51 changed dose dependently. However, the effects were not statistically significant.

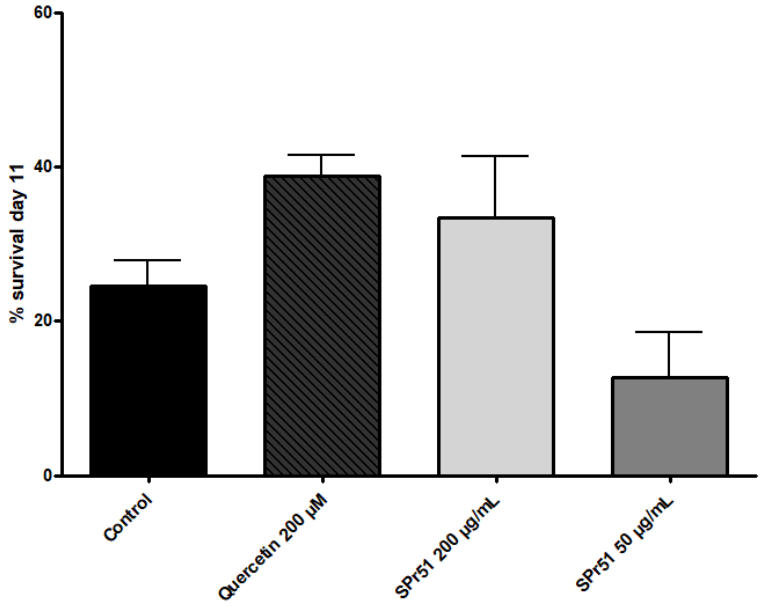

Figure 22. Effects of *Centella asiatica* (SPr51) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Convolvulus prostrates* Forssk.**

The hydroethanolic extract of *Convolvulus prostrates* herba (SPr52) was tested in the final concentrations of 50 µg/ml and 200 µg/ml (Figure 23). Worms treated with the vehicle control exhibited a survival rate of 24.62% ± 5.64. Conversely, 200 µg/ml of the extract led to an enhanced survival rate of 26.57% ± 3.18 compared with the control group. In worms which were treated with 50 µg/ml of the extract a slightly declined survival rate compared with the vehicle group could be observed reaching a value of 22.45% ± 17.25. The effects measured were not of statistical significance.

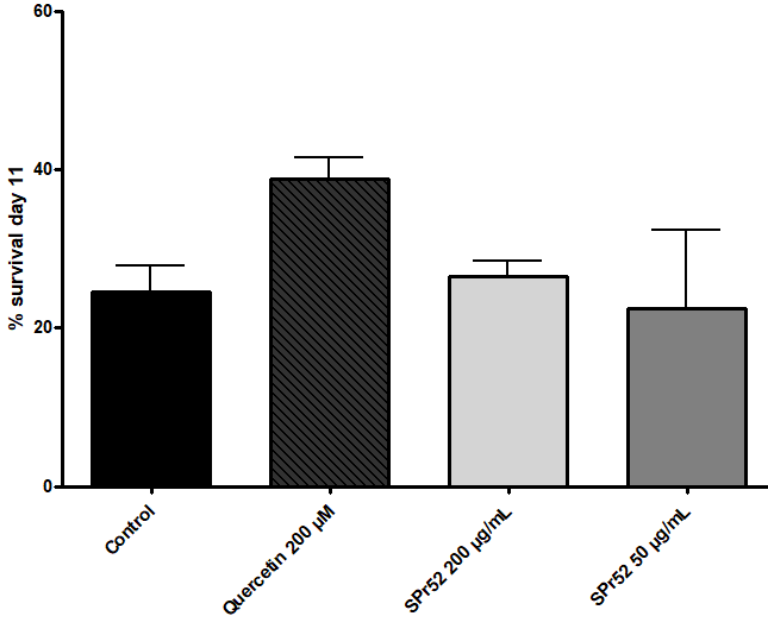

Figure 23. Effects of *Convolvulus prostrate*s (SPr 52) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Passiflora incarnata* L.**

The hydroethanolic *Passiflora incarnata* herba (SPr53) extract was tested in final concentrations of 50 µg/ml and 200 µg/ml (Figure 24). The SPr53 treatment caused an increased survival rate of the worms in both concentrations, in a dose dependent manner, compared to the DMSO vehicle control (22.45% ± 10.58). Worms, which were treated with 200 µg/ml of the extract exhibited a survival rate of 40.40% ± 19.76, 50 µg/ml SPr53 led to a survival rate of 30.90% ± 6.92. However, calculations with One Way ANOVA and Dunnett's post-test revealed no statisitcal significance of the effect.

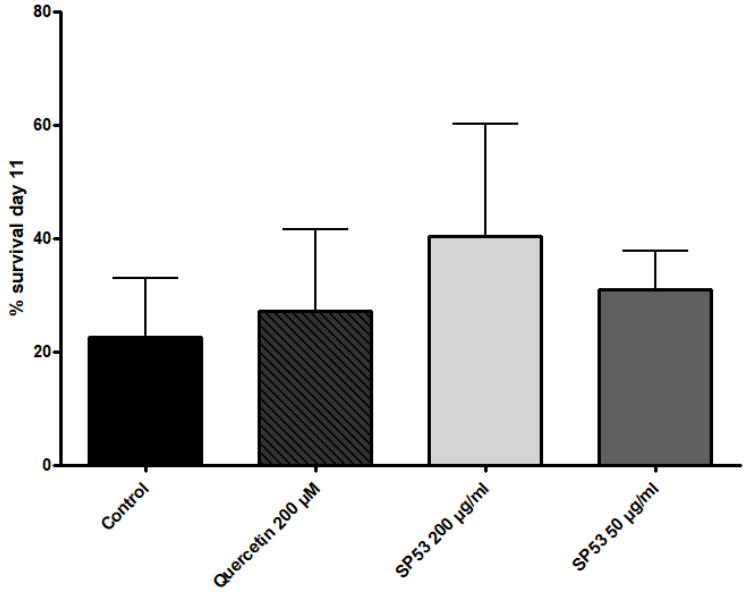

Figure 24: Effects of *Passiflora incarnata* (SPr53) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Salvia officinalis* L.**

The hydroethanolic extract of *Salvia officinalis* folium (SPr54) was tested in final concentrations of 50 µg/ml and 200 µg/ml in the *C. elegans* based survival assay (Figure 25). The worms hereby treated with the vehicle control of 0.7% DMSO displayed a survival rate of 22.45% ± 10.58. Concentrations of 200 µg/ml did however contribute to a slightly enhanced survival rate, compared to the vehicle group, reaching a value of 28.88% ± 15.55. The final concentration of 50 µg/ml exhibited a survival rate of the worms of 19.76% ± 12.84. The effects change in a dose-dependent manner. However, the results did not show any statisitcal significance.

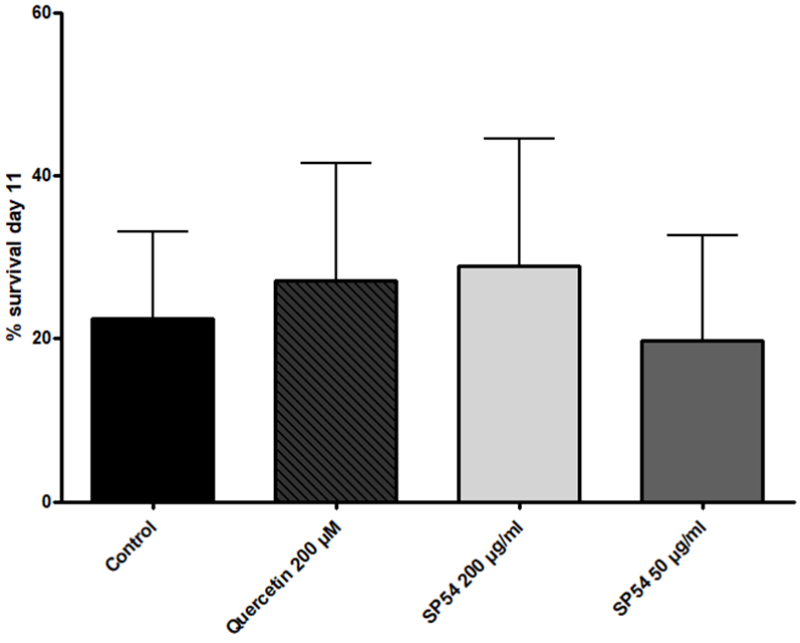

Figure 25: Effects of *Salvia officinalis* (SPr54) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Cynara scolymus* L.**

Final concentrations of 50 µg/ml and 200 µg/ml of the hydroethanolic extract of *Cynara scolymus* folium (SPr55) were tested in the *C. elegans* based survival assay (Figure 26). Worms which were treated with the vehicle control displayed a survival rate of 24.62% ± 5.64, while 200 µg/ml and 50 µg/ml of SPr55 enhanced the survival rate of the group to 33.53% ± 11.05 and 26.03% ± 4.15, respectively. Despite the visibly dose-dependently alleviating effect of SPr55 on the survival rate, the effects were not statistically significant.

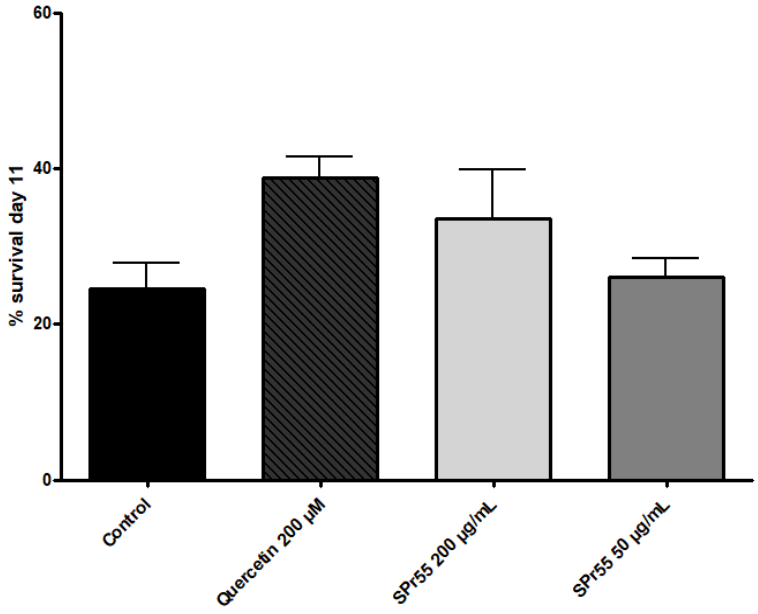

Figure 26. Effects of *Cynara scolymus* (SPr55) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Scutellaria barbata* D. Don.**

The hydroethanolic extract of *Scutellaria barbata* herba (SPr56) was tested in concentrations of 50 µg/ml and 200 µg/ml. In both concentrations, the extract led to a highly significant increase in the worm's survival rate, compared to the DMSO 0.7% control group ($p<0.01$) (Figure 28). Worms treated with the DMSO vehicle control had a survival rate of 12.04% ± 0.59, while worms treated with 200 µg/ml and 50 µg/ml displayed survival values of 57.85% ± 5.50 and 43.37% ± 4.31, respectively. *Scutellaria barbata* herba extract shows a potent dose dependent effect on the survival rate of the worms. Motility was measured on the 7th day of the experiment using the worm tracker. Figure 27, graphically presents the increased motility of the worms treated with SPr56 in both concentrations again in comparison with the DMSO vehicle control.

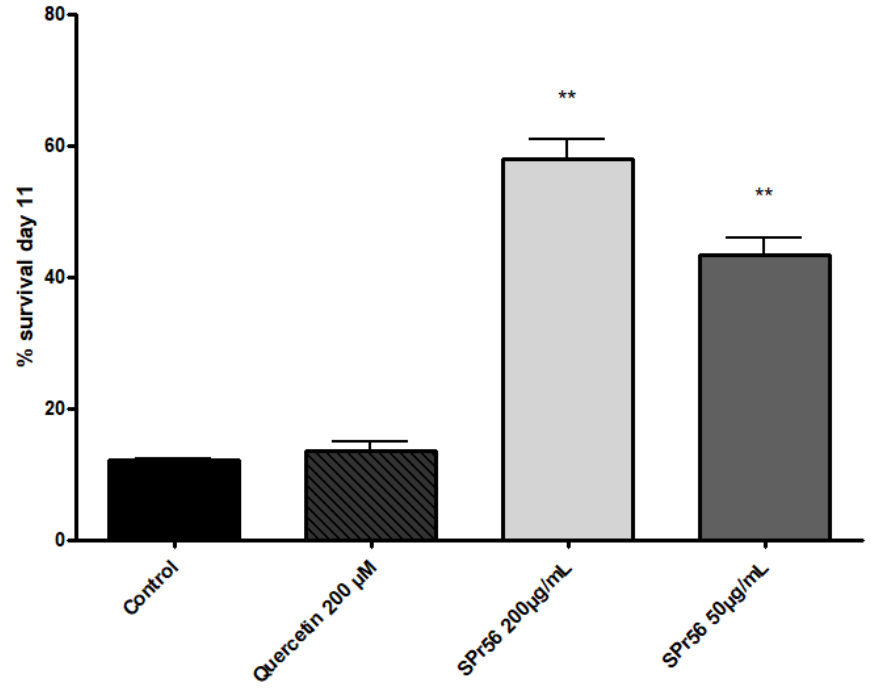

Figure 28. Effects of *Scutellaria barbata* (SPr56) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

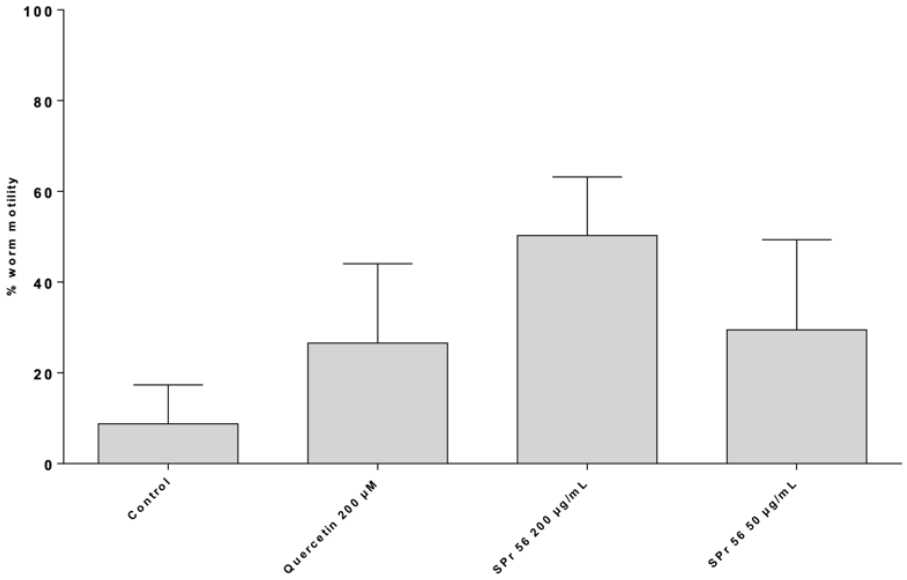

Figure 27: Motility of worms treated with *Scutellaria barbata* extracts. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Gynostemma pentaphyllum (Thunb.) Makino

The survival rate of *C. elegans* treated with a hydroethanolic extract of *Gynostemma pentaphyllum* herba (SPr57) was tested in the following final concentrations: 50 µg/ml and 200 µg/ml (Figure 29). In comparison with the vehicle control (12.04% ± 0.59) SPr57 proves to have decreasing effects on the survival rate of the worms in both concentrations. The survival rate of those worms which were treated with 200 µg/ml SPr57 amounted to 8.05% ± 3.14. Worms which were treated with 50 µg/ml on the other hand, exhibited a survival rate of 8.79% ± 2.67. The observed effects were not statistically significant.

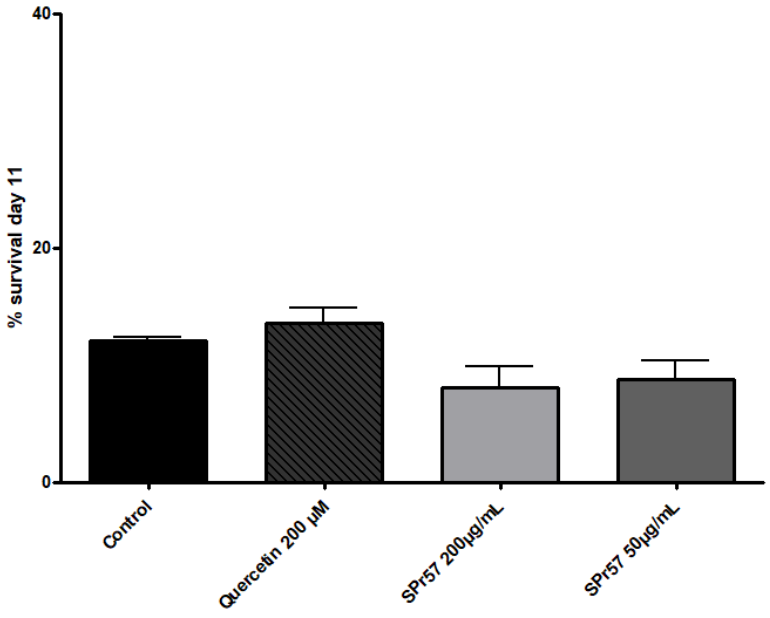

Figure 29. Effects of *Gynostemma pentaphyllum* (SPr57) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

Cistus creticus L.

The survival rate of *C. elegans* treated with a hydroethanolic extract of *Cistus creticus* herba (SPr58) was tested in final concentrations of 50 µg/ml and 200 µg/ml (Figure 30). 200 µg/ml SPr58 had a strongly attenuating effect on the survival rate (12.31% ± 10.71) while 50 µg/ml only slightly affected the survival rate of the worms (25.91% ± 13.99) compared to the vehicle control (24.62% ± 5.64). The effects on the survival rate of the worms changed in a revers dose dependent manner. Despite the visible

decreasing effect of SPr58 in the concentration of 200 µg/ml on the survival rate of the worms, the effects were not statistically significant.

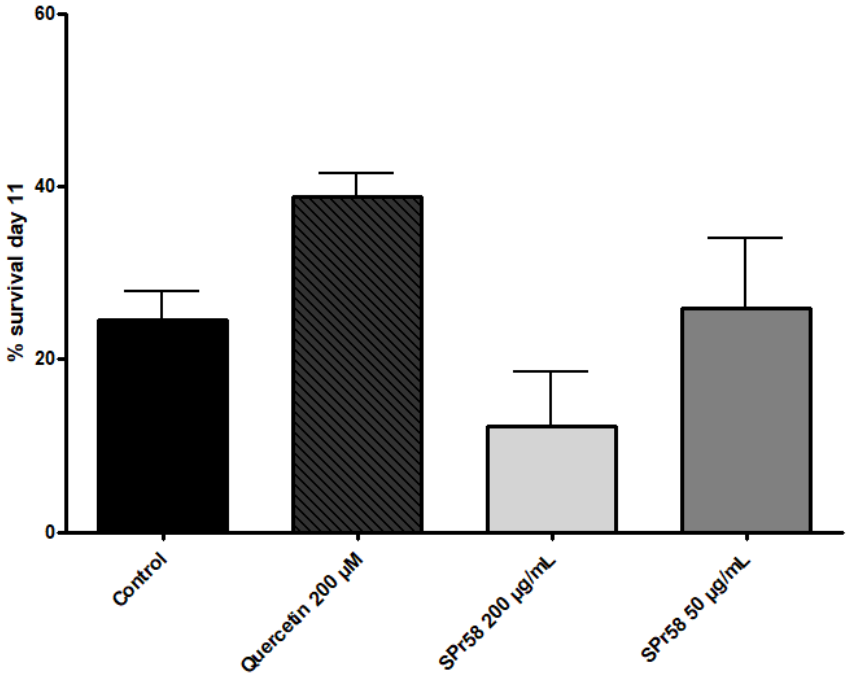

Figure 30: Effects of *Cistus criticus* (SPr58) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Ranunculus ternatus* Thunb.**

The hydroethanolic extract of *Ranunculus ternatus* radix (SPr59) was tested in final concentrations of 50 µg/ml and 200 µg/ml (Figure 31). SPr59 hardly affected the survival rate of the worms in both concentrations comparing the data attained to the vehicle control (12.04% ± 0.59). A concentration of 200 µg/ml SPr59 led to a survival rate of 11.96% ± 4.03 and 50 µg/ml exhibited a worm survival rate of 10.58% ± 3.60. Thus follows that calculations with One Way ANOVA and Dunnett's post-test revealed no statisitcal significance of the effects.

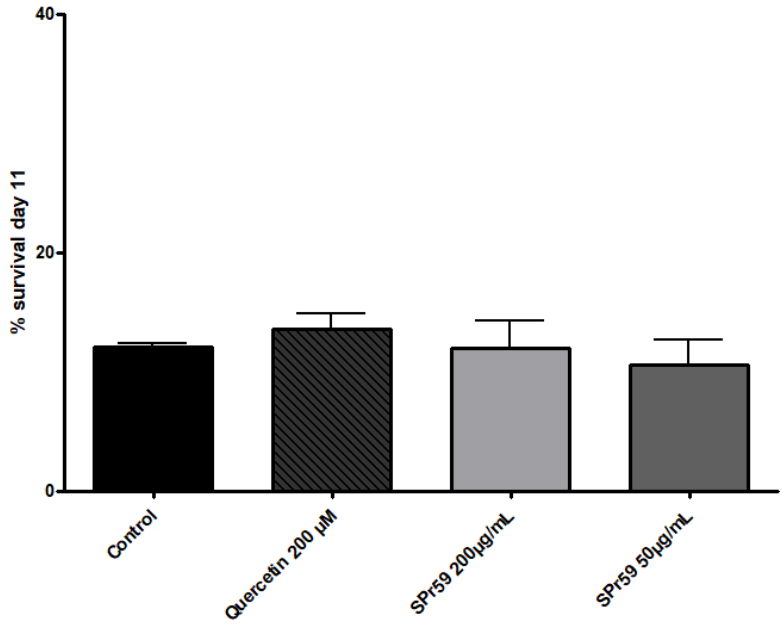

Figure 31: Effects of *Ranunculus ternatus* (SPr59) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Polygala tenuifolia* Willd.**

The effects on the survival rate of *C. elegans* treated with a hydroethanolic extract of *Polygala tenuifolia* radix (SPr60) were tested. In final concentrations of 200 µg/ml and 50 µg/ml (Figure 32). Worms which were treated with 0.7% DMSO showed a survival rate of 12.04% ± 0.59. 200 µg/ml of SPr60 slightly affected the survival rate of the worms compared to the vehicle group (11.55% ± 2.45) and 50 µg/ml of the extract led to a slight decrease in survival rate of the worms (9.06% ± 4.32), comparing the results measured to the 0.7% DMSO group. These effects, however, were not statistically significant.

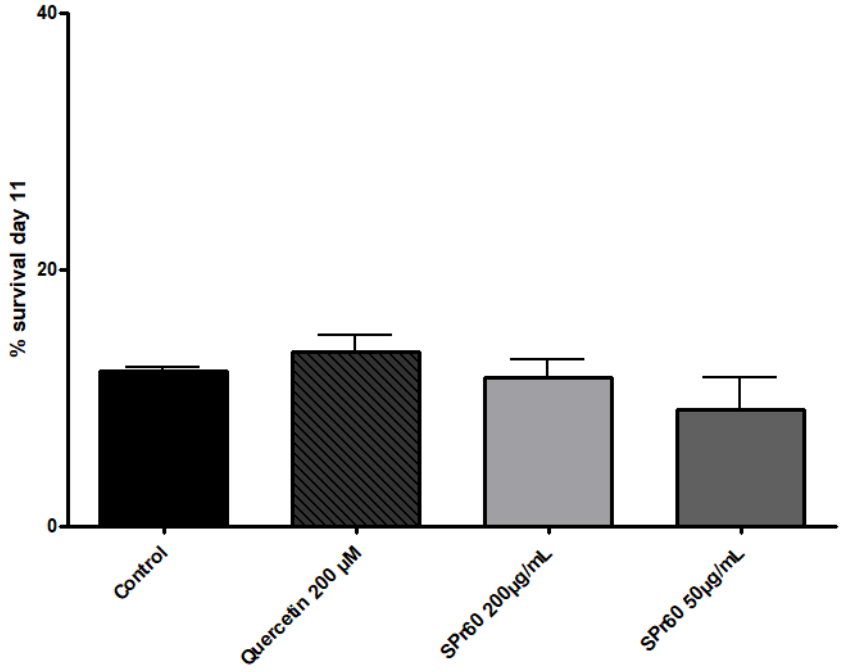

Figure 32: Effects of Polygala tenuifolia (SPr60) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Fomitopsis betulina* (Bull.) B. K. Cui, M. L. Han & Y. C. Dai**

Final concentrations of 50 µg/ml and 200 µg/ml of the hydroethanolic extract of *Fomitopsis betulina* karposoma (SPr61) were tested in the *C. elegans* based survival assay. Results indicated a significantly decreasing effect of SPr61 in 200µg/ml on the worm survival rate in comparison with the 0.7% DMSO vehicle control ($p<0.01$) (Figure 33). Worms which were treated with 0.7 % DMSO exhibited a survival rate of 24.60% ± 0.79, while 200 µg/ml of SPr61 tremendously decreased the survival rate of the worms of 1.483% ± 1.40. The decreasing effect of SPr61 on the survival rate could also be observed by worms, treated with 50 µg/ml extract (17.18% ± 9.94), however without statistical significance. The nematotoxic effect of the extract shows a dose-dependent relationship. In accordance with the survival data, motility measurement on the 7th day of the experiment revealed no motility of the worms in the final concentration of 200 µg/ml and a decreased motility of the worms in the final concentration of 50 µg/ml of *Fomitopsis betulina* as illustrated in Figure 34.

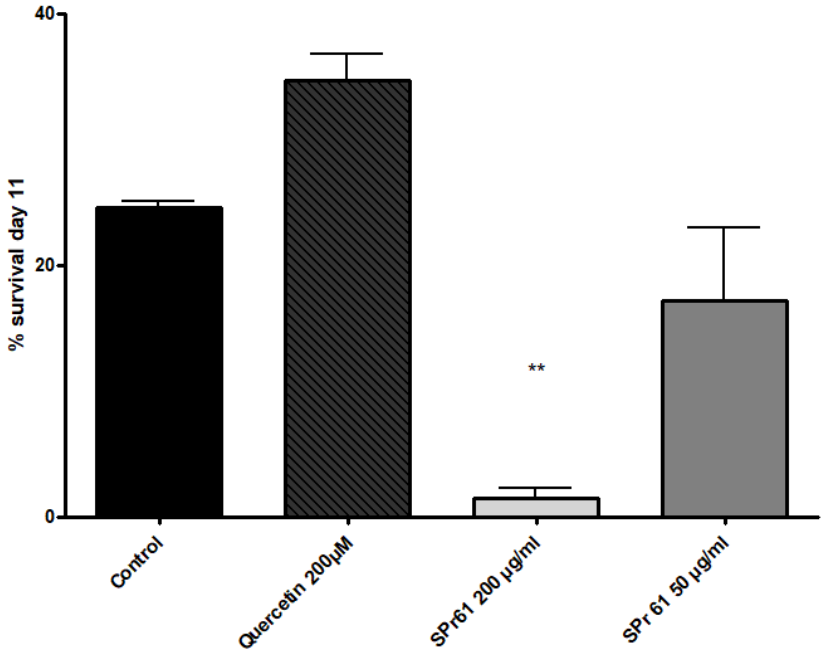

Figure 33: Effects of *Fomitopsis betulina* (SPr61) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

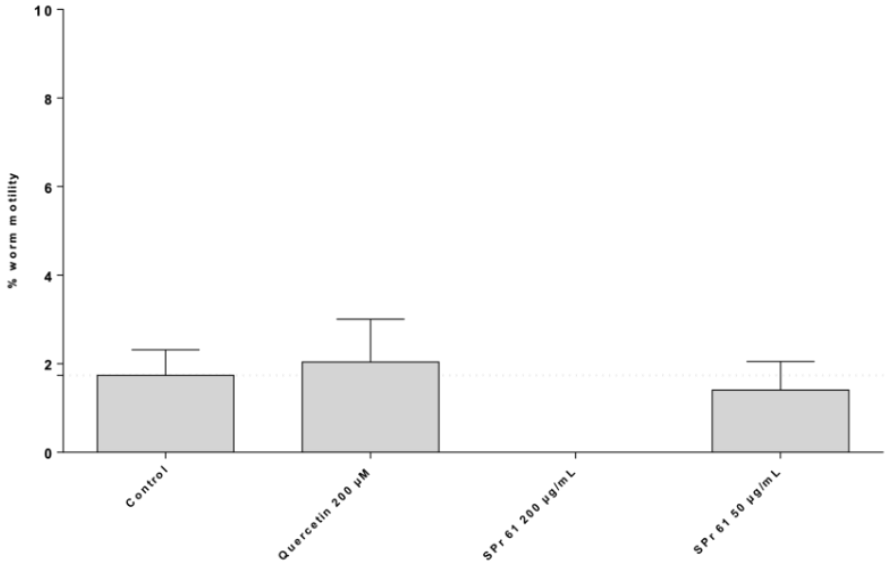

Figure 34: Motility of worms treated with *Fomitopsis betulina* extract. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

***Ganoderma lucidum* (Curtis) P. Karst.**

The hydroethanolic extract of *Ganoderma lucidum* karposoma (SPr62) was tested in final concentrations of 50 µg/ml and 200 µg/ml. Both concentrations of SPr62 had a significantly increasing effect on the survival rate of the worms in comparison with the DMSO 0.7% vehicle control ($p<0.01$) (Figure 35). Worms treated with 0.7% DMSO exhibited a survival rate of 24.60% ± 0.79. The survival rate of the worms treated with 200 µg/ml SPr69 was 69.44% ± 3.32. The worms which were treated with the extract of *Ganoderma lucidum* exhibited a survival rate of 57.59% ± 3.47 in the final concentration of 50 µg/ml. The effect of *Ganoderma lucidum* extract on survival rate of the worms in concentrations 50 µg/ml and 200 µg/ml changed in a dose-dependent manner. The motility of the worms was measured on the 7th day of the experiment using the worm tracker. Motility values as illustrated in Figure 36 were in both concentrations increased compared with the vehicle control.

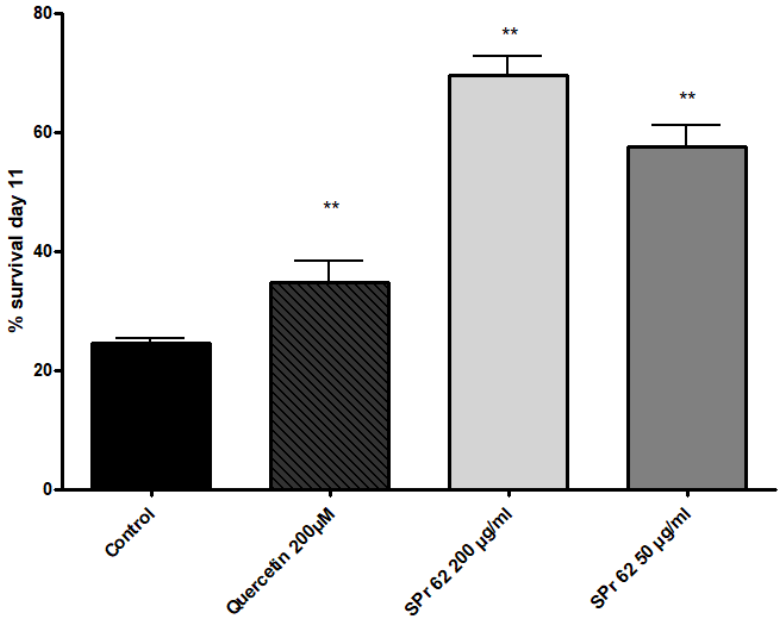

Figure 35: Effects of *Ganoderma lucidum* (SPr62) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

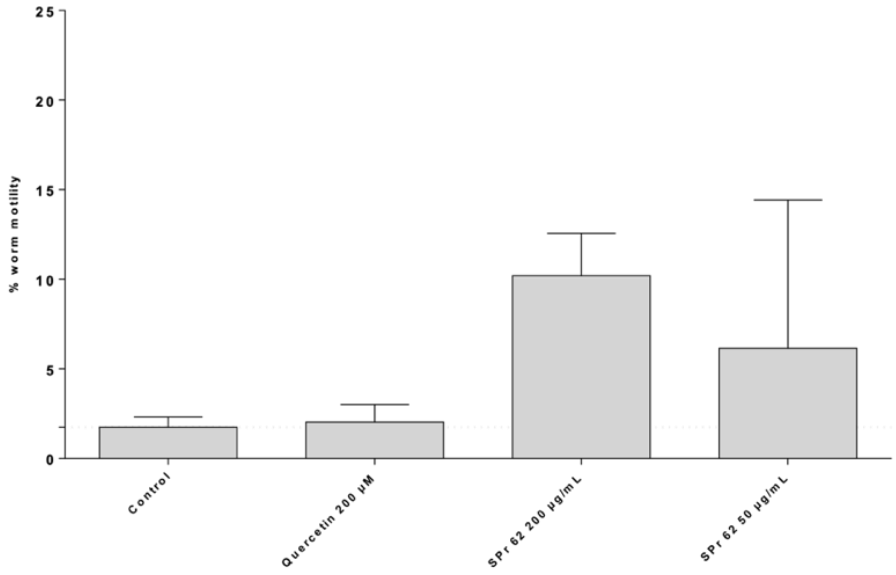

Figure 36: Motility of worms treated with *Ganoderma lucidum* extract. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Lignosus rhinocerus (*Cooke) Ryvarden*:

Final concentrations of 50 µg/ml and 200 µg/ml of the hydroethanolic extract of *Lignosus rhinocerus* sclerotium (SPr63) were tested. In both concentrations, the extract led to a significant increase in survival rate of the worms comparing the results to the DMSO 0.7% vehicle control ($p<0.01$) (Figure 37). Worms, which were treated with 0.7% DMSO exhibited a survival rate of 24.60% ± 0.79, while 200 µg/ml and 50 µg/ml SPr63 enhanced the survival rate dose-dependently to a value of 58.70% ± 2.90 and 43.90% ± 9.92, respectively. In accordance with the survival data, motility measurement on the 7th day of the experiment revealed an increase in the motility of the worms treated with SPr63 in both concentrations compared to the control group (Figure 38).

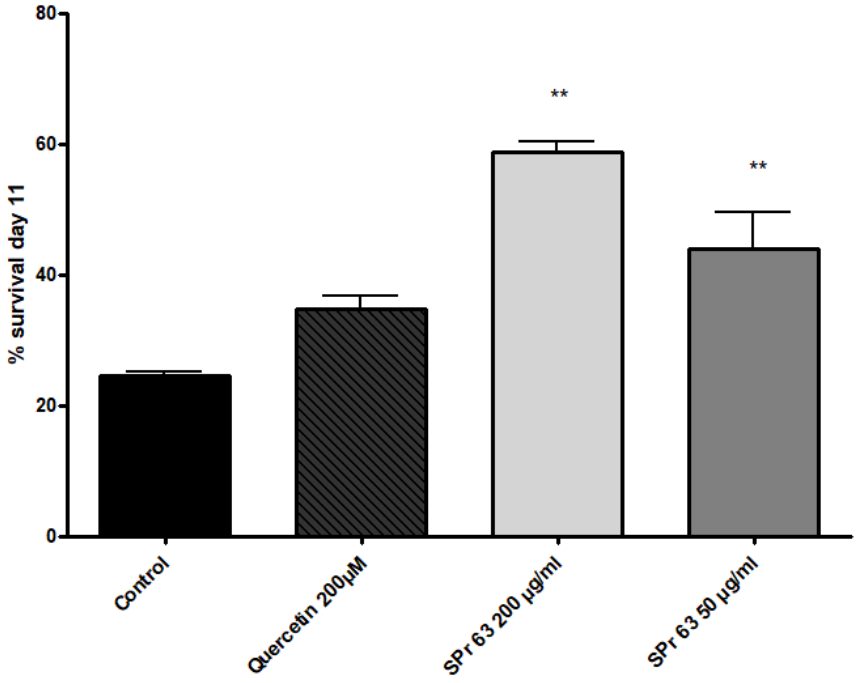

Figure 37: Effects of *Lignosus rhinoceros* (SPr63) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

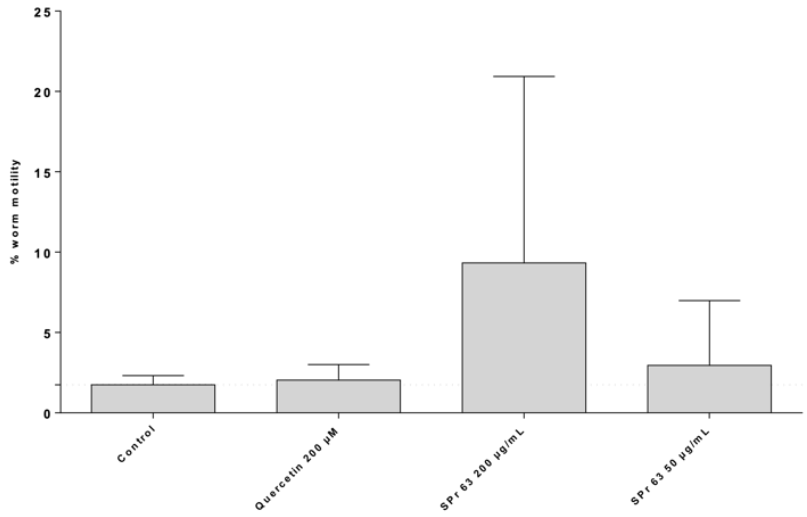

Figure 38: Motility of worms treated with *Lignosus rhinocerus* extract. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Spermidine

The survival rate of the worms which were treated with Spermidine (SPr64) was evaluated, using SPr64 in following final concentrations: 10 µM, 50 µM, 100 µM and 200 µM (Figure 39). Control worms, treated with 0.7% DMSO showed a survival rate of 25.76 % ± 0.79. 50 µM Spermidine led to a significant decrease in survival rate of the worms, compared to the DMSO 0.7% control ($p < 0.05$), showing a nematotoxic tendency by reaching a value of 11.48% ± 8.73. 200 µM Spermidine led to a slightly decreased survival rate (23.17% ± 3.12) while 100 µM showed a slightly increase in the survival rate of *C. elegans* (28.69% ± 3.35). 10 µM SPr64 resulted in a survival rate of the worms of 38.09% ± 6.44, and therefore led to the strongest increase of the survival rate among all tested concentrations, compared to the vehicle group. Although the survival rate enhancing tendency was cognizable in 10 µM, the effects of SPr64 observed in concentrations of 10 µM, 100 µM and 200 µM were not statistically significant.

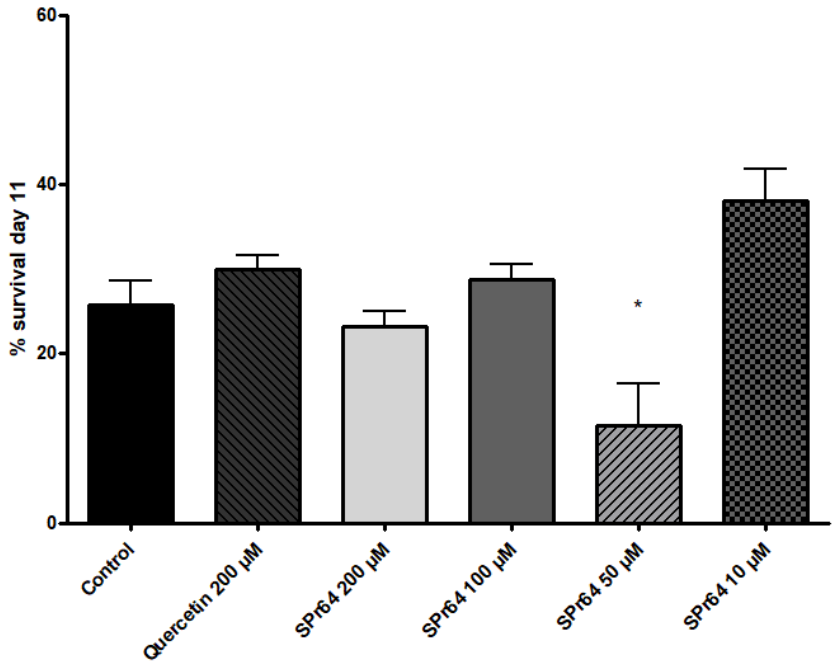

Figure 39: Effects Spermidine (SPr64) on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

CBD/CBG oil (12.5% CBD, 2.5% CBG)

The effects of CBD/CBG oil (SPr65) on the survival rate of the worms were assessed in concentrations of 50 µg/ml and 200 µg/ml (Figure 40). 200 µg/ml and 50 µg/ml of SPr65 have proven to have a decreasing effect on the survival rate of the worms in comparison with the vehicle group (12.04% ± 0.59). As the CBD/CBG oil led to survival rates of the worms of 6.37% ± 5.06 and 8.73% ± 0.95, respectively. Despite the visibly alleviating effect of SPr65 on the survival rate, the effects observed were not statistically significant.

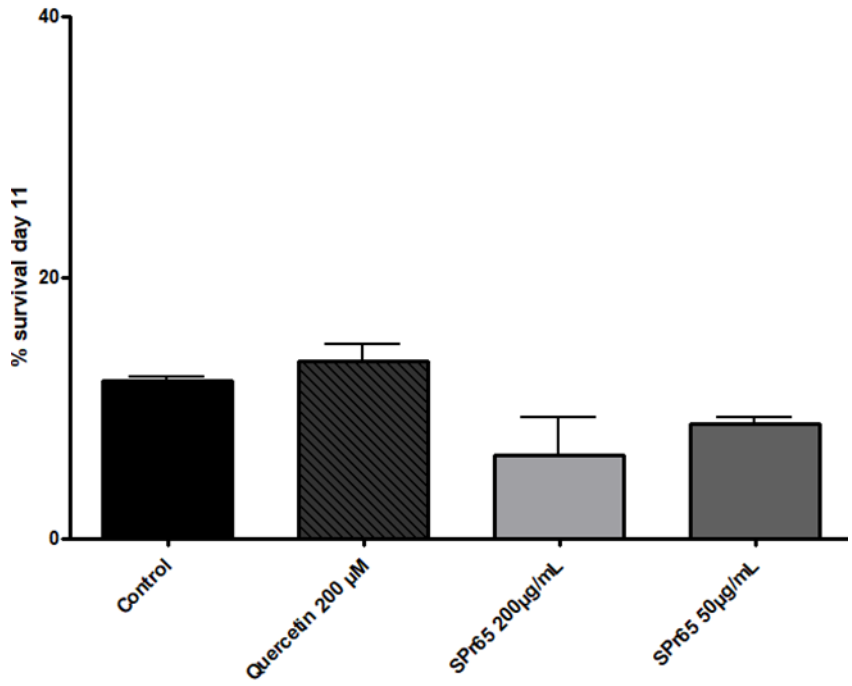

Figure 40: Effects of CBD/CBG (SPr65) oil on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

CBD extract from University of Vienna (10% CBD)

The effects of CBD extract (SPr66) on the survival rate of *C. elegans* were tested in final concentrations of 50 µg/ml and 200 µg/ml. The worms which were treated with the vehicle control exhibited a survival rate of 12.04% ± 0.59. 200 µg/ml SPr66 showed nematotoxic effects and decreased the survival rate of the worms to 1.94% ± 0.25 ($p<0.01$). Likewise, 50 µg/ml SPr66 had a decreasing effect on the survival rate with a

value of 8.57% ± 4.12, however the effect was not statistically significant (Figure 41). Moreover, nematotoxic effect of SPr66 seems to diminish with decreasing concentrations.

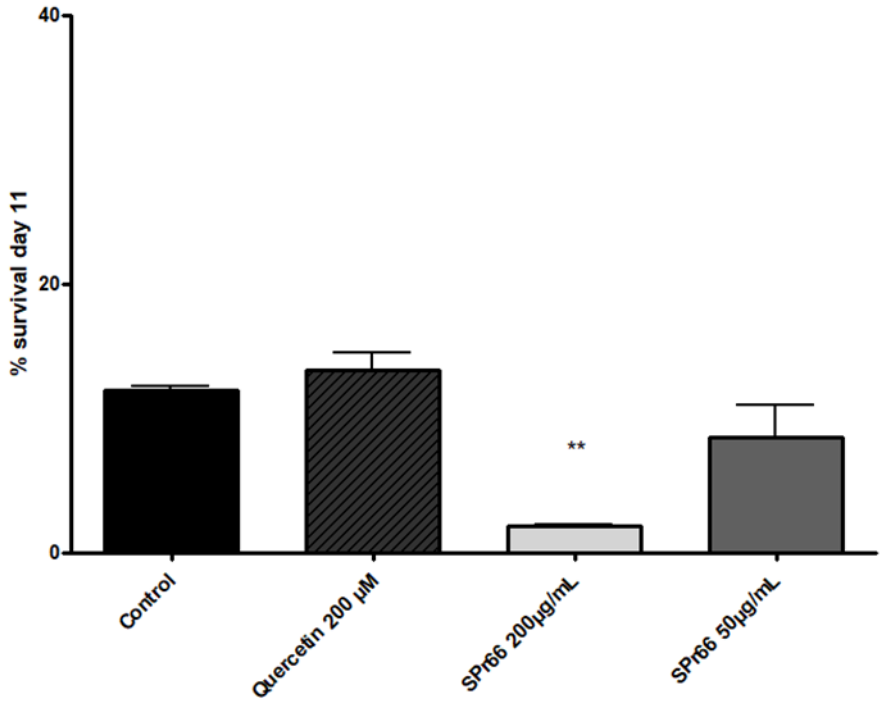

Figure 41: Effects of CBD (SPr66) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

Salicylic acid

Final concentrations of 50 µM and 200 µM of salicylic acid (SPr67) were tested in a *C. elegans* based survival assay (Figure 42). Worms which were treated with 0.7% DMSO displayed a survival rate of 22.45% ± 10.58. SPr67 slightly decreased the survival rate of the worms in both concentrations compared to the vehicle group. 200 µM slightly decreased the survival rate of the worms with a value of 20.42% ± 13.66, while worms treated with 50 µM exhibited a survival rate of 21.37% ± 5.07. Despite the visibly decreasing effect of SPr67 on the survival rate in both concentrations, the results were not statistically significant (which is shown in Figure 42).

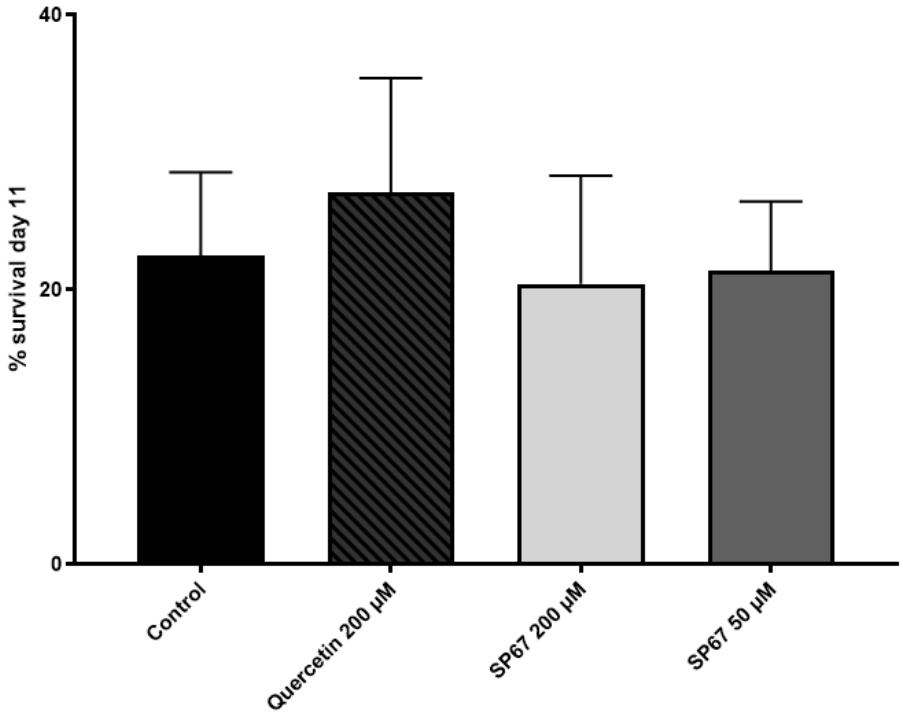

Figure 42 Effects of Salicylic acid (SPr67) on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

Salicin

Salicin (SPr68) was tested in final concentrations of 10 µM, 50 µM, 100 µM, 200 µM in *C. elegans* survival assay (Figure 43). The experiment showed no statistically significant effects ($p>0.05$), however, an enhancing effect on the survival rate could be shown in concentrations of 100 and 50 µM. The highest increase of the survival rate was observed at 50 µM, enclosed by a bell-shaped dose response curve. Worms which were treated with 0.7% DMSO displayed a survival rate of 25.76% ± 5.02, whereas 200 µM salicin reduced the survival rate of the worms compared to the vehicle group, resulting in a survival value of 21.70% ± 4.98. 50 µM SPr68 led to the highest increase of the survival rate of the worms compared with the vehicle control with a value of 42.19% ± 7.54. Concentrations of 100 and 10 µM slightly affected the survival rate of the worms, displaying values of 32.69% ± 12.86 and 28.69% ± 9.67, respectively.

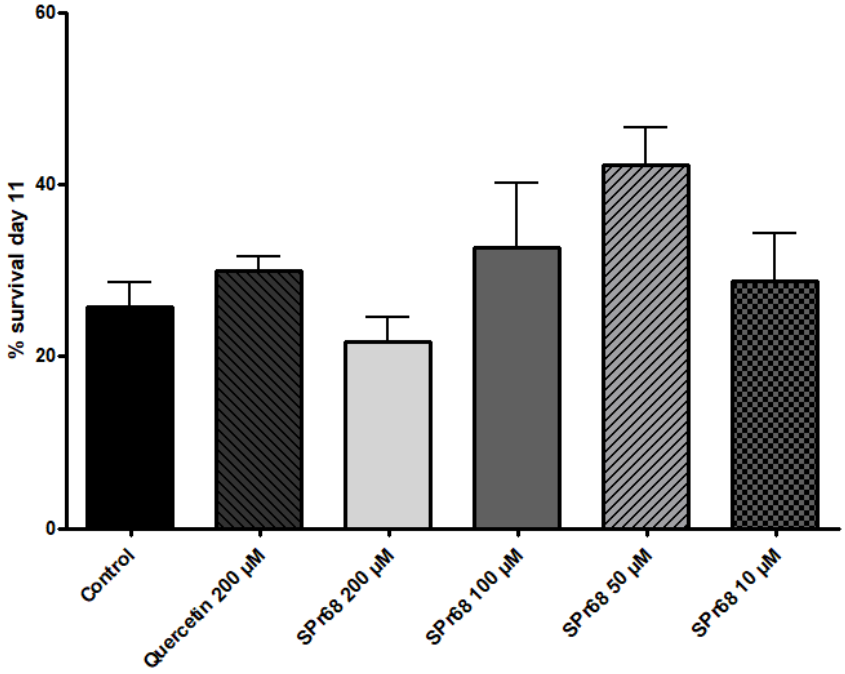

Figure 43: Effects of Salicin (SPr68) on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test.

***Genista tinctoria* L.**

The hydroethanolic extract of *Genista tinctoria* herba (SPr69) was tested in final concentrations of 50 µg/ml and 200 µg/ml (Figure 45). In both concentrations, the extract led highly significant increase of the *C. elegans* survival rate, compared to DMSO 0.7% control ($p<0.01$). The experiment showed a stronger effect of SPr69 on the survival rate in 50µg/ml, indicating a reversed dose response. The survival rate of worms treated with 200 µg/ml was 56.43% ± 8.47. Moreover, 50 µg/ml SPr69 caused an even higher enhancement of the survival rate, leading to a value of 66.59% ± 4.97, compared to the vehicle control (22.45% ± 10.58). Motility measurement on the 7th day of the experiment delivered congruent results, as presented in Figure 44, illustrating the increase in motility in the worms treated with SPr69 in both concentrations. The motility measurement revealed a strongly increased motility in the worms treated with

200 µg/ml and a moderately enhanced motility in the worms treated with 50 µg/ml SPr69.

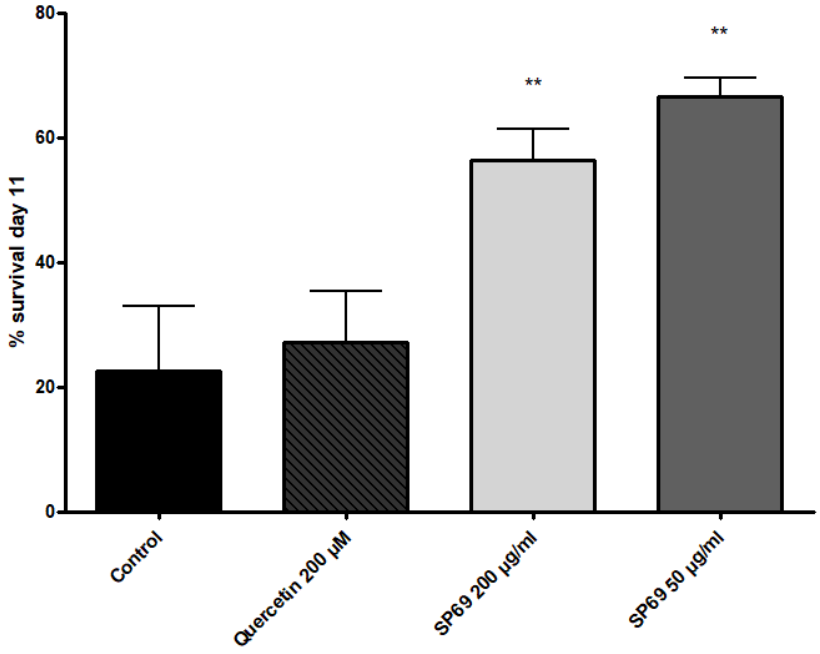

Figure 45: Effects of *Genista tinctoria* (SPr69) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

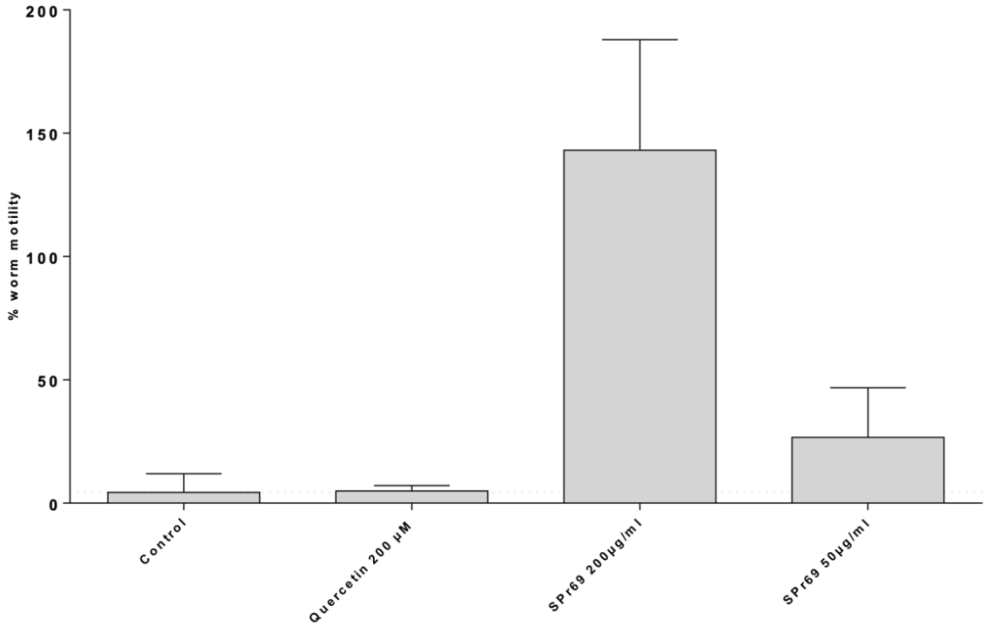

Figure 44: Motility of worms treated with *Genista tinctoria* extract. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Macrocybe titans (H.E. Bigelow & Kimbr.) Pegler, Lodge & Nakasone

The hydroethanolic extract of *Macrocybe titans* karposoma (SPr70) was tested in final concentrations of 50 µg/ml and 200 µg/ml (Figure 46). Both concentrations of SPr70 significantly increased the worm's survival rate compared with the DMSO 0.7% control (200 µg/ml $p<0.05$, 50 µg/ml $p<0.01$). The survival rate of the worms treated with 200 µg/ml was 35.16% ± 7.92, while 50 µg/ml SPr70 led to a survival rate of 42.77% ± 1.99. The motility of the worms was measured using the worm tracker on the 7th day of the experiment. Results as can be seen in Figure 47 indicate an increased motility of the worms treated with SPr70 in both concentrations.

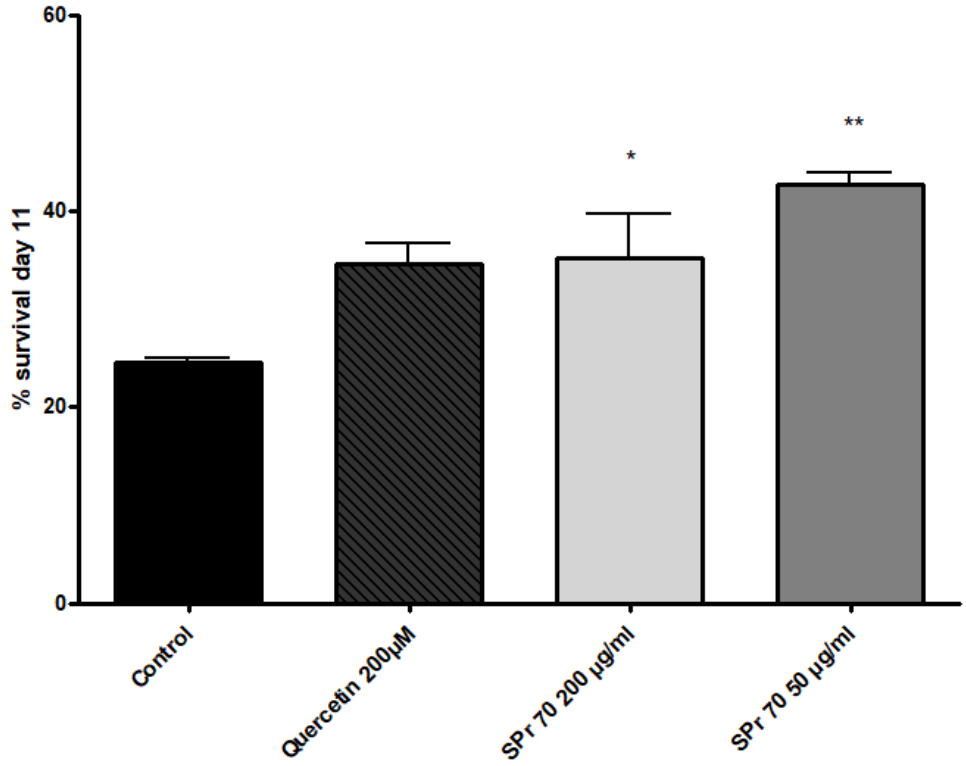

Figure 46: Effects of Macrocybe titans (SPr70) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

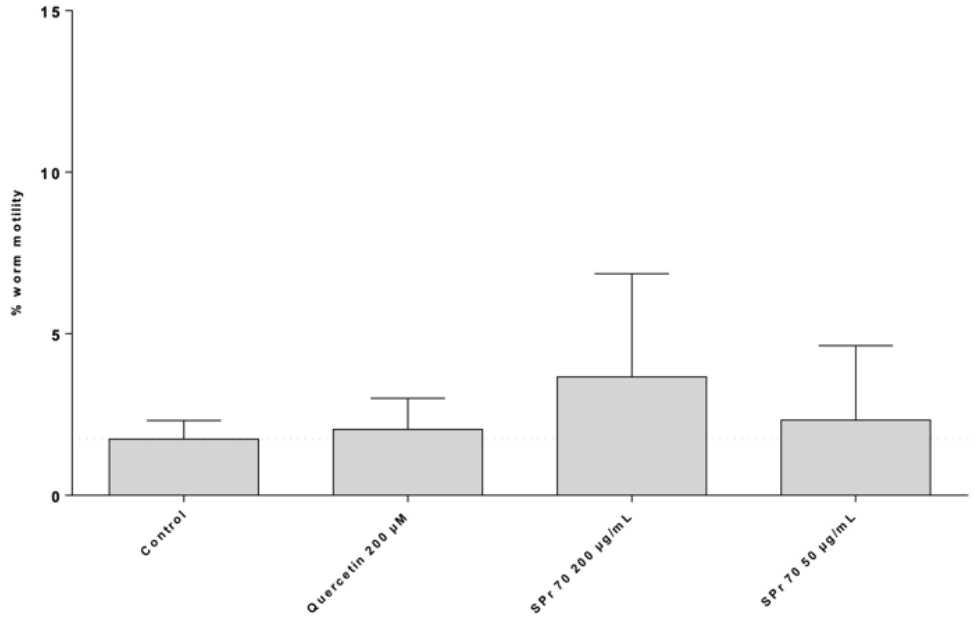

Figure 47: Motility of worms treated with *Macrocybe titans* extract. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Chlorogenic acid

The effects of chlorogenic acid (SPr71) on *C. elegans* were tested in a C. *elegans* survival assay in concentrations of 50 µM and 200 µM (Figure 48). The results show the survival rate enhancing effects of chlorogenic acid, which increases in a dose-dependent manner, compared to the vehicle control. Worms which were treated with 0.7% DMSO exhibited a survival rate of 24.60% ± 0.79. The significant increase in survival rate could be achieved with 200 µM SPr71 ($p<0.05$), leading to a value of 43.83% ± 10.78. 50 µM chlorogenic acid exhibited a survival rate of the worms of 42.77% ± 1.99 As another health parameter motility was measured using the worm tracker on the 7th day of the experiment. Results as illustrated in Figure 49 indicate only a slightly increased motility of the worms treated with SPr71 in both concentrations compared to the vehicle control.

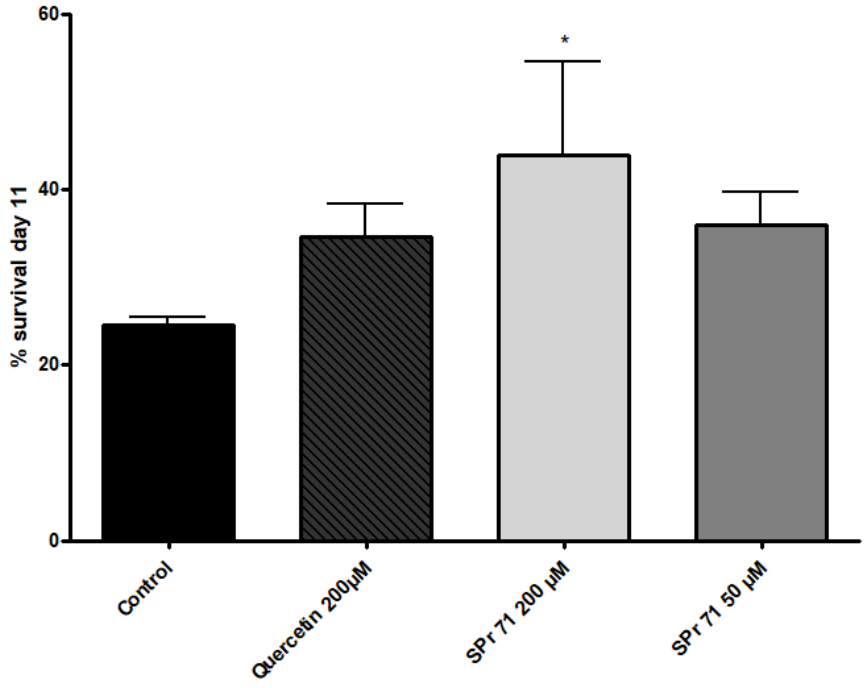

Figure 48: Effects of chlorogenic acid (SPr71) on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

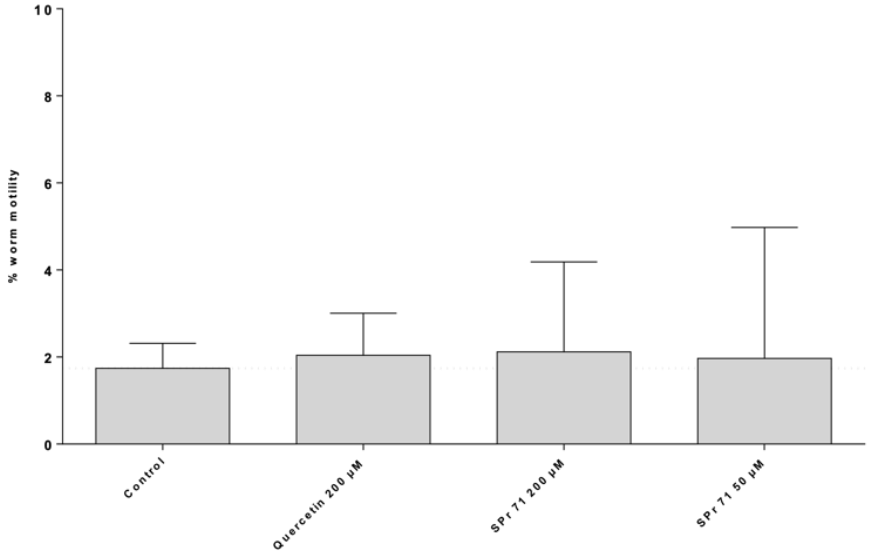

Figure 49: Motility of worms treated with chlorogenic acid. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Pterostilbene

Final concentrations of 50 µM and 200 µM of Pterostilbene (SPr72) were tested in the *C. elegans* based survival assay. The experiment showed a statistically significant increased survival rate of worms treated with 50 µM SPr72 compared to the control ($p<0.05$). Worms treated with the vehicle control exhibited a survival rate of 24.60% ± 0.79. 200 µM SPr72 led to a decreased survival rate, compared to the control, reaching a mean value of 21.39% ± 0.86. However, the decreasing effect was not statistically significant. 50 µM on the other hand, increased the survival rate of the worms to a value of 29.83% ± 1.88 (Figure 50). The experiment showed an enhancing effect of SPr72 on the survival rate only in 50 µM, indicating a reversed dose response. In accordance with the survival data, motility measurement on the 7th day of the experiment revealed an increase in the motility of the worms treated with 50 µM SPr72 and a decreased motility of worms, treated with 200 µM compared to the control group (Figure 51).

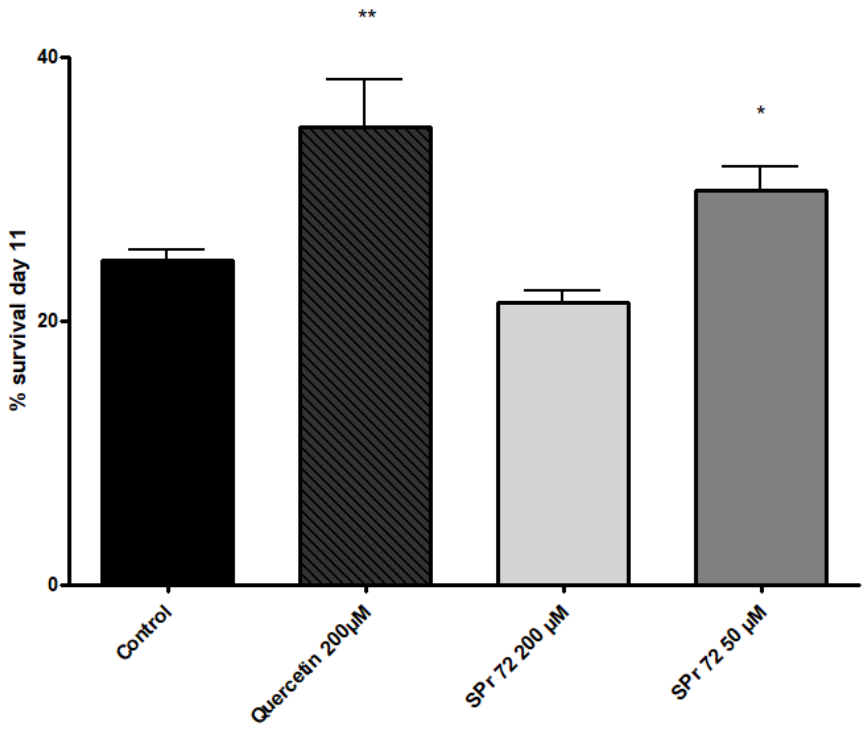

Figure 50: Effects of pterostilbene (SPr72) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

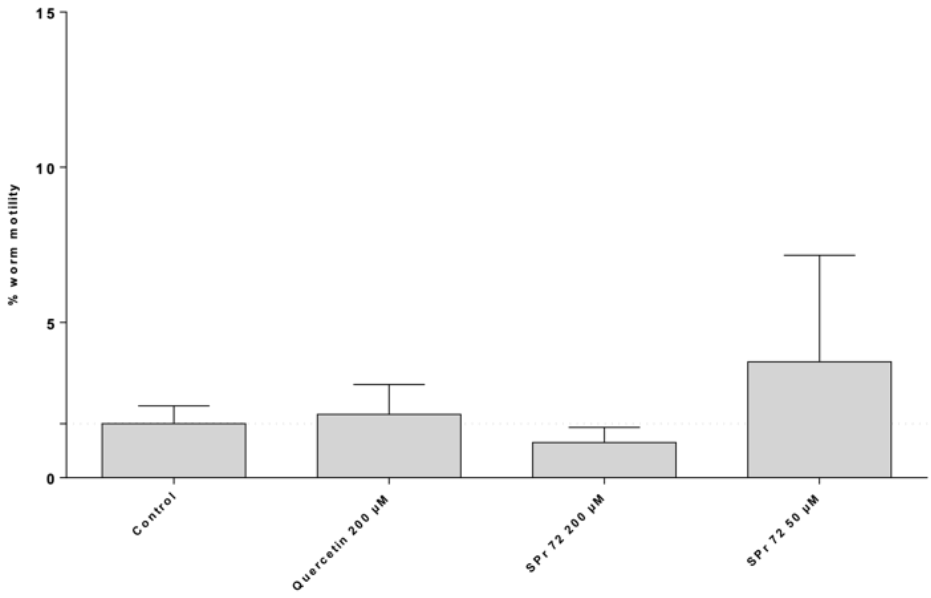

*Figure 51:*Motility of worms treated with pterostilbene. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Ascorbic acid:

Final concentrations of 50 µM and 200 µM of ascorbic acid (SPr74) were tested in the survival assay. Vitamin C had an enhancing effect on the survival rate, which slightly intensify with decreasing concentration. A significant increase of the *C. elegans* survival rate, compared to the 0.7% DMSO control (24.60% ± 0.79) was achieved in the group treated with of 50 µM ascorbic acid, exhibiting a survival value of 35.70% ± 6.12 ($p<0.05$) (Figure 52). 200 µM of ascorbic acid led to an increased survival rate of the worms, compared to the vehicle group, by attaining a value of 31.55% ± 3.43. Nevertheless, no statistical significance could be recorded using the effects of 200 µM. As additional health parameter motility was measured using the worm tracker on the 7th day of the experiment. Figure 53 illustrates these results in detail. A slightly increased motility of the worms treated with SPr74 in both concentrations was achieved compared to the control group.

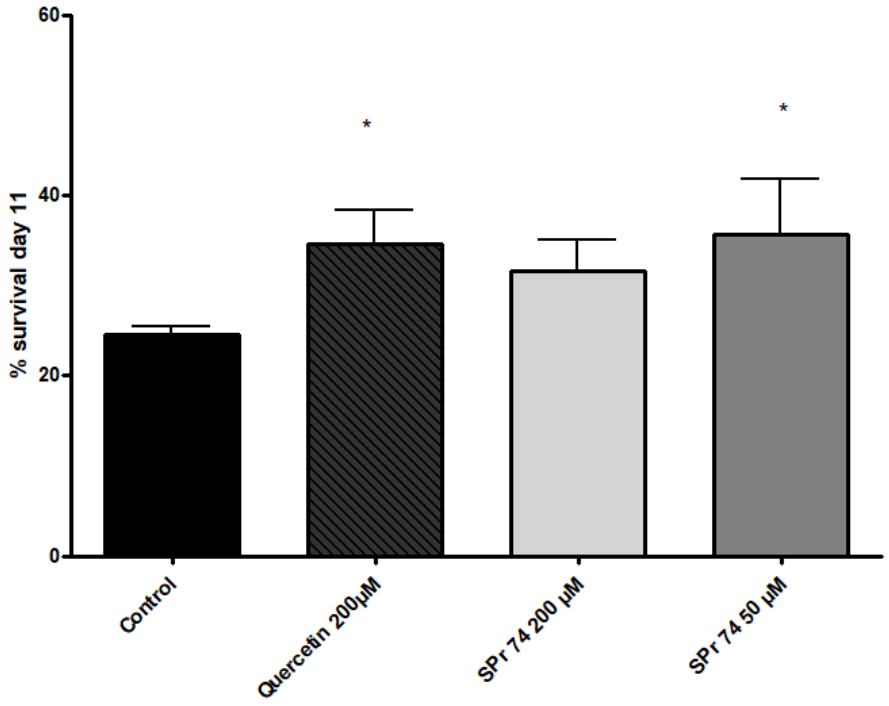

Figure 52: Effects of ascorbic acid (74) extract on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

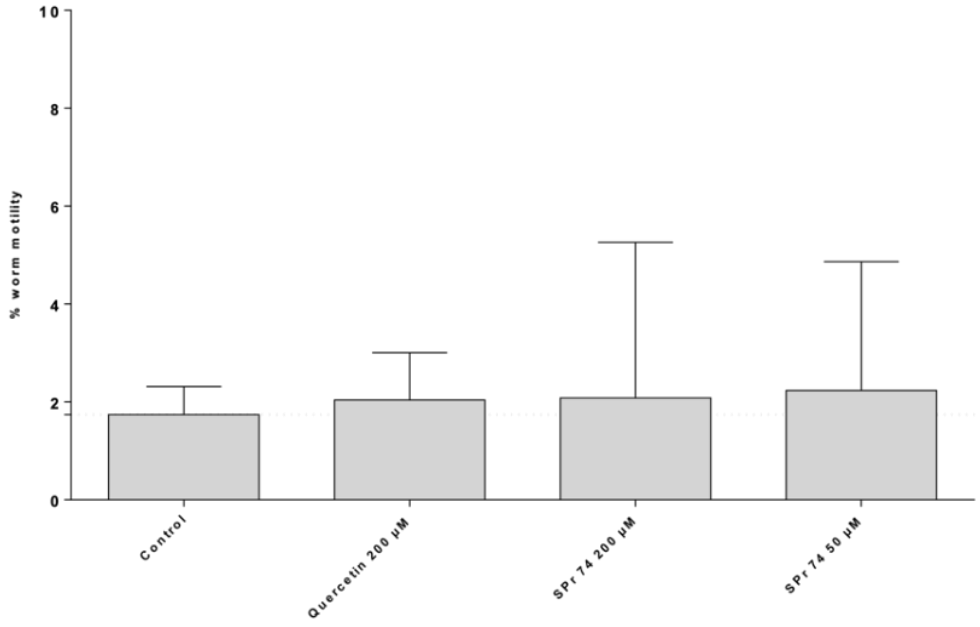

Figure 53: Motility of worms treated with ascorbic acid extract. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Resveratrol

The effects of resveratrol were tested in a *C. elegans* based survival assay. In both concentrations, 50 µM and 200 µM resveratrol led to a significant increase in survival rate of the worms comparing the data attained to the DMSO 0.7% control ($p<0.01$). Worms treated with the vehicle control exhibited a survival rate of 24.60% ± 0.79. The survival rate of the worms treated with 200 µM was 37.21% ± 3.90. 50 µM of resveratrol led to a survival rate of the worms of 41.28% ± 1.47 (Figure 54). The motility of the resveratrol treated worms measured on the 7th day of the experiment was enhanced compared to the vehicle control. As presented in Figure 55 50 µM resveratrol caused a strongly increased motility of the worms, while the motility of worms treated with 200 µM resveratrol showed a smaller increase, compared to the vehicle control.

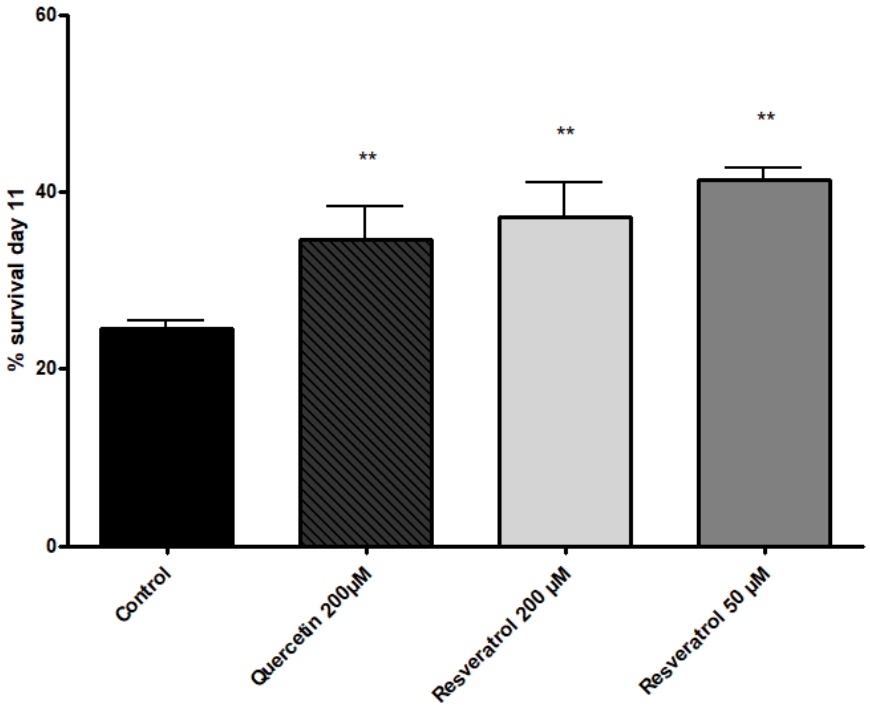

Figure 54: Effects of resveratrol on the survival rate of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean survival rate on day 11 of adulty, of three parallel experiments, expressed as mean % of live worms ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$).

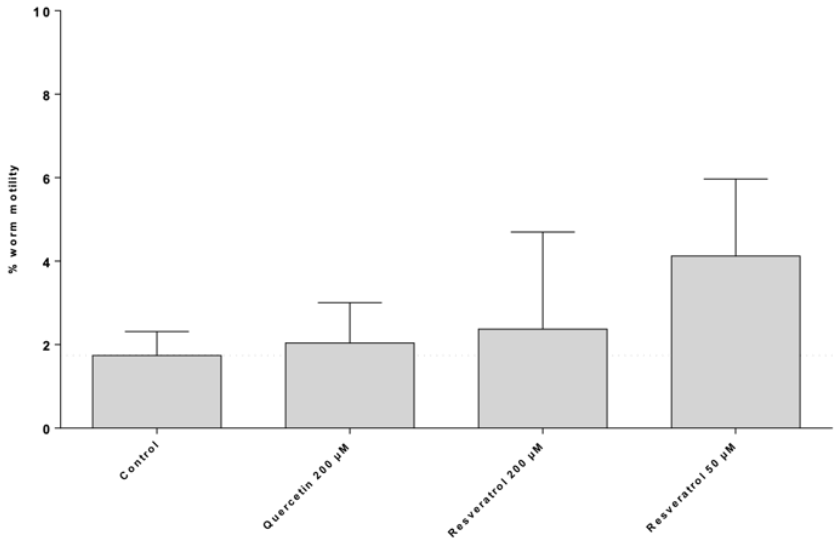

Figure 55: Motility of worms treated with resveratrol. Bars represent the mean worm motility (%) on the 7th day of the experiment ± SD of three parallel experiments.

Taken together, in the survival assay 19 herbal, four fungal extracts, seven NCs and two CBD formulations were tested. Five herbal and three fungal extracts, and four NCs showed a significant increase in survival rate of the worms compared to the 0.7% DMSO control group at least in one of the final concentrations. In order to gain more information and to select the most promising samples for lifespan studies, a detailed literature search on the current state of knowledge was conducted.

Considering those samples which drastically decreased the nemtodes' lifespan, at the current state of scientific knowledge, there is no data on nematotoxic effects of *Fomitopsis betulina*, as well as on the bioactive compounds responsible for this effect. Moreover, there is no data on nematotoxic effects on parasitic worm species, making our results an important starting point for the search of novel anthelmintic drugs. Contrarily to literature data, the study showed that a cannabis extract with 10% CBD content, led to a significant decrease of the survival rate in *C. elegans*. CBD is used in the treatment of pain and sleeping disorders and as an additional therapy in cancer treatment and is therefore associated with a healthspan prolonging effect. Studies testing CBD in higher concentrations showed no toxic effect on *C. elegans* [118]. Therefore, the question arises whether the effect observed in our study was caused by the CBD content or other NCs in the multicomponent mixture, which have to be investigated in further studies.

Since the investigated samples were selected based on their traditional use and ethnopharmacological data, it is not surprising that a large number of extracts have led

to a significant increase in survival rate of the worms. The results obtained from two extracts were outstanding, namely *Genista tinctoria* herba extract SPr69 and the extract of *Ganoderma lucidum* SPr62 due to their strong increase in *C. elegans* survival rate.

From the current state of research, there are no publications on life prolonging or health promoting effects of *Genista tinctoria* in *C. elegans*. The lack of studies could be due to toxic effects attributed to members of the Genista species, which are caused by the content of quinolizidine alkaloids. However, our results are of high interest and importance for further phytochemical and bioactivity-guided investigations. In accordance to the literature, we could show similar lifespan promoting effects in C. elegans after treatment with an extract of *Ganoderma lucidum*. *Ganoderma lucidum* has already been well studied for its health promoting properties. These properties include, for example, enhancing processes like autophagy[119].

On the 7th day of the experiment, the motility of the worms was semi-automatically measured. The motility data showed to be congruent with the survival data in more than 70%. The results indicate the suitability of this assay for the high throughput screening (HTS). In our study, the combination of survival assay and motility assay proved to be a perfect combination for the first screening, providing information on life-prolonging effects and the health status of the worms. In the survival assay, manual counting of each individual enables the contemporaneous, visual assessment of the health condition of the worms, by observing behaviour and size. A big advantage of the Wormtracker, however, is its independence from the operator. Due to the semi-automatic mode of operation, the results are less susceptible to manipulation. Table 3 illustrates a summary of the results gained through the first screening.

Table 3: Results of survival rate and motility rate. Survival rate: + means an increased survival rate compared to the control group DMSO 0.7%, - means a decreased survival rate. * $p < 0.05$, ** $p < 0.01$. Motility rate: ⇓ means a decreased motility rate compared to the control group DMSO 0.7%, ⇑ stands for an increased motility rate compared to the control group and ⇔ means the motility rate of the extract/NPs is approximately the same as the control group; n.t., not tested.

Extract-ID	Plant/Natural compound	Family	Organ	Survival rate		Motility rate	
				200 ug/ml	50 ug/ml	200 ug/ml	50 ug/ml
SPr41	*Lycium barbarum* Mill.	Solanaceae	Cortex	+	+	⇑	⇑
SPr42	*Lonicera japonica* Thunb.	Caprifoliaceae	Caulis	+**	+**	⇑	⇑
SPr43	*Panax ginseng* C.A.Mey	Araliaceae	Radix	+	+	⇔	⇓
SPr45	*Punica granatum* L.	Lythraceae	Semen	+	+	⇑	⇑
SPr46	*Panax notoginseng* (Burkill) F.H.Chen	Araliaceae	Radix	+	+	⇑	⇑
SPr47	*Astragalus mongholicus* (Bunge) and *membranaceus* Fisch. Ex Bunge	Fabaceae	Radix	+	+	⇑	⇑
SPr48	*Angelica sinensis* (Oliv.) Diels.	Apiaceae	Radix	+**	+	⇑	⇔

SPr49	*Withania somnifera* L. Dunal.	Solanaceae	Radix	+	+	⇑	⇑
SPr51	*Centella Asiatica* (L.) Urb.	Apiaceae	Herba	+	-	⇔	⇑
SPr52	*Convolvulus prostratus* Forssk.	Convolvulaceae	Herba	+	-	⇓	
SPr53	*Passiflora incarnata* L.	Passifloraceae	Herba	+	+	⇑	⇔
SPr54	*Salvia officinalis* L.	Lamiaceae	Folium	+	-	⇔	⇑
SPr55	*Cynara scolymus L.*	Asteraceae	Folium	+ **	+	⇑	⇑
SPr56	*Scutellaria barbata* D. Don	Lamiaceae	Herba	+ **	+ **	⇑	⇑
SPr57	*Gynostemma pentaphyllum* (Thunb.) Makino	Cucurbitaceae	Herba	-	-	⇓	⇑
SPr58	*Cistus creticus* L.	Cistaceae	Herba	-	+	⇔	⇑
SPr59	*Ranunculus ternatus Thunb.*	Ranunculaceae	Radix	-	-	⇔	⇔

SPr60	*Polygala tenuifolia* Willd/sibirica	Polygalaceae	Radix	-	-	⇔	⇓
SPr61	*Fomitopsis betulina* (Bull.) B. K. Cui, M. L. Han & Y. C. Dai	Fomitopsidaceae	Karposoma	- **	-	⇓	⇓
SPr62	*Ganoderma lucidum* (Curtis) P. Karst.	Ganodermataceae	Karposoma	+ **	+ **	⇑	⇑
SPr63	*Lignosus rhinocerus* (Cooke) Ryvarden	Polyporaceae	Sclerotium	+ **	+ **	⇑	⇑
SPr64	Spermidine	/	/	-	- **	n.t.	n.t.
SPr64	Spermidine	/	/	100 µM: +	10 µM: +	n.t.	n.t.
SPr65	CBD/CBG oil (12.5 % CBD, 2.5 % CBG)	/	/	-	-	⇓	⇓
SPr66	CBD extract (10%) – University of Vienna	/	/	- **	-	⇑	⇔
SPr67	Salicylic acid	/	/	-	-	⇔	⇓

SPr68	Salicin	/	/	-	+	⇓	⇔
SPr68	Salicin	/	/	100 μM: +	10 μM: +	⇔	⇓
SPr69	*Genista tinctoria* L.	Fabaceae	Herba	+ **	+ **	⇑	⇑
SPr70	*Macrocybe titans* (H.E. Bigelow & Kimbr.) Pegler, Lodge & Nakasone	Tricholomataceae	Karposoma	+ *	+ **	⇑	⇑
SPr71	*Chlorogenic acid*	/	/	+ *	+	⇑	⇑
SPr72	Pterostilbene	/	/	-	+ *	⇓	⇑
SPr74	Ascorbic acid	/	/	+	+ *	⇑	⇑
	Resveratrol	/	/	+ **	+ **	⇑	⇑

5.3 Lifespan assay

Based on the survival and motility data archived in the previous screening, four herbal extracts and three mushroom extracts were tested in *C. elegans* lifespan assay to gain more detailed information on the life extending effects (Table 4). The extracts were tested at 50 µg/ml and 200 µg/ml. This assay was performed in three parallel experiments using 0.7% DMSO as vehicle control and 200 µM quercetin as positive control. The number of live worms was counted every second day until the proportion of live worms fell beyond 30%. Survival curves for each plate were created by plotting the percentage of living worms per condition against the time using GraphPad PRISM 4.0. DT_{50} was calculated based on the survival curves. The mean DT_{50} of the vehicle control was set to day 20 and DT_{50} values of each treatment were normalized to day 20. The deviation of lifespan was expressed as percentage of increase compared to the vehicle control. Statistical significance was calculated using One Way ANOVA and Dunnett's post-test.

Out of the seven different extracts tested in the lifespan assay, four extracts led to a statistically significant increase of the DT_{50} in both concentrations relative to the control, namely the extracts of *Lonicera japonica* (SPr42), *Scutellaria barbata* (SPr56), *Lignosus rhinoceros* (SPr63) and *Genista tinctoria* (SPr69) ($p<0.01$). The strongest life prolonging effect was achieved by 200 µg/ml SPr63, which increased the DT_{50} by 41.15%, relative to the vehicle control. The extracts of *Angelica sinensis* (SPr48) and *Ganoderma lucidum* (SPr62) exhibited a statistically significant increase of the DT_{50} in a concentration of 200 µg/ml ($p<0.01$), while 50 µg/ml showed an increase in DT_{50}, however without a statistical significance ($p>0.05$). *Macrocybe titans* (SPr70) showed life prolonging effects at both concentrations relative to the vehicle control, however without statistical significance ($p>0.05$). All observed effects in this assay changed in a dose dependent manner, as presented in Figure 56, which graphically illustrate the results of the lifespan assay.

Table 4: Summarized results from lifespan assay

Sample	Concentration	Mean	SD	DT_{50} normalized	DT_{50} increased	P Value
Quercetin	200 µM	13.52	0.49	22.42	12.10%	>0.05
SPr42	50 µg/ml	14.69	0.42	24.37	21.85%	<0.01
SPr42	200 µg/ml	15.44	0.83	25.62	28.10%	<0.01
SPr48	50 µg/ml	13.58	0.71	22.53	12.65%	>0.05
SPr48	200 µg/ml	14.80	0.42	24.56	22.8%	<0.01
SPr56	50 µg/ml	14.21	0.60	23.57	17.85%	<0.01
SPr56	200 µg/ml	15.47	1.27	25.65	28.10%	<0.01
SPr62	50 µg/ml	13.57	0.82	22.51	12.55%	>0.05
SPr62	200 µg/ml	15.18	0.46	25.18	25.90%	<0.01
SPr63	50 µg/ml	14.31	042	23.73	18.65%	<0.01
SPr63	200 µg/ml	15.55	0.41	25.80	29.00%	<0.01
SPr69	50 µg/ml	15.57	0.74	25.82	29.10%	<0.01
SPr69	200 µg/ml	17.02	1.16	28.23	41.15%	<0.01
SPr70	50 µg/ml	12.83	0.66	21.28	6.40%	>0.05
SPr70	200 µg/ml	12.97	0.64	21.52	7.60%	>0.05

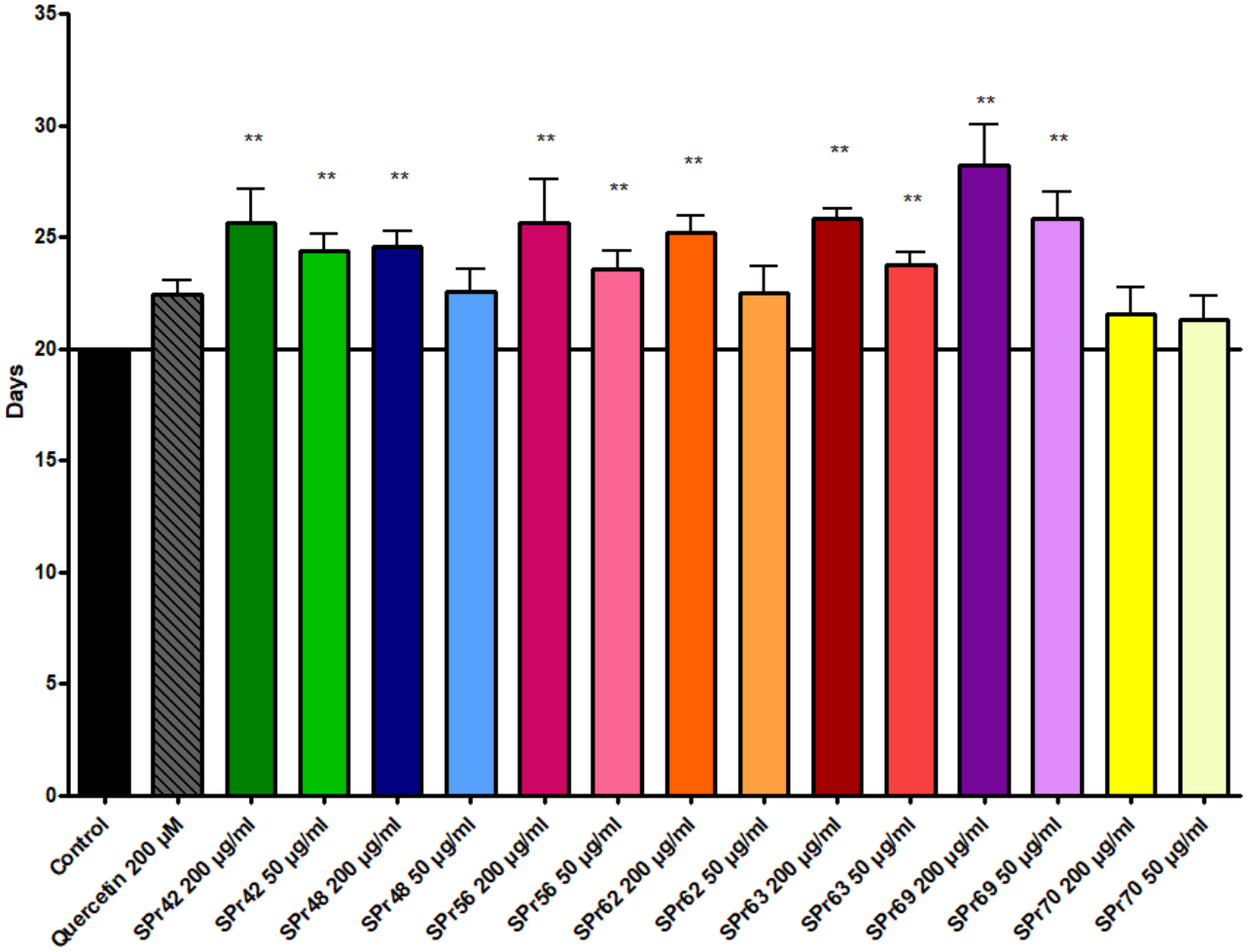

Figure 56: The effects of seven different extracts on the DT_{50} of adult N2 wildtype worms in the given concentrations. Bar charts represents the mean DT_{50} of three parallel experiments, expressed as mean days of reaching DT_{50} ± standard deviation (SD). Statistical significance was assessed by One Way ANOVA and Dunnett post-test (* $p < 0.05$, ** $p < 0.01$). *Lonicera japonica* (SPr42), *Angelica sinensis* (SPr48), *Scutellaria barbata* (SPr56), *Ganoderma lucidum* (SPr62), *Lignosus rhinocerus* (SPr63), *Genista tinctoria* (SPr69), *Macrocybe titans* (SPr70).

6 Conclusion and Outlook

In this study 19 plant extracts, four mushroom extracts, seven natural compounds and two CBD formulations were screened in *C. elegans* survival and motility assays. Results from this screening indicated that five plant extracts, three fungal extracts and four NCs caused a statistical increased survival rate of the worms. Furthermore, one plant extract (SPr61), one NC (SPr64) and one CBD formulation (SPr66) resulted in a significantly decreased survival rate of the worms, making the samples interesting candidates for further investigation in anthelmintic screenings. Seven hits from the motility and survival screening were selected and tested in *C. elegans* lifespan assay. Results of this assay confirmed the expected life prolonging properties of all tested samples, showing statistical significance in six of seven extracts. Findings from our screening are consistent with the *C. elegans* based data in terms of lifespan increase from the literature [119–123]. So far, mainly extracts of the selected material have been studied in *C. elegans.* Therefore, the results of our study constitute an important starting point for further studies on the bioactive natural compounds and bioactivity-guided isolation. Moreover, we could show a correlation of survival and motility data of more than 70%. This proves the suitability of the motility tracker for quick, preliminary HTS. The combination of survival and motility assay revealed to be highly suitable for providing a broad spectrum of health and lifespan information, which can be entered into the assessment of hits to be further tested.

The second part of this work was the development and optimization of the stress protection assay by integrating a semi-automatic wormtracker. In this study we could successfully determine the following parameters: 96-well plate type, worm density and stressor. Integrating the semi-automatic wormtracker accelerates the screening and reduces the susceptibility to undesired manipulation by the operator's way of working, building an important base for the routine use of this assay in *C. elegans* healthspan screenings.

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