

REVIEW

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Caenorhabditis elegans as a powerful tool in natural product bioactivity research

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Abstract

In addition to synthetic and semisynthetic compounds, natural products have received much attention as prolific sources of lead compounds with therapeutic effects on various diseases. In the process of screening the bioactivities of potential candidates, an in vivo assay is very important for providing meaningful insight into the efficacy, adverse effects, and modes of action that are relevant to humans. Among the many experimental models, *Caenorhabditis elegans* is particularly efficient due to its advantages in morphology, behavior, and genetic aspects. This review summarizes some basic and useful techniques commonly used in screening the bioactivities of natural products. Recent studies of naturally occurring extracts as well as bioactive compounds in various areas, namely, anti-aging, anti-neurodegeneration, anti-obesity, anti-infection, and gut health, are reviewed as examples of the applicability of the *C. elegans* model. Technological developments that incorporate *C. elegans* in other fields, such as instrumental analysis and emerging methods, are also discussed in this paper.

Keywords: Anti-aging, Anti-infection, Anti-obesity, *Caenorhabditis elegans*, Neuroprotection, Gut health, High-throughput screening, Natural products

Introduction

Caenorhabditis elegans is a small, transparent, free-living nematode that lives in soil [1]. The organism has attracted attention for its use as an alternative in vivo model, especially in studies involving the potential biological activity of natural products (NPs). Due to its small size (approximately 1 mm in length for adults), *C. elegans* and its behavior, such as moving, eating, mating, and laying eggs, can be easily observed by microscopy. When comparing the genomes of humans and *C. elegans*, it is evident that many human disease genes and disease pathways are present in the worm. A total of 40–80% of human genes have orthologous genes in the *C. elegans* genome [2, 3], and 40–50% of human disease-associated genes have orthologs in the worm genome [3, 4]. Human disease-related and lipid metabolism genes and signaling

pathways including the insulin signaling pathway are highly conserved in *C. elegans* [5, 6]. Because of these significant points, the nematode model has been utilized in a wide range of evaluations for therapeutic effects using bioactive NPs.

In this review, we described some basic *C. elegans* procedures in terms of morphological analysis, behavior analysis, biochemical analysis, and molecular assays. Many promising candidates in various areas from natural sources that have successfully been discovered using the *C. elegans* model are also summarized.

General information of *C. elegans*

Life cycle and anatomy

C. elegans are either self-fertilizing hermaphrodites or males; however, males account for approximately 0.1% of the population. Hermaphrodites that self-fertilize can produce approximately 300 offspring, whereas male-fertilized hermaphrodites can produce more than 1000 offspring. *C. elegans* has a short life cycle (3 days at 20 °C from eggs to gravid adults), and its life cycle consists of

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four larval stages (L1, L2, L3, and L4) and adulthood [7]. Under optimal culture conditions, the average lifespan of *C. elegans* is 2 to 3 weeks [8]. Figure 1 illustrates the life cycle of *C. elegans*.

The anatomy of *C. elegans* is simple and consists of the mouth, pharynx, intestine, gonad, and cuticle. It has a nervous system containing 302 neurons that are completely connected. The digestive system of *C. elegans* includes a pharynx, intestine, and rectum. The *C. elegans* intestine has various functions, such as food digestion by enzymes, nutrient absorption, and the synthesis and storage of various macromolecules. The intestine includes approximately 20 cells arranged to form a tube with a central lumen. The surface of intestinal cells carries numerous microvilli to increase the absorption surface [9].

Database of *C. elegans*

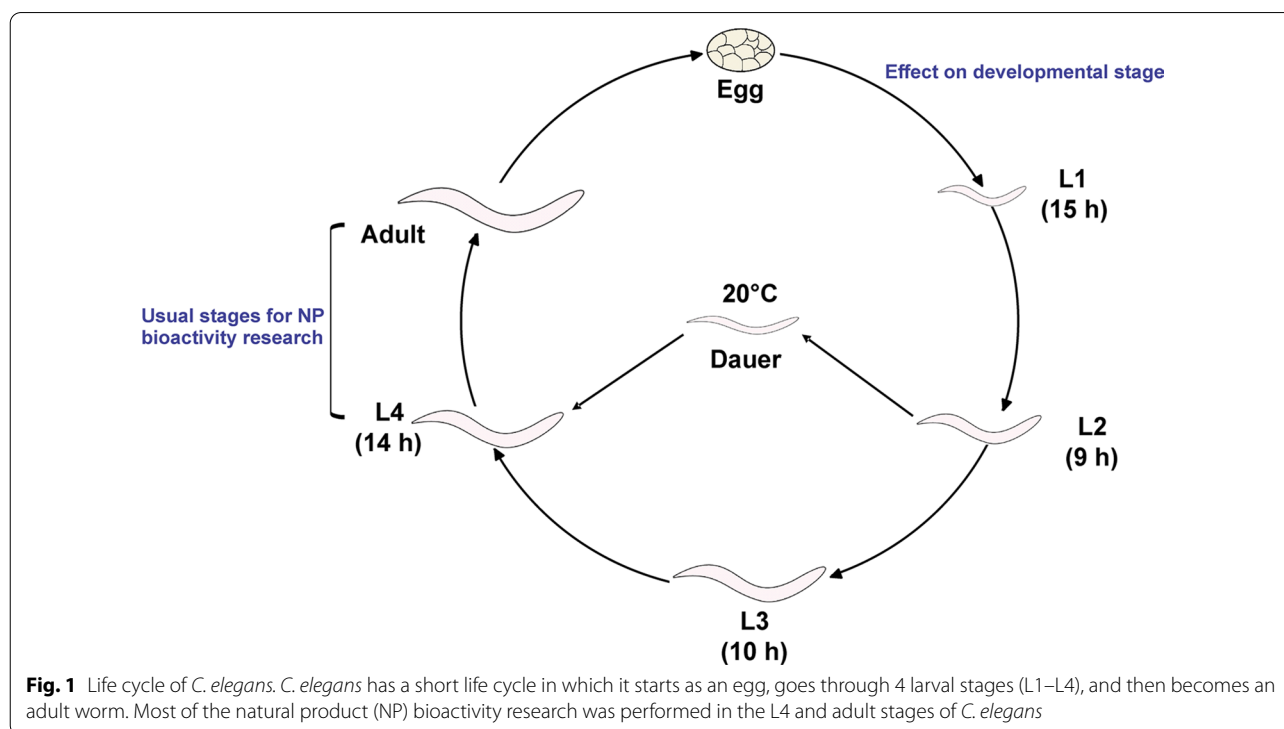
Currently, there are many databases available for research using the *C. elegans* model. WormAtlas (<https://www.wormatlas.org/>) is a valuable online database of nematode behavior and structural anatomy, anatomical methods, cell function, and cell identification. When examining the genomics of *C. elegans*, WormBase (<http://www.wormbase.org/>) is a helpful tool that provides information on the genetics, genomics, and biology of *C. elegans*, including gene sequences, gene expression patterns, loss-of-function mutants, RNAi phenotypes, and

genetic maps. Many other online databases related to *C. elegans*, such as WormImage (<http://www.wormimage.org/>), which is a database of *C. elegans* electron micrographs and associated data, and WormBook (<http://www.wormbook.org/>), which contains basic reviews of *C. elegans* biology and methodology.

The advantages and disadvantages of the *C. elegans* model

Overall, *C. elegans* is a powerful model organism for research involving the biological activity of NP. This is not only because of the convenience of maintaining *C. elegans* in the laboratory but also because of its high fertility rates and short life cycle [10]. In addition, the availability of its complete genome sequence makes *C. elegans* a valuable model for investigating the molecular basis of human diseases [11]. Currently, with the development of many high-throughput screening (HTS) methods, thousands to millions of natural compounds can be simultaneously screened for targeted bioactivity. *C. elegans*, with its many advantages, such as its small size, short generation time with a high number of eggs, and short life cycle, has become a suitable candidate for HTS methods for new drug development from natural sources. Moreover, experiments with *C. elegans* do not have ethical concerns [10, 12].

However, using *C. elegans* still has some limitations compared to other mammalian animal models because *C. elegans* has a simple anatomy and lacks some mammalian



organs or tissues, such as lungs, livers, kidneys, and blood transport systems. Therefore, it is not appropriate to use *C. elegans* as a research model for human diseases directly related to these organs [12]. In addition, the small size (only 1.1 mm) of *C. elegans* can cause difficulties for inexperienced researchers in experimental manipulation. Each animal model has its own advantages and disadvantages, and the selection of model animals to use as a research model depends on many factors. Table 1 compares some of the advantages and limitations of commonly used model organisms including *C. elegans*, fruit fly (*Drosophila melanogaster*), zebrafish (*Danio rerio*), mouse (*Mus musculus*), and human cells.

Experimental techniques using *C. elegans*






Lifespan assay

Lifespan assays play an important role as markers for screening and elucidating the underlying molecular mechanism in studies involving aging, stress resistance, and toxicology [13]. Due to its advantage of a relatively short life expectancy, a lifespan assay using *C. elegans* lasts for 21 days, and resistance assays with heat, chemical, or oxidative stress could last for a shorter duration [8, 13]. Lifespan assays can be conducted in both solid and liquid media. The traditional workflow involves counting the live and dead worms from an initial synchronized population over a period of time. The live and dead worms are recorded based on their responses to being touched with platinum wire in agar plates, shaking, or exposure to light or by the fluorescence signal from viable

staining dye in the case of liquid media [13]. Recently, a system using vibrotaxis with a controllable angular speed and a controllable duration of stimulus application was suggested to enhance the sensitivity and to minimize the mechanical damage to worms [14]. The lifespan curve was then constructed based on the percentage of live worms. A detailed method was described in the review paper by Park et al. [13]. This commonly used approach is labor intensive because it is necessary to regularly and manually transfer worms to fresh plates; thus, it is time consuming and inappropriate for assessing a large population of worms [15]. Therefore, significant efforts have been made to provide increasingly automated approaches.

The last decade has shown an ever-increasing development of lifespan assays assisted by lab-on-chip methods. The general concept of this method is the fabrication of many chambers for housing worms on polymers, such as polydimethylsiloxane [15]. Valves, pumps, and branching channels accurately facilitate all processes, such as feeding *E. coli* OP50, supplementing media or reagents, disposing waste and loading, sorting animals across progeny and immobilizing worms for imaging. The system is incorporated with an image acquisition module and software that allows real-time and automated scoring. Another automated technology that has been developed is the lifespan assay machine using a platform of standard Petri dishes or microfabricated well plates, which uses cessation in worm movement as a live or dead criterion. Some representative designs of microfluidic and

Table 1 Comparison of commonly used animal models

	Human cell culture	<i>Caenorhabditis elegans</i>	<i>Drosophila melanogaster</i>	<i>Danio rerio</i>	<i>Mus musculus</i>	References
						
Lifespan	N/A	18–20 days	60–80 days	3–5 years	3–4 years	[12, 142–144]
Life cycle	N/A	2.5–4 days	~10 days	~90 days	6–8 weeks	[12, 142, 144, 145]
Reproduction	N/A	140 eggs/day	100 eggs/day	200 eggs/week	5–8 pups/month	[142, 144, 146, 147]
Adult size	N/A	~1.0 mm	~3.0 mm	2.0–5.0 cm	7.5–10.0 cm	[12, 142, 148, 149]
Number of neurons	N/A	302	~100,000	~10 millions	~70 millions	[150–153]
Percentage of human genes with orthologs ^a	100%	38.1%	40.3%	55.2%	80.5%	[3]
Percentage of human disease genes with orthologs ^b	100%	48.6%	53.4%	72.9%	97.0%	[3]
High-throughput screening	++	++	+	+	–	
Ethical issue	++	++	++	+	–	

N/A not applicable, ++ good, + partly suitable, – not good

^a Calculated as the total number of human genes with orthologs present in animals divided by the total number of genes in humans

^b Calculated as the total number of human disease genes with orthologs present in animals divided by the total number of genes in humans

automatic system, such as NemaLife chip, WorMotel, and WormBot, were previously thoroughly reviewed [15, 16]. All these approaches can be complementary to conventional methods that provide more reliable results.

Growth-rate assay

Our group has reported a protocol for measuring the growth retardation of *C. elegans* treated with chemicals [17, 18]. Beginning with synchronized eggs, worms were incubated at 20 °C for 4 days. Microscopic images were taken every day for 4 days to exclude the possibility of starvation. Starvation was a considered possibility in the toxicity tests and was minimized by feeding worms a sufficient amount of *E. coli* or by adjusting the initial eggs. The growth rate assay can also be conducted in liquid with a 96-well plate platform using a high-throughput imaging analysis system to obtain microscopic pictures [19]. Growth rate assays are especially useful in the assessment of toxins or side effects of bioactive substances at different developmental stages. Wittkowsky et al. demonstrated that correlation existed between the growth retardation effect of toxic chemical substances on *C. elegans* and a reduction in liver weight in rats [20].

Locomotion assay

Locomotion is a remarkable phenotype in studying aging, neuronal behavior, and metabolism in *C. elegans*. Worms have distinct forms of locomotion including swimming, burrowing, or crawling in response to complex environments, and these forms have a pattern that is classified into the following categories: forward locomotion, backward locomotion, dwelling, and quiescence [21]. Despite some different patterns of motor control, swimming, and crawling are not qualitatively different, as there is a linear correlation among frequency and wavelength amplitude. In the laboratory, locomotive behaviors focus on kinematic parameters, especially forward locomotion, in the form of worm trails and shape. The basic assay follows the steps of transferring a number of worms into agar plates or liquid media and recording their movement under a microscope [22]. The parameters of locomotion, such as body bends and velocity, can be calculated based on the frame-to-frame image of recorded video. The movement of the tip of the tail from one side to the other is counted as one body bend. The straightness rate was represented by the ratio of distance traveled to track the length. The dwelling time periods when the worms moved less than one body bend forward or backward are also determined. In addition to the manual count of body bends, models and software have been used to automatically analyze other motion parameters, such as wave initiation rate,

asymmetry, stretch, and curling [23]. For large-scale screening purposes, microfluidics technology has also been applied to evaluate worm locomotion in liquid environments. Recently, a 3D system to perform burrowing assays on Pluronic gel was reported [24].

Pharynx pumping assay

Caenorhabditis elegans is famous for its neural circuits underlying behavior, especially its feeding motion. The rhythmic feeding motions, termed pharyngeal pumping, are controlled by an autonomous network of 20 neurons of 14 types. Therefore, pharynx pumping is not only correlated with the rate of food intake and the rate of growth but is also connected with various chronic diseases, such as obesity, type 2 diabetes, cardiovascular diseases, and cancers [25, 26]. Similar to the locomotion assay, the conventional pharynx pumping assay relies on visually scoring pumps of short recordings during a particular time [27]. Counting the number of pumps, which is one complete cycle of contraction and relaxation of the corpus and the terminal bulb, per minute is the simplest way to determine the pumping rate. Pharynx pumping assays are often used to estimate eating behavior changes and healthspan extension [28].

Egg laying assay

Caenorhabditis elegans egg laying is another established rhythmic behavior of interest in neuroscience and signaling research, as it is controlled by neural circuits and turns in behaviors [29]. After egg synchronization, initial plates with and without the compound of interest that contained five L4 worms were prepared. Those worms were left to lay eggs and were transferred daily to fresh plates alongside the count of egg number laid on previous plates. In addition, the worms that crawled off, died, or were internally hatched were counted. The process continued until the adults stopped laying eggs, and approximately five days for the wild-type worms and total eggs at the end of the experiment were recorded [17]. Egg laying assays can be used to evaluate the reproductive toxicity of chemicals [17, 18].

Another aspect of egg-laying behavior is the egg-in-worm assay, which counts the number of eggs retained in the uterus of *C. elegans* [30]. Egg laying retention is an effort to protect their progeny until the environment becomes more favorable, which indicates the toxicity of substances or drugs that affect neurotransmitter signaling. This assay takes advantage of bleaching solution to dissolve the cuticle, thus making the eggs visible.

Reactive oxygen species (ROS) measurement

ROS levels are a remarkable indicator of redox status, giving clues to inflammatory or aging processes [31]. Due

to transparency, using fluorescent or chemiluminescent probes that can be taken up by cells and emit detectable signals is a common method to measure ROS in *C. elegans*.

Generally, synchronized worms are routinely grown in nematode growth medium plates coated with *E. coli* OP50 as a food source. ROS measurement is often conducted under stress conditions such as toxins, heat, or supplementation with chemicals. On the day of the assessment, the worms were incubated with the target dye and permitted to eat the dye or transferred to a multiwell plate containing dye. After destaining the dye by washing or allowing animals to forage on seeded plates, fluorescence or chemiluminescence was measured in a microplate reader or by mounting the worms and was visualized under a fluorescence microscope. Confocal microscopy, electron spin resonance, or high-performance liquid chromatography (HPLC) with detection by absorbance, fluorescence, or mass spectrometry (MS) are also used for quantification [31].

Each dye has a specific mechanistic target for different types of ROS, enabling the tracking of ROS formation in specific compartments. For example, 2',7'-dichlorodihydrofluorescein diacetate, the most common reporter for intracellular ROS, enters the membrane and is then converted to 2',7'-dichlorodihydrofluorescein and then to 2',7'-dichlorofluorescein, which is impermeable and highly fluorescent, upon oxidation [31, 32]. Regarding mitochondrial ROS, the MitoTracker Red CM-H₂Xros or MitoSOX are useful probes for detecting O₂•⁻ or H₂O₂. Although MitoTracker Red has greater sensitivity, MitoSOX would be preferable over MitoTracker Red in certain cases due to its lower non-specific fluorescence [33]. Other fluorescent probes have been used, such as peroxy orange I, 2-methyl-6-(p-methoxyphenyl)-3,7-dihydroimidazole[1,2-a]pyrazin-3-one (MCLA) or CellROX Green [34–37]. CellROX Green was used by Min et al. to study the influence of maternal nicotinamide supplementation on mitochondrial ROS in aged *C. elegans* [37]. Most recently, a novel fluorogenic probe (CBH) [38], a nuclear-localized fluorescent probe (Nuclear Peroxy Emerald 1) [39], and a near-infrared fluorescent probe (DCHP) [40] with high sensitivity and selectivity have been introduced as promising analysis tools in studying oxidative-related conditions such as inflammation or Parkinson's disease. The detailed characteristics of the probes used in *C. elegans* are summarized in Table 2.

Lipid quantification

The lipid content is an important indicator in elucidating metabolic disorders such as obesity [41]. Although many microscopy techniques are able to visualize cellular lipids, such as conventional light microscopy, Raman

microscopy, and anti-Stokes Raman microscopy, imaging techniques using fluorescent probes remain powerful to quantify lipid content and identify their distribution [42]. Sudan Black, Oil Red O, boron dipyrromethene (BODIPY), and Nile red are common dyes that have been used in cells as well as *C. elegans* to stain lipid droplets, which are cellular organelles for lipid storage containing neutral lipid cores [43, 44]. Sudan Black and Oil Red O are azo dyes used to stain fixed animals. The fixation step takes place with the addition of isopropanol solution to the synchronized population. Worms are allowed to settle down before incubation with staining dyes such as Oil Red O or Sudan black. Finally, the staining solution was removed, and the fixed worms were mounted onto agarose pads for visualization under a microscope [42, 45]. The fixed staining procedure can be time-consuming and inconsistent due to artifacts during the fixation, permeabilization, and washing steps [46]. The drawback of Oil Red O staining is that it is unable to visualize all fat stores in worms, and the signal from autofluorescence granules or lipofuscin and other nonspecific cellular organelles may decrease the accuracy [43]. In contrast, vital dyes such as Nile red and BODIPY that stain living worms when mixed with *E. coli* as a food source examine fat content in intact living animals, which may analyze epistasis in large-scale analyses. This approach also incorporates RNA interference screening to demonstrate the roles of hundreds of fat-regulatory genes [47]. However, the signal may be different with different endogenous uptake and transport pathways, affecting the conciseness in certain cases [41]. For example, Nile red stains only lysosome-related organelles by accumulating in gut granules instead of all lipid stores. The result yielded by BODIPY staining was also variable because of the changes in dye uptake during food consumption. In addition to the common commercial lipid staining dyes mentioned, fluoranthene FLUN-550 and 7-((4-methoxyphenyl)benzo[c]-[1,2,5]thiadiazol-4-yl)amino)-4-methyl-2H-chromen-2-one (BTD-Lip) were developed as new fluorescent probes for the selective quantification of intracellular lipid droplets [48, 49]. The staining pattern from BTD-Lip displayed a much more intense and highly specific signal without background noise than that of BODIPY [49].

Fluorescent protein expression

Since the first introduction of the gene for green fluorescent protein, *gfp*, in *C. elegans* by Dr. Chalfie in 1994, these genetically encoded sensors have become one of the standard tools of research in *C. elegans*. This technique is of significance in worms because of their transparency and thin diameter that allow live visualization under a fluorescence microscope [50]. Moreover,

Table 2 Available probes for detecting ROS in C. elegans

Probe	Reaction	Target	Pros	Cons	Refs
2,7'-Dichlorofluorescein diacetate	Oxidation of 2,7'-dichlorodihydrofluorescein to fluorescent 2,7'-dichlorofluorescein	H ₂ O ₂ , •HO, and ROO•	Detects ROS in the whole body	- Exerts free radical properties itself - Lacks specificity	[31]
Amplex red	Oxidation of 10-acetyl-3,7-dihydroxyphenoxazine by horseradish peroxidase in the presence of H ₂ O ₂ that produces a red fluorescence	H ₂ O ₂	- Both fluorometric and spectrophotometric measurement - Detect ROS in the surrounding medium	- Causes autooxidation and a self-amplification of the fluorescent signal - Forms superoxide in the presence of NADH	[154, 155]
MitoSOX	Oxidation of dihydroethidium by O ₂ •− to fluorescent ethidium	Mainly O ₂ •−	- Targets the mitochondria - Provides less background fluorescence signal	- Possibly reacts with cell components like cytochrome C	[31]
MitoTracker Red CM-H ₂ Xros	Oxidation of the reduced form to the red-fluorescent rosamine	H ₂ O ₂	Mitochondria selectivity	- Poor detection of other ROS - Background signal generated from nonspecific lipid uptake of dye	[33]
Nuclear Peroxy Emerald 1	H ₂ O ₂ -mediated conversion of arylboronates to phenol	H ₂ O ₂	Measuring nuclear H ₂ O ₂ fluxes		[39]
Peroxy orange I	Oxidation of the fluorophore masked by boronate moiety by H ₂ O ₂ to unmasked fluorescence compound	H ₂ O ₂		- Competes with H ₂ O ₂ -scavenging enzymes - Reacts with ONOO−	[35]
MCLA	Forms N-methylacridone upon oxidation by O ₂ •−	O ₂ •− and singlet oxygen	Does not undergo redox cycling	- Low specificity - High background noise	[31, 36]
CellROX	Bright green fluorescence upon oxidation by ROS and subsequent binding to mitochondrial DNA	O ₂ •− and •HO	Detects many types of ROS		[34, 37]
CBH	Oxidizes the benzeneboronic group to phenol after reacting with H ₂ O ₂	H ₂ O ₂	High sensitivity and selectivity to H ₂ O ₂		[38]
DCHP	DCHP is oxidized to a fluorescent dicyanomethylene-4H-pyran derivative (DCM)	H ₂ O ₂	High sensitivity and selectivity to H ₂ O ₂		[40]

fluorescent proteins can be targeted to specific locations, which is useful in many highly specific biological processes, such as ROS production. In particular, *C. elegans* possesses much larger gene families related to oxidative status and pathways than other model organisms and humans [51]. The typical example is the group of superoxide dismutases (SODs). While most organisms have three SODs, five SODs are expressed in *C. elegans*.

A typical example of a protein probe in ROS evaluation is hydrogen peroxide sensor (HyPer), an H_2O_2 -specific probe constructed by combining the H_2O_2 -sensitive regulatory domain of the *E. coli* transcription factor OxyR and circularly permuted yellow fluorescent protein [52]. When exposed to H_2O_2 , an intramolecular cysteine disulfide bridge is formed, leading to a conformational change near the yellow fluorescent protein chromophore. HyPer has been applied in many studies in *C. elegans*. HyPer was modulated in the muscle cells of *C. elegans*, and therefore, the production patterns of H_2O_2 during the entire life span of wild-type N2, *daf-2*, and *daf-16* mutants were revealed, in which the developmental stage had higher levels of H_2O_2 , and this level was reduced at the start of the reproductive phase [53]. This study showed that a major advantage is the monitoring of real-time H_2O_2 levels during aging, which is currently not possible in other model animals. One consideration when using HyPer is its sensitivity to pH changes and its overexpression.

In addition to direct ROS measurement, GFP mutant worms such as SKN-1::GFP or GST-4::GFP strains have been used as an indicator of oxidative state. As SKN-1 (mammalian Nrf2 homolog) induces phase II detoxification gene expression, which is required for oxidative stress resistance and longevity, nuclear localization assays and downstream gene expression using those mutant worms have been utilized to elucidate the signaling pathways of natural product bioactivities [54–56].

RNA interference (RNAi) assay

RNAi serves as a simple and quick tool for assessing genetic interactions by introducing a specific double-stranded RNA to worms to silence a particular gene. The loss-of-function phenotype observations may then reveal gene functions [57, 58]. In *C. elegans*, RNAi experiments can be performed in worms using several different protocols, including microinjection, feeding, and soaking. A detailed description can be found in the review of Zhuang et al. [57].

The choice of method for introducing RNA depends on the experimental purpose. Although microinjection may be more technically difficult than other approaches, it may also yield rapid results using in vitro double-stranded RNA through polymerase chain reaction. The

simultaneous inhibition of two or more genes is also possible in this approach [58]. In contrast, the feeding of double-stranded RNA in microtiter format is simple and requires no specific technical system [59]. Despite its advantages, several important factors must be considered when interpreting RNAi results. The differences in the downregulation of gene expression depending on RNAi methods remain unclear. Moreover, there are differences between the knockdown phenotypes produced by RNAi and the genetic mutant phenotype [60].

Caenorhabditis elegans as useful tool in NP bioactivity research

Because of the advantages and available techniques mentioned above, many NP studies have been conducted using the *C. elegans* model. Table 3 summarizes some representative examples of the biological activities of NPs and probiotics in the *C. elegans* model.

Anti-aging

Many potential bioactive NPs that extend the worm lifespan have been investigated. Plant extracts serve as important sources of potential lifespan extension materials such as *Commiphora leptophloeos*, tart cherry, *Hibiscus sabdariffa* L., ginseng, *Glochidion zeylanicum*, and *Caesalpinia sappan* L. [54–56, 61–63]. Phenethylamine and N-acetylphenethylamine are metabolites from the oral commensal bacterium *Corynebacterium durum* that induce a significant and dose-dependent increase in the lifespan of *C. elegans* [64]. Among anti-aging and antioxidant NPs, many well-known anti-aging bioactive compounds are phenolic compounds such as myricetin, rutin, vitexin, quercetin, naringin, curcumin, epicatechin, and phenolic acids (protocatechuic, gallic, and vanillic acid) [63, 65, 66]. Curcumin, the pigment component from spices turmeric, is a well-known bioactive compound that has strong antioxidant activity due to the presence of phenolic hydroxyl groups at its active sites that quench ROS. Curcumin increased mean lifespan by 1.39 days under normal conditions as well as the survival rate during juglone-induced oxidative stress compared to those of the control group [67]. Another example is myricetin, a widely distributed substance found in tea, berries, fruits, vegetables, and medicinal herbs. Myricetin enhanced both the lifespan and health span of *C. elegans*, as evidenced by the prolongation of the mean adult lifespan by 32.9% without an increase in the pharyngeal pumping rate and motility of aged worms [68].

A variety of high-throughput assays have been developed to analyze the molecular mechanism of NP bioactivity. Nonsense mutants of *age-1*, *daf-2*, and *daf-16* are commonly used to identify biological pathways with lifespan-extending effects. The curcumin-mediated

Table 3 Representative bioactivities of natural products and probiotic bacteria in the *C. elegans* model

Category	Natural products	Main finding	Molecular pathway	References
Anti-aging	<i>Hibiscus sabdariffa</i> L. extract	↑ Lifespan by 24% ↓ Lipofuscin ↑ Intracellular ROS levels	DAF-16 and SKN-1	[54]
	Ginsenosides	↑ Lifespan by 14.02%	SKN-1, SIR-2.1, and DAF-16	[55]
	<i>Glochidion zeylanicum</i> leaf extracts	↓ Mortality by juglone by 42.23% ↓ Intracellular ROS by 41.33%	SOD-3, and GST-4 HSP-16.2 DAF-16 and SKN-1	[56]
	<i>Corynebacterium durum</i> metabolites	↑ Lifespan up to 21.6%	SIR-2.1	[64]
	Protocatechuic, gallic, and vanillic acid	↑ Lifespan and heat stress resistance	<i>sir-2.1</i> expression ATP depletion in aged nematodes	[66]
	Myricetin	↑ Lifespan and body bending in age worms	SIR-2.1	[72]
Anti-obesity	Luteolin	↓ Fat accumulation up to approximately 35%	Serotonin synthesis in ADF neurons to promote lipolysis and fatty acid β-oxidation	[82, 156]
	Chrysin	↓ Fat accumulation by 38.9%	DAF-16	[83]
	<i>trans</i> -Trismethoxy resveratrol	↓ Triglyceride accumulation by 14%	Downregulated stearoyl-CoA desaturase genes, <i>fat-6</i> and <i>fat-7</i>	[84]
	Bitter melon polysaccharides	↓ Triglyceride content	<i>daf-2</i> , <i>fat-5</i> , <i>fat-6</i> and <i>fat-7</i> mediated fatty acid desaturases pathways	[87]
Neuroprotective effect	Magnolol	↓ Paralyzed phenotype by 20%	PPAR-γ	[90]
	Hydroxytyrosol and oleuropein aglycone	↓ α-Synuclein accumulation by 14% at day 12	Proteasome activity	[98]
	<i>Mucuna pruriens</i> seed extract	↑ Median (25%) and maximum survival (47.8%) in dopaminergic neurotoxin treatment		[93]
	Olive polyphenols	↑ The activity index by 142% in a rotenone-induced PD model ↓ α-Synuclein by 17%		[156]
Gut health improvement	3,3'-Diindolylmethane	↓ <i>C. elegans</i> intestinal permeability triggered by <i>P. aeruginosa</i> infection by 14.81%		[100, 101]
	<i>L. casei</i>	↑ Egg laying and pharyngeal pumping in sick worms ↓ <i>K. pneumoniae</i> colonization in the intestine	TLR, RACK-1 and p38 MAPK	[105]
	<i>E. coli</i> strain Nissle 1917	↓ Relative permeability of a pathogenic <i>E. coli</i> infected gut ↓ Body-cavity leakage by approximately 20%	ZOO-1	[106]
Anti-infective effect	<i>Diplocyclos palmatus</i>	↑ Survival time by 50% ↓ Colonization of <i>S. marcescens</i>	<i>daf-16</i> and immune-related genes (<i>clec-60</i> , <i>clec-87</i> , <i>lys-7</i> and <i>bec-1</i>)	[117]
	5-Hydroxymethyl-2-furaldehyde	↑ Survival time by 24 h	Multidrug resistance gene expression	[118]
	<i>Bifidobacterium</i> spp.	↑ Survival rate against enterohemorrhagic <i>E. coli</i>	Transcription of the genes encoding virulence factors	[121]
	Broccoli extract	↑ Survival rates of <i>C. elegans</i> increase by 28.5%	<i>luxS</i> and <i>pfs</i> genes QS-mediated bacterial virulence	[122]
	Honokiol and magnolol	↓ Lethality rate in <i>C. elegans</i> during MSSA infection	Modulation of immune response IFN-β and IFN-λ	[123]
	Hypericin and Ampicillin Cotreatment	Recovered body sizes that are similar to vehicle control worms		[125]
	<i>Tripterygium wilfordii</i> extract	↑ Survivability of <i>S. pyogenes</i> infection		[126]

lifespan-extending effects mentioned were modulated by *age-1* and *skn-1* [67]. A study demonstrated that fullereneol decreased the endogenous ROS levels and protected *C. elegans* by upregulating stress-related genes in a DAF-16-dependent manner, thus improving lifespan [69]. In the case of myricetin, studies have suggested that the regulation of transcription factors DAF-16 (mammalian FOXO homolog) and SKN-1, the promotion of mitochondrial function via SIRT, and the inhibition of protein misfolding through protein aggregation are possible mechanisms of myricetin's effects [68, 70–72]. SIRT was also found to be involved in the activity of other NPs, such as curcumin, monoamines, oligonol, and 5,5'-diferyllic acid, suggesting that epigenetic mechanisms are potential targets in screening anti-aging compounds [64, 73–75].

Somatic aging is related to reproductive aging, which is the earliest aging phenotype in *C. elegans* [76]. Reproductive aging begins in day-3 adult hermaphrodites, whereas intestinal aging begins in day-10 adult hermaphrodites [77]. Furthermore, in the germ line of *C. elegans* hermaphrodites, the entire process of germ cell development, including germ cell proliferation, gametogenesis, and germ cell death, can be observed at the same time [78]. Therefore, *C. elegans* is an excellent model organism to investigate reproductive capacity with age in association with somatic aging. It was previously reported that nicotinamide supplementation improves oocyte quality in an aging *C. elegans* model [37], suggesting that the roles of potential anti-aging NPs can be investigated to understand the molecular link between soma and the germ line in the process of aging using *C. elegans* as a model.

Anti-obesity

C. elegans stores fat in the form of lipid droplets in its intestinal and hypodermal cells instead of adipose tissue in mammals [41]. With the ease of visualization under microscopy by using lipid staining dyes, *C. elegans* is useful in the screening of therapeutic compounds that potentially decrease body weight through the reduction in lipid droplets. Many plant extracts (*Momordica charantia*, *Ilex paraguariensis*, and chia seed oil) as well as compounds such as flavonoids (baicalein, chrysin, scutellarein, 6-hydroxyflavone, apigenin, chrysin, luteolin, kaempferol, myricetin, and quercetin) have been shown to reduce fat accumulation [79–83]. L1 worms treated with *Ilex paraguariensis* extract had 63.36% less intestinal fat than that of the control worms in the BODIPY fat staining assay [79]. *trans*-Trismethoxy resveratrol, a methyl analog of resveratrol at a concentration of 200 μ M, significantly reduced triglyceride accumulation by 20% without interfering with nematode growth, food

intake, and fecundity [84]. Similarly, luteolin showed a potent anti-fat effect, and the effect is mediated by the induction of lipolysis and fatty acid β -oxidation that is triggered by central serotonin signaling [82]. Based on natural sources, the lipid-reducing efficacy of many nanoconstructions was also tested in the *C. elegans* model. Curcumin-loaded nanoemulsions and liposomes loaded with ethanolic extract of purple pitanga, exerted a significant fat reduction in *C. elegans* [85, 86]. In addition to the lipid-reducing effects in wild-type worms under normal conditions, diet-induced obesity and associated metabolic disorders were demonstrated. *Momordica* saponin extract significantly decreased the Oil Red O staining intensities by 21% and 24% in worms fed a normal diet and a glucose diet, respectively, which indicates the preventive and therapeutic effects of *Momordica* saponin extract [81]. Barley β -glucan, one kind of polysaccharide from bitter melon, could reduce fat accumulation in *C. elegans* with excessive glucose intake via the insulin/IGF-1 and fatty acid desaturase-dependent pathways. It was also found that barley β -glucan reduced fat accumulation by affecting *fat-5*, *fat-6*, and *fat-7*, which mediated fatty acid desaturase pathways in *C. elegans* [87].

Anti-neurodegeneration

To date, many neurodegenerative disease models have been established using *C. elegans*, including models for Alzheimer's disease (AD), Parkinson's disease (PD), and polyglutamine expansion diseases [88]. Through the overexpression of neurodegeneration-associated genes, such as beta amyloid (A β) peptides and neurofibrillary tangles of hyperphosphorylated tau proteins in AD or alpha-synuclein and other genes such as Lewy bodies in PD, clues were revealed through the correlations between the genotype of human diseases and the phenotypes of transgenic *C. elegans*. Based on this principle, the neuroprotective effects of various natural compounds, including *Cleistocalyx nervosum* var. *paniala* extract, magnolol from *Magnolia officinalis*, and caffeine were documented in the assessment of various worm strains [89–91]. Selvaraj et al. demonstrated the neuroprotective effect of a chalcone derivative in 6-hydroxyl dopamine-injured wild-type *C. elegans* N2, which is an experimental model of oxidative stress-induced dopaminergic neurodegeneration [92]. CL4176 is a transgenic worm that possesses an A β -dependent paralysis phenotype due to the expression of the human A β 1–42 peptide in muscle cells. Supplementation with magnolol (2.5–10 μ M) delayed the onset of the paralyzed phenotype, in which the time to paralyze 50% of worms treated with 5 μ M magnolol was 10 h, 20% longer than that in worms treated with accepted anti-AD drug [90]. Caffeic acid (300 μ M)

prolonged the mean lifespan by 15.57%, and *daf-16* expression was significantly upregulated in caffeic acid-treated CL4176 worms [91]. Similarly, using another A β -expressing worm, CL2006, *Cleisto calyx nervosum* var. *paniala* extract (10 μ g/mL) reduced A β toxicity by increasing the median lifespan to 28 days compared to the untreated control of 22 days. The effect was further explained through the involvement of the DAF-16 pathway, in which *daf-16* was upregulated, while *daf-2*, *age-1*, and *utx-1* were downregulated significantly [89].

In the PD model, the marker is the degeneration of dopaminergic neurons and the accumulation of Lewy bodies containing aggregated α -synuclein protein [88]. Many extracts, including red seaweed *Chondrus crispus*, *Sorbus alnifolia*, *Mucuna pruriens* seed extract, *Dioscorea alata* L. tubers, and *Holothuria leucospilota*, were reported to reduce the aggregation of α -synuclein in a transgenic model expressing “human” α -synuclein worms [93–97]. Two polyphenols from olive oil, namely, hydroxytyrosol and oleuropein aglycone, attenuated the α -synuclein-induced locomotion impairments, in which the movement indexes, such as the wave initiation rate and body wave number on day-7 of adulthood. The increase in degenerated neurons

with age was also completely blocked by 250 μ g/mL of hydroxytyrosol [98]. Worms grown from L1 on an *E. coli* diet supplemented with probiotic *Bacillus subtilis* crude extracts or vegetative pellets showed a reduction in α -synuclein aggregation, partially demonstrating the anti-Parkinson effect of active and stable *B. subtilis* metabolites [99].

Gut health improvement

C. elegans can be a good animal model for in vivo experiments to evaluate the effects of NPs on intestinal permeability and gut health. Le et al. established a high-throughput image analysis system that screens intestinal permeability alterations by various chemicals and pathogenic bacteria in *C. elegans* [100]. Kim et al. demonstrated that 3,3'-diindolylmethane, a digestive metabolite from broccoli, ameliorated intestinal permeability dysfunction and extended the lifespan of *C. elegans* fed the intestinal pathogen *P. aeruginosa* PAO1 [101]. The mean lifespan of 3,3'-diindolylmethane-treated worms (10.8 ± 1.3 days) was higher than that of the vehicle control worms (9.7 ± 1.1 days). The general working scheme of the phenotype-based gut permeability HTS of NPs against gut pathogens is illustrated in Fig. 2.

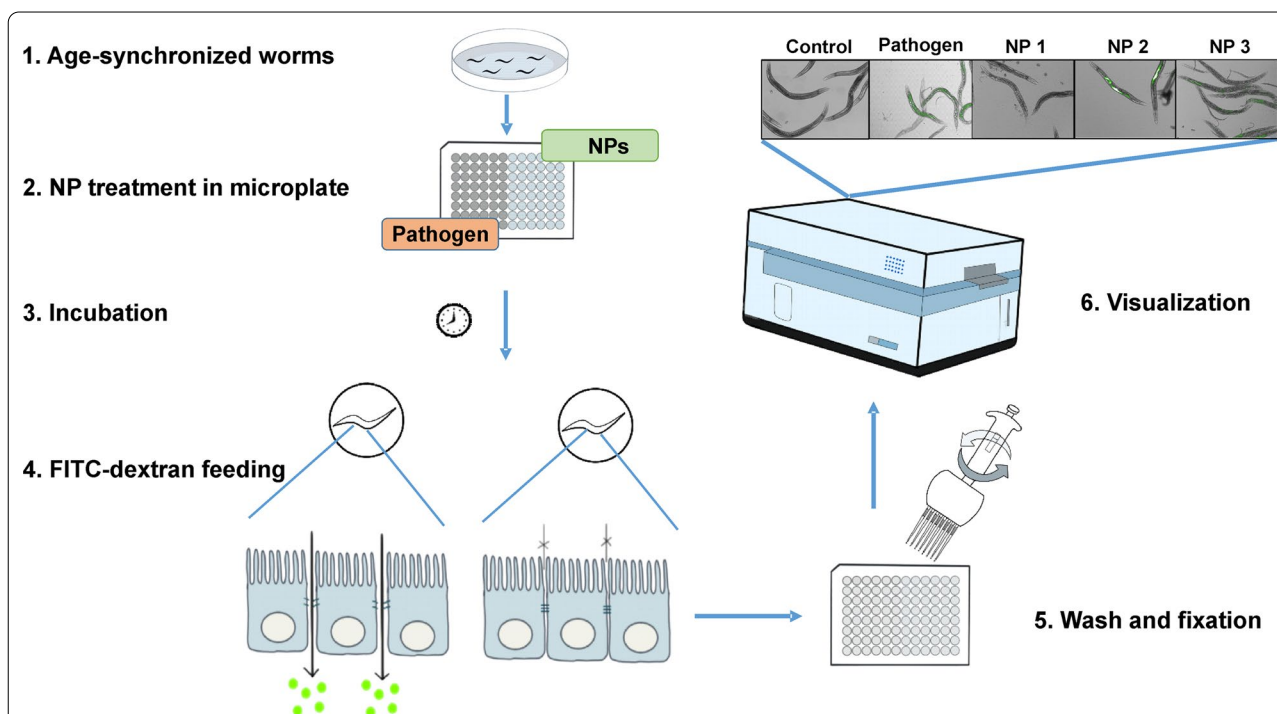


Fig. 2 Working scheme of HTS for screening natural products (NPs) that improve gut permeability. Age synchronized worms are infected with the pathogen and cotreated with NPs for an appropriate time. Worms are then fed with FITC-dextran as an indicator of gut permeability. After washing and fixing in 96-well plates, a high-throughput Operetta machine is used to measure the intestinal permeability of *C. elegans*. Infected worms show higher fluorescence intensity due to intestinal damage. Effective compounds are screened as having lower fluorescence intensity, indicating the improving effect against gut barrier dysfunction

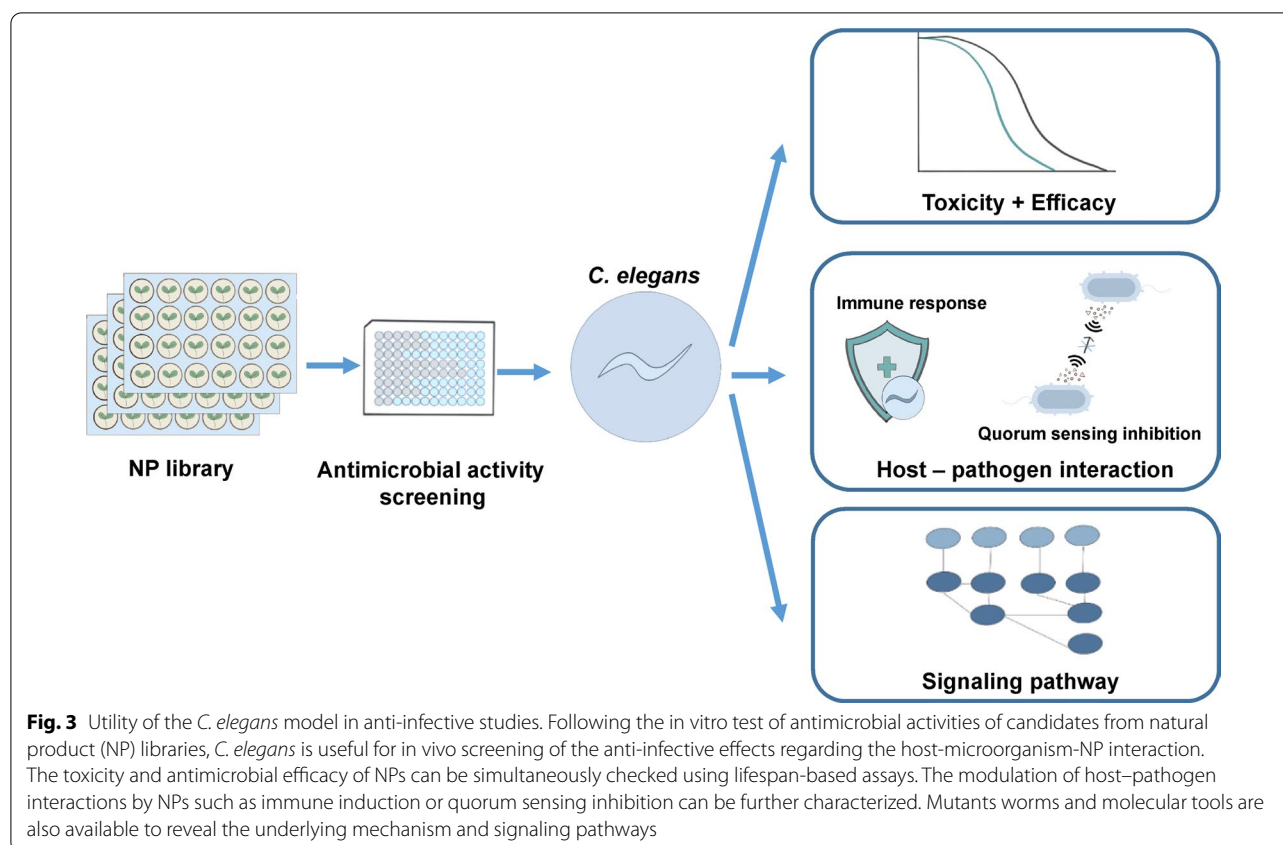
In addition, as bacteria associated with the animal gut are important for gastrointestinal function, *C. elegans* is a meaningful model to study the interaction among microbiota, pathogen, and food: the worms use bacteria as food, and the laboratory culture is a mono-association. Han et al. have suggested that the beneficial effects on longevity in worms may be exerted through modulation of the gut microbiota. *E. coli* mutants deficient in some biochemical components can extend *C. elegans* longevity. They reported that the increased secretion of the polysaccharide colanic acid by *E. coli* mutants extended the lifespan and decreased age-related pathologies by regulating mitochondrial dynamics and the unfolded protein response in *C. elegans* [102]. Similarly, metformin, a synthetic derivative of guanidine, which is a drug for treating type 2 diabetes, can extend the lifespan and regulate lipid metabolism via production of agmatine, a metabolite derived from the gut microbiota [103]. In addition to the metabolites from microbes, *C. elegans* was utilized to assess the effect of probiotic strains [104]. Studies have shown that a strain of probiotic *Lactobacillus rhamnosus* or *Weissella* bacteria activates the DAF-16 signaling pathway and extends the lifespan of *C. elegans* compared to feeding with a normal diet of *E. coli*. *L. casei* rescued worms against *K. pneumoniae* infection by strengthening host resistance in a p38 MAPK-dependent manner [105]. A study showed that a nonpathogenic strain of *E. coli* can increase the survival of enteropathogenic *Escherichia coli*-infected worms and interfere with pathogen colonization through a decrease in the luminal level of GFP-labeled enteropathogenic *E. coli* in the worm intestines. This protective effect that resulted from the improvement of epithelial cell integrity was also confirmed using two markers of tight junction protein, ZOO-1 (human ZO-1 homolog) and F10A3.1 (human claudin homolog) [106]. Recently, Kim et al. reported that *L. casei* HY2782 treatment prevented a particulate matter-induced decline in reproduction and locomotion activity in *C. elegans* via the inhibition of intestinal cell death [107]. However, because the main difference between the microbiota in *C. elegans* and humans is that the gut microbiota composition in the *C. elegans* experiment is a single bacterial species, further studies are needed to fill this gap.

Anti-infective effect

Using a basic lifespan assay in a *C. elegans* pathogen infection model, many NPs exhibited antivirulence effects against a wide range of pathogens, such as *Phyllanthus emblica*, EGCG, lignans (sesamin and sesamol) from *Sesamum indicum*, and clove bud oil against *P. aeruginosa* [108–111]. An in vivo assay using *C. elegans* has advantages over a conventional anti-infective screening approach because it can assess efficacy and toxicity at the

same time, which eliminates compounds that are toxic to the host at early stages or have poor drug-like properties, while the latter identifies only direct antimicrobial compounds [112]. The basic scheme is based on an excess of live worms compared to nontreated pathogen-infected nematodes to exclude both ineffective compounds and hit compounds that are highly toxic to worms. Based on this principle, Kong et al. successfully revealed that plant extract of *Orthosiphon stamineus* leaves and its active compound eupatorin improved the survival of *S. aureus*-infected worms through immunomodulation. They also found that liquid-based assays are more sensitive than conventional agar-based assays in detecting hit compounds [113]. In addition, Yang et al. reported that the three major active compounds from rhubarb (emodin, rhein, and aloe-emodin) increased the survival of worms infected with *S. aureus* and inhibited the growth of *S. aureus* replication by using an integrated microfluidic platform [114]. Toxicity tests serve as tools in discovered antihelminthic agents apart from antibacterial and antifungal compounds, and motility/mortality assays or locomotion bioassays have been used with wild-type and mutant *C. elegans* to screen nematocide compounds from traditional Chinese medicines [115].

When testing whole live *C. elegans*, the interaction between compounds and pathogens as well as the host immune system are easily observed, contributing to the identification of underlying anti-infective mechanisms. Using a worm model also enables the screening of bioactive compounds that only exhibit anti-infective effects in a host pathogen factor modulation-dependent manner. Figure 3 illustrates the use of *C. elegans* in revealing the mode of action in anti-infective studies. One strategy of bioactive compounds to suppress pathogen infection is to control the virulence of pathogens, such as bacterial membrane microdomains, toxin neutralization, biofilm inhibition, and quorum-sensing (QS) interference [116]. QS is a complicated cell-to-cell communication system that regulates the expression of various virulence factors in gram-positive and negative bacteria, making it an attractive target for antivirulence treatment. It is also believed that QS-inhibiting agents could disrupt the protective biofilms of bacteria, leading to an increase in antimicrobial efficacy. Many natural inhibitors acting on QS and biofilms that have been tested using *C. elegans* are essential oils from *Cymbopogon* spp. and *Cinnamomum verum* against *E. coli* O157:H7, *Diplocyclos palmatus* against *Serratia marcescens*, 5-hydroxymethyl-2-furaldehyde from marine bacterium *B. subtilis* and *Hibiscus sabdariffa* extract against *Candida albicans* [117–120]. In the worm-*S. marcescens* infection model, 600 µg/mL *D. palmatus* extract extended the lifespan by 140 h compared with the 70 h control, which clearly confirmed the



in vivo disease protection efficacy of *D. palmatus* extract. The results also proved that *D. palmatus* extract has anti-QS activity that further exhibits anti-adhesion activity on *S. marcescens*-infected *C. elegans*. through microscopic images and colony forming unit counting assays [117]. Similarly, both broccoli extract and *Bifidobacterium longum* extract increase the survival of sick *C. elegans* by inhibiting QS signaling molecule-autoinducer-2 activity [121, 122].

Another mechanism of interest is the activation of the worm immune system. The polyphenols isolated from magnolia plants, i.e., honokiol and magnolol, rescued worms from *S. aureus* infection [123]. The induced expression of *lys-7*, p38 MAP kinase, and insulin-like signaling pathways using GFP worms was evidence for the induction of innate defense by the plant extracts *O. stamineus* and *D. palmatus* [113, 117].

Antimicrobial photodynamic therapy (APDT) is an alternative therapeutic method for the control and treatment of pathogen infections. APDT is based on the use of photoactive dye molecules, which are widely known as photosensitizers. Upon irradiation with a specific wavelength of light, photosensitizers produce ROS that can destroy biomolecules such as lipids and proteins, causing microbial cell death [124]. The APDT effects

using plant-derived photosensitizers, hypericin and plant extract of *Tripterygium wilfordii* were evaluated on *C. elegans* infected with various pathogenic bacteria and fungi. After APDT using natural compounds and extracts, *C. elegans* survived without significant side effects, and the growth retardation induced by pathogen infections was reversed [125, 126].

Instrumental method for NP research using *C. elegans*

High-throughput screening using *C. elegans*

With the diversity of origin and structure of natural compounds, the screening of bioactive compounds is an emerging issue that needs to be investigated. Nowadays, with the development of many HTS methods, thousands to millions of natural compounds can be screened simultaneously for targeted bioactivity. Among many model animals, *C. elegans* is one of the most suitable for organism-level phenotype-based HTS because of its advantages such as small size, transparent body, cost effectiveness, maintainability, and speed. Natural compounds and extracts were assessed for bioactivity by evaluating various phenotypic characteristics, such as growth, lifespan, reproduction, movement of the worm, or intestinal permeability.

Moy et al. have developed an HTS method to find compounds that enhance the survival of *C. elegans* infected with *E. faecalis*. A total of 37,200 compounds and natural extracts were screened in this study, and 28 compounds and extracts were reported to have antimicrobial activity, but they did not affect the growth of the pathogen in vitro [127]. A library of 1280 compounds was screened by Ye et al. to identify compounds that increase the lifespan of *C. elegans*. Sixty compounds were found to increase the longevity of worm, 33 of which also increased the oxidative stress resistance of *C. elegans*. Many of the candidate compounds are drugs approved for human use, such as minocycline hydrochloride, cinnarizine, and vincristine sulfate [128]. Lucanic et al. screened over 300,000 compounds to identify new chemical structures that extend the lifespan of *C. elegans* through a dietary restriction mechanism. They described that out of 57 compounds found to prolong *C. elegans* lifespan, 3 compounds contained a nitrophenyl piperazine backbone and induced a significant lifespan extension [129]. Taki et al. developed an HTS method to establish the effect of small molecules on the motility of *C. elegans* using infrared light interference. A total of 14,400 compounds were screened by this method, and the results showed that 43 compounds decreased worm motility by $\geq 70\%$, equating to a hit rate of 0.3% [130].

High-performance liquid chromatography (HPLC) for evaluating NP metabolism in *C. elegans*

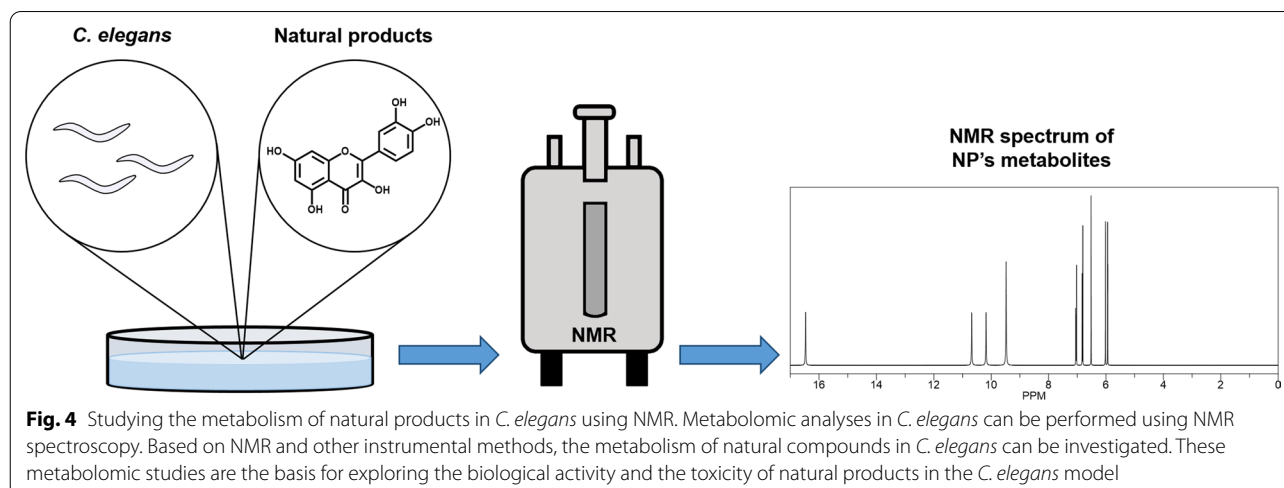
HPLC is a simple, convenient method used for quantifying natural compounds. Zheng et al. evaluated the metabolism of resveratrol, a natural phenolic compound with a good antioxidant effect on *C. elegans* by HPLC. The results showed that the rate of metabolism of resveratrol was dependent on both dose and time.

The concentration of resveratrol in worms ranged from approximately 300 to 600 mg/kg after treatment with 100 μM resveratrol, which was comparable with studies in mice, which ranged from 4.9 to 400 mg/kg [131]. The HPLC–UV method was developed by Stupp et al. to evaluate the metabolism of two toxicants, 1-hydroxyphenazine and indole, released by *P. aeruginosa* and *E. coli*, respectively. The analysis results show that the worms can glycosylate both toxins, a metabolic modification that significantly decreases their toxicity [132].

Nuclear magnetic resonance (NMR) and mass spectrometry (MS) using *C. elegans*

NMR and MS have already been demonstrated to be effective tools for metabolic profiling in *C. elegans* by investigating the metabolism of amino acids, organic acids, choline, sugars, nucleotides, or cofactors [133]. Figure 4 illustrates the use of NMR to study the metabolism of NP. NMR and MS have been used to investigate the metabolic changes in *C. elegans* exposed to toxicity. The metabolic change in *C. elegans* exposed to the heavy metal nickel, the pesticide chlorpyrifos, and their mixture was reported using gas chromatography–MS and NMR. It has been reported that novel metabolic profiles are associated with both exposure and dose levels. In addition, changes in branch chain amino acids and tricarboxylic acid cycle intermediates were also observed [134].

Ascarosides are a group of water-soluble small molecules secreted by *C. elegans* for chemical communication to control certain behaviors, such as mating attraction, aggregation, and avoidance. Zhang et al. reported an experimental method for analyzing the types and concentration of ascaroside in *C. elegans* by NMR and liquid chromatography–tandem mass spectrometry LC–MS/MS [135]. Stasiuk et al. reported the biotransformation of five benzimidazole



anthelmintics, namely, albendazole, mebendazole, thia-bendazole, oxfendazole, and fenbendazole, in *C. elegans* by LC–MS/MS analysis. The results showed that glucose conjugation is the primary biotransformation pathway for benzimidazole drugs in *C. elegans*. The biotransformation of albendazole by *C. elegans* reduced drug efficacy and was inhibited by the UGT inhibitor chrysin [136]. Interestingly, Nguyen et al. demonstrated real-time metabolomics changes in *C. elegans* by using in-organism NMR analysis [137]. Currently, the applications of NMR and MS are mainly in studies of the metabolism of *C. elegans*. Therefore, these methods will be useful for evaluating the pharmacokinetics (absorption, distribution, metabolism, elimination, and toxicity) of natural compounds in the *C. elegans* model.

Emerging technologies in *C. elegans* studies

Currently, an increasing number of techniques have been developed to support research on the *C. elegans* model. Modern technologies, such as artificial intelligence, machine learning, and computational techniques, have been applied to simulate the body structure, nervous system, and behaviors of *C. elegans*. OpenWorm is one of the first projects to simulate *C. elegans* at the cellular level. This project's long-term goal is to model all 959 cells of *C. elegans*, and users can access datasets about *C. elegans* neuronal structure [138].

An automatic pipeline, CShaper, was developed by Cao J et al. [139]. It was applied to quantify the morphological parameters of cells in 17 developing *C. elegans* embryos. A time-lapse 3D atlas of cell morphology for the worm embryo from the 4- to 350-cell stages has been generated, which consists of cell shape, cell volume, cell surface area, cell migration, nucleus position, and cell-to-cell contact.

Xu et al. investigated the chemotaxis behaviors of *C. elegans* through association with biological nerve connections [140]. These behaviors include food attraction, toxin avoidance, and mixed behaviors (finding food and avoiding toxins simultaneously). Eight dynamic neural network models, two artificial models, and six biological models were used to understand the chemotaxis behaviors of worm. The results showed that the developed models could effectively simulate the real chemotaxis behaviors of *C. elegans* in different environments.

Martineau et al. reported a multidimensional phenotyping method that predicts lifespan and quantifies healthspan in *C. elegans* [141]. Multiple phenotypes at the organismal scale were characterized to measure the aging process, including the morphological, postural, and behavioral changes extracted from high-resolution videos. In a total of 1019 features extracted, 896 aging biomarkers correlated with relative age, and vector regression was used to predict the age, remaining life, and lifespan

expectancy of worms. More features added to the model lead to an increase in the quality of the prediction.

Concluding remarks and future prospective

This article has included some general information about *C. elegans* and the advantages of applying the *C. elegans* model in NP bioactivity research. The mechanism of action at the cellular and molecular levels of natural compounds has also been elucidated through studies based on the *C. elegans* model. Techniques to evaluate the effects of NPs in the *C. elegans* model are also being improved and developed. Many valuable biological activities of NPs have been discovered and studied using *C. elegans* models, such as anti-aging, antiobesity, anti-neurodegeneration, gut health improvement, and anti-infective effects. With the diversity in the number and structure of natural compounds, HTS methods are essential to shorten research time and save costs for screening and discovering natural compounds with interesting biological effects. With the advantages of their body composition, short life cycle, and rapid reproduction, *C. elegans* has become a particularly useful tool in HTS methods for the discovery of NP bioactivity. Moreover, research on the *C. elegans* model is also developing daily with the effective support of modern technologies. Therefore, the *C. elegans* model will shortly become an effective tool to screen before conducting studies in mammalian animal models to provide sufficient scientific evidence for the efficacy and safety of natural compounds before preclinical and clinical trials. In addition, *C. elegans* can be a convenient and consumer-friendly experimental model for elucidating the molecular genetic mechanism underlying the bioactivity of popular commercial nutraceuticals with unclear modes of action, and dealing with animal ethical issues is not a problem. Establishing theories of the NP bioactivity and performing experimental verification with the *C. elegans* model will provide valuable information for NP science and technology.

Abbreviations

AD: Alzheimer's disease; APDT: Antimicrobial photodynamic therapy; A β : Amyloid beta peptide (A β); BODIPY: Boron dipyrromethene; BTD-Lip: 7-((7-(4-Methoxyphenyl)benzo[c]-[1,2,5]thiadiazol-4-yl)amino)-4-methyl-2H-chromen-2-one; GFP: Green fluorescent protein; HPLC: High-performance liquid chromatography; HTS: High-throughput screening; LC–MS/MS: Liquid chromatography-tandem mass spectrometry; MCLA: 2-Methyl-6- (4-methoxyphenyl) -3,7-dihydroimidazo [1,2-a] pyrazin-3(7H)-one; MS: Mass spectrometry; HyPer: Hydrogen peroxide sensor; NMR: Nuclear magnetic resonance; NP: Natural product; PD: Parkinson's disease; QS: Quorum sensing; ROS: Reactive oxygen species; SOD: Superoxide dismutase.

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Authors' contributions

Conceptualization, NMH, SHT, and KK; Original draft writing, NMH, SHT, and YHS; Review and editing, NMH, YHS, and KK. All authors read and approved the final manuscript.

Declarations

Competing interests

The authors declare that they have no conflicts of interest.

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References

- Kenyon C (1988) The nematode *Caenorhabditis elegans*. *Science* 240:1448–1453
- Kaletta T, Hengartner MO (2006) Finding function in novel targets: *C. elegans* as a model organism. *Nat Rev Drug Discov* 5:387–398
- Forslund K, Schreiber F, Thanintorn N, Sonnhammer EL (2011) OrthoDisease: tracking disease gene orthologs across 100 species. *Brief Bioinform* 12:463–473
- Culetto E, Sattelle DB (2000) A role for *Caenorhabditis elegans* in understanding the function and interactions of human disease genes. *Hum Mol Genet* 9:869–877
- Corsi AK, Wightman B, Chalfie M (2015) A transparent window into biology: a primer on *Caenorhabditis elegans*. *Genetics* 200:387–407
- Kim Y, Park Y, Hwang J, Kwack K (2018) Comparative genomic analysis of the human and nematode *Caenorhabditis elegans* uncovers potential reproductive genes and disease associations in humans. *Physiol Genomics* 50:1002–1014
- Byerly L, Cassada RC, Russell RL (1976) The life cycle of the nematode *Caenorhabditis elegans*. I. Wild-type growth and reproduction. *Dev Biol* 51:23–33
- Strange K (2006) An overview of *C. elegans* biology. *Methods Mol Biol* 351:1–11
- Kormish JD, Gaudet J, McGhee JD (2010) Development of the *C. elegans* digestive tract. *Curr Opin Genet Dev* 20:346–354
- Johnson TE (2003) Advantages and disadvantages of *Caenorhabditis elegans* for aging research. *Exp Gerontol* 38:1329–1332
- Consortium CeS (1998) Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* 282:2012–2018
- Zhang S, Li F, Zhou T, Wang G, Li Z (2020) *Caenorhabditis elegans* as a useful model for studying aging mutations. *Front Endocrinol* 11:554994
- Park HH, Jung Y, Lee SV (2017) Survival assays using *Caenorhabditis elegans*. *Mol Cells* 40:90–99
- Puchalt JC, Layana Castro PE, Sánchez-Salmerón AJ (2020) Reducing results variance in lifespan machines: an analysis of the influence of vibrotaxis on wild-type *Caenorhabditis elegans* for the death criterion. *Sensors* 20:5981
- Felker DP, Robbins CE, McCormick MA (2020) Automation of *C. elegans* lifespan measurement. *Transl Med Aging* 4:1–10
- Puchalt JC, Sánchez-Salmerón AJ, Ivorra E, Llopis S, Martínez R, Martorell P (2021) Small flexible automated system for monitoring *Caenorhabditis elegans* lifespan based on active vision and image processing techniques. *Sci Rep* 11:12289
- Lee SY, Kang K (2017) Measuring the effect of chemicals on the growth and reproduction of *Caenorhabditis elegans*. *J Vis Exp* 128:e56437
- Lee SY, Kim JY, Jung YJ, Kang K (2017) Toxicological evaluation of the topoisomerase inhibitor, etoposide, in the model animal *Caenorhabditis elegans* and 3T3-L1 normal murine cells. *Environ Toxicol* 32:1836–1843
- Partridge FA, Brown AE, Buckingham SD, Willis NJ, Wynne GM, Forman R, Else KJ, Morrison AA, Matthews JB, Russell AJ, Lomas DA, Sattelle DB (2018) An automated high-throughput system for phenotypic screening of chemical libraries on *C. elegans* and parasitic nematodes. *Int J Parasitol Drugs Drug Resist* 8:8–21
- Wittkowski P, Marx-Stoelting P, Violet N, Fetz V, Schwarz F, Oelgeschläger M, Schönfelder G, Vogl S (2019) *Caenorhabditis elegans* as a promising alternative model for environmental chemical mixture effect assessment—a comparative study. *Environ Sci Technol* 53:12725–12733
- Gjorgjieva J, Biron D, Haspel G (2014) Neurobiology of *Caenorhabditis elegans* locomotion: where do we stand? *Bioscience* 64:476–486
- Lüersen K, Gottschling DC, Döring F (2016) Complex locomotion behavior changes are induced in *Caenorhabditis elegans* by the lack of the regulatory leak K⁺ channel TWK-7. *Genetics* 204:683–701
- Ibáñez-Ventoso C, Herrera C, Chen E, Motto D, Driscoll M (2016) Automated analysis of *C. elegans* swim behavior using CeleST software. *J Vis Exp* 118:e54359
- Lesanpezeshki L, Hewitt JE, Laranjeiro R, Antebi A, Driscoll M, Szweczyk NJ, Blawdziewicz J, Lacerda CMR, Vanapalli SA (2019) Pluronic gel-based burrowing assay for rapid assessment of neuromuscular health in *C. elegans*. *Sci Rep* 9:15246
- Blancas-Velazquez A, Mendoza J, Garcia AN, la Fleur SE (2017) Diet-induced obesity and circadian disruption of feeding behavior. *Front Neurosci* 11:23
- Diomedea L, Rognoni P, Lavatelli F, Romeo M, del Favero E, Cantù L, Ghibaldi E, di Fonzo A, Corbelli A, Fiordaliso F, Palladini G, Valentini V, Perfetti V, Salmons M, Merlini G (2014) A *Caenorhabditis elegans*-based assay recognizes immunoglobulin light chains causing heart amyloidosis. *Blood* 123:3543–3552
- Hao L, Buttner EA (2014) Methods for studying the mechanisms of action of antipsychotic drugs in *Caenorhabditis elegans*. *J Vis Exp* 84:e50864
- Kozlova AA, Lotfi M, Okkema PG (2019) Cross talk with the GAR-3 receptor contributes to feeding defects in *Caenorhabditis elegans* EAT-2 mutants. *Genetics* 212:231–243
- Koelle MR, Horvitz HR (1996) EGL-10 regulates G protein signaling in the *C. elegans* nervous system and shares a conserved domain with many mammalian proteins. *Cell* 84:115–125
- Gardner M, Rosell M, Myers EM (2013) Measuring the effects of bacteria on *C. elegans* behavior using an egg retention assay. *J Vis Exp* 80:e51203
- Labuschagne CF, Brenkman AB (2013) Current methods in quantifying ROS and oxidative damage in *Caenorhabditis elegans* and other model organism of aging. *Ageing Res Rev* 12:918–930
- Kang K, Jho EH, Lee HJ, Oidovsambuu S, Yun JH, Kim CY, Yoo JH, Kim YJ, Kim JH, Ahn SY, Nho CW (2011) *Youngia denticulata* protects against oxidative damage induced by tert-butylhydroperoxide in HepG2 cells. *J Med Food* 14:1198–1207
- Hicks KA, Howe DK, Leung A, Denver DR, Estes S (2012) In vivo quantification reveals extensive natural variation in mitochondrial form and function in *Caenorhabditis briggsae*. *PLoS ONE* 7:e43837
- Min H, Youn E, Shim YH (2020) Maternal caffeine intake disrupts eggshell integrity and retards larval development by reducing yolk production in a *Caenorhabditis elegans* model. *Nutrients* 12:1334
- Fu X, Tang Y, Dickinson BC, Chang CJ, Chang Z (2015) An oxidative fluctuation hypothesis of aging generated by imaging H₂O₂ levels in live *Caenorhabditis elegans* with altered lifespans. *Biochem Biophys Res Commun* 458:896–900
- Dikalov SI, Harrison DG (2014) Methods for detection of mitochondrial and cellular reactive oxygen species. *Antioxid Redox Signal* 20:372–382
- Min H, Lee M, Cho KS, Lim HJ, Shim YH (2021) Nicotinamide supplementation improves oocyte quality and offspring development by modulating mitochondrial function in an aged *Caenorhabditis elegans* model. *Antioxidants* 10:519
- Zhang G, Li Z, Chen F, Zhang D, Ji W, Yang Z, Wu Q, Zhang C, Li L, Huang W (2020) A novel fluorogenic probe for visualizing the hydrogen peroxide in Parkinson's disease models. *J Innov Opt Health Sci* 13:2050013
- Dickinson BC, Tang Y, Chang Z, Chang CJ (2011) A nuclear-localized fluorescent hydrogen peroxide probe for monitoring sirtuin-mediated oxidative stress responses in vivo. *Chem Biol* 18:943–948
- He Y, Miao L, Yu L, Chen Q, Qiao Y, Zhang J-F, Zhou Y (2019) A near-infrared fluorescent probe for detection of exogenous and endogenous hydrogen peroxide in vivo. *Dye Pigment* 168:160–165
- Jones KT, Ashrafi K (2009) *Caenorhabditis elegans* as an emerging model for studying the basic biology of obesity. *Dis Model Mech* 2:224–229

42. Escorcia W, Ruter DL, Nhan J, Curran SP (2018) Quantification of lipid abundance and evaluation of lipid distribution in *Caenorhabditis elegans* by Nile Red and Oil Red O staining. *J Vis Exp* 133:e75352
43. Yen K, Le TT, Bansal A, Narasimhan SD, Cheng JX, Tissenbaum HA (2010) A comparative study of fat storage quantitation in nematode *Caenorhabditis elegans* using label and label-free methods. *PLoS ONE* 5:e12810
44. Zhang SO, Trimble R, Guo F, Mak HY (2010) Lipid droplets as ubiquitous fat storage organelles in *C. elegans*. *BMC Cell Biol* 11:96
45. Brooks KK, Liang B, Watts JL (2009) The influence of bacterial diet on fat storage in *C. elegans*. *PLoS ONE* 4:e7545
46. Fam TK, Klymchenko AS, Collot M (2018) Recent advances in fluorescent probes for lipid droplets. *Materials* 11:1768
47. Ashrafi K, Chang FY, Watts JL, Fraser AG, Kamath RS, Ahringer J, Ruvkun G (2003) Genome-wide RNAi analysis of *Caenorhabditis elegans* fat regulatory genes. *Nature* 421:268–272
48. Mota AAR, Correa JR, de Andrade LP, Assumpção JAF, de Souza Cintra GA, Freitas-Junior LH, da Silva WA, de Oliveira HCB, Neto BAD (2018) From live cells to *Caenorhabditis elegans*: selective staining and quantification of lipid structures using a fluorescent hybrid benzothiadiazole derivative. *ACS Omega* 3:3874–3881
49. Goel A, Sharma A, Kathuria M, Bhattacharjee A, Verma A, Mishra PR, Nazir A, Mitra K (2014) New fluoranthene FLUN-550 as a fluorescent probe for selective staining and quantification of intracellular lipid droplets. *Org Lett* 16:756–759
50. Hobert O, Loria P (2006) Uses of GFP in *Caenorhabditis elegans*. *Methods Biochem Anal* 47:203–226
51. Braeckman BP, Smolders A, Back P, De Henau S (2016) In vivo detection of reactive oxygen species and redox status in *Caenorhabditis elegans*. *Antioxid Redox Signal* 25:577–592
52. Belousov VV, Fradkov AF, Lukyanov KA, Staroverov DB, Shakhbazov KS, Tersikh AV, Lukyanov S (2006) Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. *Nat Methods* 3:281–286
53. Knoeffler D, Thamsen M, Koniczek M, Niemuth NJ, Diederich AK, Jakob U (2012) Quantitative in vivo redox sensors uncover oxidative stress as an early event in life. *Mol Cell* 47:767–776
54. Koch K, Weldle N, Baier S, Büchter C, Wätjen W (2020) *Hibiscus sabdariffa* L. extract prolongs lifespan and protects against amyloid- β toxicity in *Caenorhabditis elegans*: involvement of the FoxO and Nrf2 orthologues DAF-16 and SKN-1. *Eur J Nutr* 59:137–150
55. Wang H, Zhang S, Zhai L, Sun L, Zhao D, Wang Z, Li X (2021) Ginsenoside extract from ginseng extends lifespan and health span in *Caenorhabditis elegans*. *Food Funct* 12:6793–6808
56. Duangjan C, Rangsinth P, Gu X, Zhang S, Wink M, Tencomnao T (2019) *Glochidion zeylanicum* leaf extracts exhibit lifespan extending and oxidative stress resistance properties in *Caenorhabditis elegans* via DAF-16/FoxO and SKN-1/Nrf-2 signaling pathways. *Phytomedicine* 64:153061
57. Zhuang JJ, Hunter CP (2012) RNA interference in *Caenorhabditis elegans*: uptake, mechanism, and regulation. *Parasitology* 139:560–573
58. Dudley NR, Labbé JC, Goldstein B (2002) Using RNA interference to identify genes required for RNA interference. *Proc Natl Acad Sci USA* 99:4191–4196
59. Maeda I, Kohara Y, Yamamoto M, Sugimoto A (2001) Large-scale analysis of gene function in *Caenorhabditis elegans* by high-throughput RNAi. *Curr Biol* 11:171–176
60. Kamath RS, Ahringer J (2003) Genome-wide RNAi screening in *Caenorhabditis elegans*. *Methods* 30:313–321
61. Jayarathne S, Ramalingam L, Edwards H, Vanapalli SA, Moustaid-Moussa N (2020) Tart cherry increases lifespan in *Caenorhabditis elegans* by altering metabolic signaling pathways. *Nutrients* 12:1482
62. Zhao J, Zhu A, Sun Y, Zhang W, Zhang T, Gao Y, Shan D, Wang S, Li G, Zeng K, Wang Q (2020) Beneficial effects of sappanone A on lifespan and thermotolerance in *Caenorhabditis elegans*. *Eur J Pharmacol* 888:173558
63. Cordeiro M, Ribeiro ARC, de Melo LFM, da Silva LF, Fidelis GP, Silva LMP, Caland RBO, Cadavid COM, Aragão CFS, Zucolotto SM, Oliveira RP, Dos Santos D, Rocha HAO, Scortecchi KC (2021) Antioxidant activities of *Commiphora leptophloeos* (Mart.) J. B. Gillett (Burseraceae) leaf extracts using in vitro and in vivo assays. *Oxid Med Cell Longev* 2021:3043720
64. Kim JH, Bang IH, Noh YJ, Kim DK, Bae EJ, Hwang IH (2020) Metabolites produced by the oral commensal bacterium *Corynebacterium durum* extend the lifespan of *Caenorhabditis elegans* via SIR-2.1 overexpression. *Int J Mol Sci* 21:2212
65. Zhu Q, Qu Y, Zhou XG, Chen JN, Luo HR, Wu GS (2020) A Dihydroflavonoid Naringin extends the lifespan of *C. elegans* and delays the progression of aging-related diseases in PD/AD models via DAF-16. *Oxid Med Cell Longev* 2020:6069354
66. Dilberger B, Weppeler S, Eckert GP (2021) Phenolic acid metabolites of polyphenols act as inductors for hormesis in *C. elegans*. *Mech Ageing Dev* 198:111518
67. Yu CW, Wei CC, Liao VH (2014) Curcumin-mediated oxidative stress resistance in *Caenorhabditis elegans* is modulated by age-1, akt-1, pdk-1, osr-1, unc-43, sek-1, skn-1, sir-2.1, and mev-1. *Free Radic Res* 48:371–379
68. Büchter C, Ackermann D, Havermann S, Honnen S, Chovolou Y, Fritz G, Kampkötter A, Wätjen W (2013) Myricetin-mediated lifespan extension in *Caenorhabditis elegans* is modulated by DAF-16. *Int J Mol Sci* 14:11895–11914
69. Cong W, Wang P, Qu Y, Tang J, Bai R, Zhao Y, Chunying C, Bi X (2015) Evaluation of the influence of fullerene on aging and stress resistance using *Caenorhabditis elegans*. *Biomaterials* 42:78–86
70. Cuanalco-Contreras K, Park KW, Mukherjee A, Millán-Pérez Peña L, Soto C (2017) Delaying aging in *Caenorhabditis elegans* with protein aggregation inhibitors. *Biochem Biophys Res Commun* 482:62–67
71. Grünz G, Haas K, Soukup S, Klingenspor M, Kulling SE, Daniel H, Spanier B (2012) Structural features and bioavailability of four flavonoids and their implications for lifespan-extending and antioxidant actions in *C. elegans*. *Mech Ageing Dev* 133:1–10
72. Jung HY, Lee D, Ryu HG, Choi BH, Go Y, Lee N, Lee D, Son HG, Jeon J, Kim SH, Yoon JH, Park SM, Lee SV, Lee IK, Choi KY, Ryu SH, Nohara K, Yoo SH, Chen Z, Kim KT (2017) Myricetin improves endurance capacity and mitochondrial density by activating SIRT1 and PGC-1 α . *Sci Rep* 7:6237
73. Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. *Nature* 430:686–689
74. Park SK, Seong RK, Kim JA, Son SJ, Kim Y, Yokozawa T, Shin OS (2016) Oligonol promotes anti-aging pathways via modulation of SIRT1-AMPK-autophagy pathway. *Nutr Res Pract* 10:3–10
75. Xue J, Sheng X, Zhang BJ, Zhang C, Zhang G (2020) The Sirtuin-1 related antioxidant and antiaging activity of 5,5'-diferulic acid glucoside esters derived from corn bran by enzymatic method. *J Food Biochem* 44:e13519
76. Luo S, Murphy CT (2011) *Caenorhabditis elegans* reproductive aging: regulation and underlying mechanisms. *Genesis* 49:53–65
77. McGee MD, Weber D, Day N, Vitelli C, Crippen D, Herndon LA, Hall DH, Melov S (2011) Loss of intestinal nuclei and intestinal integrity in aging *C. elegans*. *Aging Cell* 10:699–710
78. Hall DH, Winfrey VP, Blaeuer G, Hoffman LH, Furuta T, Rose KL, Hobert O, Greenstein D (1999) Ultrastructural features of the adult hermaphrodite gonad of *Caenorhabditis elegans*: relations between the germ line and soma. *Dev Biol* 212:101–123
79. Machado ML, Arantes LP, Gubert P, Zamberlan DC, da Silva TC, da Silveira TL, Boligon A, Soares FAA (2018) *Ilex paraguariensis* modulates fat metabolism in *Caenorhabditis elegans* through purinergic system (ADOR-1) and nuclear hormone receptor (NHR-49) pathways. *PLoS ONE* 13:e0204023
80. Rodrigues CF, Salgueiro W, Bianchini M, Veit JC, Puntel RL, Emanuelli T, Dernadin CC, Ávila DS (2018) *Salvia hispanica* L. (chia) seeds oil extracts reduce lipid accumulation and produce stress resistance in *Caenorhabditis elegans*. *Nutr Metab* 15:83
81. Lin C, Lin Y, Chen Y, Xu J, Li J, Cao Y, Su Z, Chen Y (2019) Effects of *Momordica saponin* extract on alleviating fat accumulation in *Caenorhabditis elegans*. *Food Funct* 10:3237–3251
82. Lin Y, Yang N, Bao B, Wang L, Chen J, Liu J (2020) Luteolin reduces fat storage in *Caenorhabditis elegans* by promoting the central serotonin pathway. *Food Funct* 11:730–740
83. Guerrero-Rubio MA, Hernández-García S, García-Carmona F, Gandía-Herrero F (2021) Flavonoids' effects on *Caenorhabditis elegans*' longevity, fat accumulation, stress resistance and gene modulation involve mTOR, SKN-1 and DAF-16. *Antioxidants* 10:438
84. Yue Y, Shen P, Chang AL, Qi W, Kim KH, Kim D, Park Y (2019) trans-Tris-methoxy resveratrol decreased fat accumulation dependent on fat-6 and fat-7 in *Caenorhabditis elegans*. *Food Funct* 10:4966–4974

85. Roncato JFF, Camara D, Brussulo Pereira TC, Quines CB, Colomé LM, Denardin C, Haas S, Ávila DS (2019) Lipid reducing potential of liposomes loaded with ethanolic extract of purple pitanga (*Eugenia uniflora*) administered to *Caenorhabditis elegans*. *J Liposome Res* 29:274–282
86. Shen P, Zhang R, McClements DJ, Park Y (2019) Nanoemulsion-based delivery systems for testing nutraceutical efficacy using *Caenorhabditis elegans*: demonstration of curcumin bioaccumulation and body-fat reduction. *Food Res Int* 120:157–166
87. Zhu Y, Bai J, Zhou Y, Zhang Y, Zhao Y, Dong Y, Xiao X (2021) Water-soluble and alkali-soluble polysaccharides from bitter melon inhibited lipid accumulation in HepG2 cells and *Caenorhabditis elegans*. *Int J Biol Macromol* 166:155–165
88. Dimitriadi M, Hart AC (2010) Neurodegenerative disorders: insights from the nematode *Caenorhabditis elegans*. *Neurobiol Dis* 40:4–11
89. Prasanth MI, Brimson JM, Chuchawankul S, Sukprasansap M, Tencom-nao T (2019) Antiaging, stress resistance, and neuroprotective efficacies of *Cleistocalyx nervosum* var. *paniala* fruit extracts using *Caenorhabditis elegans* model. *Oxid Med Cell Longev* 2019:7024785
90. Xie Z, Zhao J, Wang H, Jiang Y, Yang Q, Fu Y, Zeng H, Hölscher C, Xu J, Zhang Z (2020) Magnolol alleviates Alzheimer's disease-like pathology in transgenic *C. elegans* by promoting microglia phagocytosis and the degradation of beta-amyloid through activation of PPAR- γ . *Biomed Pharmacother* 124:109886
91. Li H, Yu X, Li C, Ma L, Zhao Z, Guan S, Wang L (2021) Caffeic acid protects against A β toxicity and prolongs lifespan in *Caenorhabditis elegans* models. *Food Funct* 12:1219–1231
92. Selvaraj B, Nguyen UTT, Huh G, Nguyen DH, Mok IK, Lee H, Kang K, Bae AN, Kim DW, Lee JW (2020) Synthesis and biological evaluation of chalcone derivatives as neuroprotective agents against glutamate-induced HT22 mouse hippocampal neuronal cell death. *Bioorg Med Chem Lett* 30:127597
93. Johnson SL, Park HY, DaSilva NA, Vattam DA, Ma H, Seeram NP (2018) Levodopa-reduced mucuna pruriens seed extract shows neuroprotective effects against parkinson's disease in murine microglia and human neuroblastoma cells, *Caenorhabditis elegans*, and *Drosophila melanogaster*. *Nutrients* 10:1139
94. Liu J, Banskota AH, Critchley AT, Hafting J, Prithiviraj B (2015) Neuroprotective effects of the cultivated *Chondrus crispus* in a *C. elegans* model of Parkinson's disease. *Mar Drugs* 13:2250–2266
95. Cheon SM, Jang I, Lee MH, Kim DK, Jeon H, Cha DS (2017) *Sorbus alnifolia* protects dopaminergic neurodegeneration in *Caenorhabditis elegans*. *Pharm Biol* 55:481–486
96. Govindan S, Amirthalingam M, Duraisamy K, Govindhan T, Sundararaj N, Palanisamy S (2018) Phytochemicals-induced hormesis protects *Caenorhabditis elegans* against α -synuclein protein aggregation and stress through modulating HSF-1 and SKN-1/Nrf2 signaling pathways. *Biomed Pharmacother* 102:812–822
97. Malaiwong N, Chalorak P, Jattujan P, Manohong P, Niamnont N, Suphamungmee W, Sobhon P, Meemon K (2019) Anti-Parkinson activity of bioactive substances extracted from *Holothuria leucospilota*. *Biomed Pharmacother* 109:1967–1977
98. Brunetti G, Di Rosa G, Scuto M, Leri M, Stefani M, Schmitz-Linneweber C, Calabrese V, Saul N (2020) Healthspan maintenance and prevention of Parkinson's-like phenotypes with hydroxytyrosol and oleuropein aglycone in *C. elegans*. *Int J Mol Sci* 21:2588
99. Goya ME, Xue F, Sampedro-Torres-Quevedo C, Arnaouteli S, Riquelme-Dominguez L, Romanowski A, Brydon J, Ball KL, Stanley-Wall NR, Doitsidou M (2020) Probiotic *Bacillus subtilis* protects against α -synuclein aggregation in *C. elegans*. *Cell Rep* 30:367–380
100. Le TAN, Selvaraj B, Lee JW, Kang K (2019) Measuring the effects of bacteria and chemicals on the intestinal permeability of *Caenorhabditis elegans*. *J Vis Exp* 154:e60419
101. Kim JY, Le TAN, Lee SY, Song DG, Hong SC, Cha KH, Lee JW, Pan CH, Kang K (2019) 3,3'-Diindolylmethane improves intestinal permeability dysfunction in cultured human intestinal cells and the model animal *Caenorhabditis elegans*. *J Agric Food Chem* 67:9277–9285
102. Han B, Sivaramakrishnan P, Lin CJ, Neve IAA, He J, Tay LWR, Sowa JN, Sizovs A, Du G, Wang J, Herman C, Wang MC (2017) Microbial genetic composition tunes host longevity. *Cell* 169:1249–1262
103. Pryor R, Norvaisas P, Marinos G, Best L, Thingholm LB, Quintaneiro LM, De Haes W, Esser D, Waschina S, Lujan C, Smith RL, Scott TA, Martinez-Martinez D, Woodward O, Bryson K, Laudes M, Lieb W, Houtkooper RH, Franke A, Temmerman L, Bjedov I, Cochemé HM, Kaleta C, Cabreiro F (2019) Host-microbe-drug-nutrient screen identifies bacterial effectors of metformin therapy. *Cell* 178:1299–1312
104. Park MR, Ryu S, Maburutse BE, Oh NS, Kim SH, Oh S, Jeong SY, Jeong DY, Oh S, Kim Y (2018) Probiotic *Lactobacillus fermentum* strain JDFM216 stimulates the longevity and immune response of *Caenorhabditis elegans* through a nuclear hormone receptor. *Sci Rep* 8:7441
105. Kamaladevi A, Balamurugan K (2016) *Lactobacillus casei* triggers a TLR mediated RACK-1 dependent p38 MAPK pathway in *Caenorhabditis elegans* to resist *Klebsiella pneumoniae* infection. *Food Funct* 7:3211–3223
106. Kim J, Moon Y (2019) Worm-based alternate assessment of probiotic intervention against gut barrier infection. *Nutrients* 11:2146
107. Kim JY, Lee SY, Jung SH, Kim MR, Choi ID, Lee JL, Sim JH, Pan CH, Kang K (2020) Protective effect of *Lactobacillus casei* HY2782 against particulate matter toxicity in human intestinal CCD-18Co cells and *Caenorhabditis elegans*. *Biotechnol Lett* 42:519–528
108. Patel P, Joshi C, Kothari V (2020) The anti-infective potential of hydroalcoholic extract of *Phyllanthus emblica* seeds against selected human-pathogenic bacteria. *Infect Disord Drug Targets* 20:672–692
109. Hao S, Yang D, Zhao L, Shi F, Ye G, Fu H, Lin J, Guo H, He R, Li J, Chen H, Khan MF, Li Y, Tang H (2021) EGCG-mediated potential inhibition of biofilm development and quorum sensing in *Pseudomonas aeruginosa*. *Int J Mol Sci* 22:4946
110. Anju VT, Busi S, Ranganathan S, Ampasala DR, Kumar S, Suchiang K, Kumavath R, Dyavaiah M (2021) Sesamin and sesamol rescues *Caenorhabditis elegans* from *Pseudomonas aeruginosa* infection through the attenuation of quorum sensing regulated virulence factors. *Microb Pathog* 155:104912
111. Haripriyan J, Omanakuttan A, Menon ND, Vanuopadath M, Nair SS, Corriden R, Nair BG, Nizet V, Kumar GB (2018) Clove bud oil modulates pathogenicity phenotypes of the opportunistic human pathogen *Pseudomonas aeruginosa*. *Sci Rep* 8:3437
112. Peterson ND, Pukkila-Worley R (2018) *Caenorhabditis elegans* in high-throughput screens for anti-infective compounds. *Curr Opin Immunol* 54:59–65
113. Kong C, Tan MW, Nathan S (2014) *Orthosiphon stamineus* protects *Caenorhabditis elegans* against *Staphylococcus aureus* infection through immunomodulation. *Biol Open* 3:644–655
114. Yang J, Chen Z, Ching P, Shi Q, Li X (2013) An integrated microfluidic platform for evaluating in vivo antimicrobial activity of natural compounds using a whole-animal infection model. *Lab Chip* 13:3373–3382
115. Liu M, Kipanga P, Mai AH, Dhondt I, Braeckman BP, De Borggraeve W, Luyten W (2018) Bioassay-guided isolation of three anthelmintic compounds from *Warburgia ugandensis* Sprague subspecies *ugandensis*, and the mechanism of action of polygodial. *Int J Parasitol* 48:833–844
116. Li Z, Nair SK (2012) Quorum sensing: how bacteria can coordinate activity and synchronize their response to external signals? *Protein Sci* 21:1403–1417
117. Alexpandi R, Prasanth MI, Ravi AV, Balamurugan K, Durgadevi R, Srinivasan R, De Mesquita JF, Pandian SK (2019) Protective effect of neglected plant *Diplocyclos palmatus* on quorum sensing mediated infection of *Serratia marcescens* and UV-A induced photoaging in model *Caenorhabditis elegans*. *J Photochem Photobiol B* 201:111637
118. Subramenium GA, Swetha TK, Iyer PM, Balamurugan K, Pandian SK (2018) 5-hydroxymethyl-2-furaldehyde from marine bacterium *Bacillus subtilis* inhibits biofilm and virulence of *Candida albicans*. *Microbiol Res* 207:19–32
119. Scotti R, Stringaro A, Nicolini L, Zanellato M, Boccia P, Maggi F, Gabbianelli R (2021) Effects of essential oils from *Cymbopogon* spp. and *Cinnamomum verum* on biofilm and virulence properties of *Escherichia coli* O157:H7. *Antibiotics* 10:113
120. Dwivedi M, Muralidhar S, Saluja D (2020) *Hibiscus sabdariffa* extract inhibits adhesion, biofilm initiation and formation in *Candida albicans*. *Indian J Microbiol* 60:96–106
121. Kim Y, Lee JW, Kang SG, Oh S, Griffiths MW (2012) Bifidobacterium spp. influences the production of autoinducer-2 and biofilm formation by *Escherichia coli* O157:H7. *Anaerobe* 18:539–545

122. Lee KM, Lim J, Nam S, Yoon MY, Kwon YK, Jung BY, Park Y, Park S, Yoon SS (2011) Inhibitory effects of broccoli extract on *Escherichia coli* O157:H7 quorum sensing and in vivo virulence. *FEMS Microbiol Lett* 321:67–74
123. Choi EJ, Kim HI, Kim JA, Jun SY, Kang SH, Park DJ, Son SJ, Kim Y, Shin OS (2015) The herbal-derived honokiol and magnolol enhances immune response to infection with methicillin-sensitive *Staphylococcus aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). *Appl Microbiol Biotechnol* 99:4387–4396
124. Polat E, Kang K (2021) Natural photosensitizers in antimicrobial photodynamic therapy. *Biomedicines* 9:584
125. Alam ST, Le TAN, Park JS, Kwon HC, Kang K (2019) Antimicrobial biophotonic treatment of ampicillin-resistant *Pseudomonas aeruginosa* with hypericin and ampicillin cotreatment followed by orange light. *Pharmaceutics* 11:641
126. Alam ST, Hwang H, Son JD, Nguyen UTT, Park JS, Kwon HC, Kwon J, Kang K (2021) Natural photosensitizers from *Tripterygium wilfordii* and their antimicrobial photodynamic therapeutic effects in a *Caenorhabditis elegans* model. *J Photochem Photobiol B* 218:112184
127. Moy TI, Conery AL, Larkins-Ford J, Wu G, Mazitschek R, Casadei G, Lewis K, Carpenter AE, Ausubel FM (2009) High-throughput screen for novel antimicrobials using a whole animal infection model. *ACS Chem Biol* 4:527–533
128. Ye X, Linton JM, Schork NJ, Buck LB, Petrascheck M (2014) A pharmacological network for lifespan extension in *Caenorhabditis elegans*. *Aging Cell* 13:206–215
129. Lucanic M, Garrett T, Yu I, Calahorra F, Asadi Shahmirzadi A, Miller A, Gill MS, Hughes RE, Holden-Dye L, Lithgow GJ (2016) Chemical activation of a food deprivation signal extends lifespan. *Aging Cell* 15:832–841
130. Taki AC, Byrne JJ, Boag PR, Jabbar A, Gasser RB (2021) Practical high-throughput method to screen compounds for anthelmintic activity against *Caenorhabditis elegans*. *Molecules* 26:4156
131. Zheng SQ, Ding AJ, Li GP, Wu GS, Luo HR (2013) Drug absorption efficiency in *Caenorhabditis elegans* delivered by different methods. *PLoS ONE* 8:e56877
132. Stupp GS, von Reuss SH, Izrayelit Y, Ajredini R, Schroeder FC, Edison AS (2013) Chemical detoxification of small molecules by *Caenorhabditis elegans*. *ACS Chem Biol* 8:309–313
133. Salzer L, Witting M (2021) Quo Vadis *Caenorhabditis elegans* metabolomics—a review of current methods and applications to explore metabolism in the nematode. *Metabolites* 11:284
134. Jones OA, Swain SC, Svendsen C, Griffin JL, Sturzenbaum SR, Spurgeon DJ (2012) Potential new method of mixture effects testing using metabolomics and *Caenorhabditis elegans*. *J Proteome Res* 11:1446–1453
135. Zhang X, Noguez JH, Zhou Y, Butcher RA (2013) Analysis of ascarosides from *Caenorhabditis elegans* using mass spectrometry and NMR spectroscopy. *Methods Mol Biol* 1068:71–92
136. Stasiuk SJ, MacNevin G, Workentine ML, Gray D, Redman E, Bartley D, Morrison A, Sharma N, Colwell D, Ro DK, Gilleard JS (2019) Similarities and differences in the biotransformation and transcriptomic responses of *Caenorhabditis elegans* and *Haemonchus contortus* to five different benzimidazole drugs. *Int J Parasitol Drugs Drug Resist* 11:13–29
137. Nguyen TTM, An YJ, Cha JW, Ko YJ, Lee H, Chung CH, Jeon SM, Lee J, Park S (2020) Real-time in-organism NMR metabolomics reveals different roles of AMP-activated protein kinase catalytic subunits. *Anal Chem* 92:7382–7387
138. Sarma GP, Lee CW, Portegys T, Ghayoomie V, Jacobs T, Alicea B, Cantarelli M, Currie M, Gerkin RC, Gingell S, Gleeson P, Gordon R, Hasani RM, Idili G, Khayrulin S, Lung D, Palyanov A, Watts M, Larson SD (2018) OpenWorm: overview and recent advances in integrative biological simulation of *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 373:20170382
139. Cao J, Guan G, Ho VWS, Wong MK, Chan LY, Tang C, Zhao Z, Yan H (2020) Establishment of a morphological atlas of the *Caenorhabditis elegans* embryo using deep-learning-based 4D segmentation. *Nat Commun* 11:6254
140. Xu JX, Deng X (2013) Biological modeling of complex chemotaxis behaviors for *C. elegans* under speed regulation—a dynamic neural networks approach. *J Comput Neurosci* 35:19–37
141. Martineau CN, Brown AEX, Laurent P (2020) Multidimensional phenotyping predicts lifespan and quantifies health in *Caenorhabditis elegans*. *PLoS Comput Biol* 16:e1008002
142. Fernandez-Moreno MA, Farr CL, Kaguni LS, Garesse R (2007) *Drosophila melanogaster* as a model system to study mitochondrial biology. *Methods Mol Biol* 372:33–49
143. Kishi S, Slack BE, Uchiyama J, Zhdanova IV (2009) Zebrafish as a genetic model in biological and behavioral gerontology: where development meets aging in vertebrates—a mini-review. *Gerontology* 55:430–441
144. Perlman RL (2016) Mouse models of human disease: an evolutionary perspective. *Evol Med Public Health* 2016:170–176
145. Lebedeva L, Zhumabayeva B, Gebauer T, Kisselev I, Aitasheva Z (2020) Zebrafish (*Danio rerio*) as a model for understanding the process of caudal fin regeneration. *Zebrafish* 17:359–372
146. Muschiol D, Schroeder F, Trautspurger W (2009) Life cycle and population growth rate of *Caenorhabditis elegans* studied by a new method. *BMC Ecol* 9:14
147. Zon LI (1999) Zebrafish: a new model for human disease. *Genome Res* 9:99–100
148. Lin SY, Craythorn RG, O'Connor AE, Matzuk MM, Girdling JE, Morrison JR, de Kretser DM (2008) Female infertility and disrupted angiogenesis are actions of specific follistatin isoforms. *Mol Endocrinol* 22:415–429
149. Patton EE, Zon LI, Langenau DM (2021) Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. *Nat Rev Drug Discov* 20:611–628
150. Ero C, Gewaltig MO, Keller D, Markram H (2018) A cell atlas for the mouse brain. *Front Neuroinform* 12:84
151. Hinsch K, Zupanc GK (2007) Generation and long-term persistence of new neurons in the adult zebrafish brain: a quantitative analysis. *Neuroscience* 146:679–696
152. Scheffer LK, Xu CS, Januszewski M, Lu Z, Takemura SY, Hayworth KJ, Huang GB, Shinomiya K, Maitlin-Shepard J, Berg S, Clements J, Hubbard PM, Katz WT, Umayam L, Zhao T, Ackerman D, Blakely T, Bogovic J, Dolafi T, Kainmueller D, Kawase T, Khairi KA, Leavitt L, Li PH, Lindsey L, Neubarth N, Olbris DJ, Otsuna H, Trautman ET, Ito M, Bates AS, Goldammer J, Wolff T, Svirskas R, Schlegel P, Neace E, Knecht CJ, Alvarado CX, Bailey DA, Ballinger S, Borycz JA, Canino BS, Cheatham N, Cook M, Dreher M, Duclos O, Eubanks B, Fairbanks K, Finley S, Forknall N, Francis A, Hopkins GP, Joyce EM, Kim S, Kirk NA, Kovalyak J, Lauchie SA, Lohff A, Maldonado C, Manley EA, McLin S, Mooney C, Ndama M, Ogundeyi O, Okeoma N, Ordish C, Padilla N, Patrick CM, Paterson T, Phillips EE, Phillips EM, Rampally N, Ribeiro C, Robertson MK, Rymer JT, Ryan SM, Sammons M, Scott AK, Scott AL, Shinomiya A, Smith C, Smith K, Smith NL, Sobeski MA, Suleiman A, Swift J, Takemura S, Talebi I, Tarnogorska D, Tenshaw E, Tokhi T, Walsh JJ, Yang T, Horne JA, Li F, Parekh R, Rivlin PK, Jayaraman V, Costa M, Jefferis GS, Ito K, Saalfeld S, George R, Meinertzhagen IA, Rubin GM, Hess HF, Jain V, Plaza SM (2020) A connectome and analysis of the adult *Drosophila* central brain. *Elife* 9:e57443
153. White JG, Southgate E, Thomson JN, Brenner S (1986) The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philos Trans R Soc Lond B Biol Sci* 314:1–340
154. Zhou M, Diwu Z, Panchuk-Voloshina N, Haugland RP (1997) A stable nonfluorescent derivative of resorufin for the fluorometric determination of trace hydrogen peroxide: applications in detecting the activity of phagocyte NADPH oxidase and other oxidases. *Anal Biochem* 253:162–168
155. Zhao B, Summers FA, Mason RP (2012) Photooxidation of Amplex Red to resorufin: implications of exposing the Amplex Red assay to light. *Free Radic Biol Med* 53:1080–1087
156. Di Rosa G, Brunetti G, Scuto M, Trovato Salinaro A, Calabrese EJ, Crea R, Schmitz-Linneweber C, Calabrese V, Saul N (2020) Healthspan enhancement by olive polyphenols in *C. elegans* wild type and Parkinson's models. *Int J Mol Sci* 21:3893

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