REVIEW

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
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

|                  | Human cell culture | *Caenorhabditis elegans* | *Drosophila melanogaster* | *Danio rerio* | *Mus musculus* | 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<sup>a</sup> | 100%               | 38.1%                    | 40.3%                    | 55.2%         | 80.5%          | [3]        |
| Percentage of human disease genes with orthologs<sup>b</sup> | 100%               | 48.6%                    | 53.4%                    | 72.9%         | 97.0%          | [3]        |
| High-throughput screening | + +                | +                        | +                        | +             | –              |            |
| Ethical issue    | + +                | +                        | +                        | +             | –              |            |

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

<sup>a</sup> Calculated as the total number of human genes with orthologs present in animals divided by the total number of genes in humans

<sup>b</sup> 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 in 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].
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-[33]. Other fluorescent probes have been used, such as certain cases due to its lower non-specific fluorescence MitoSOX would be preferable over MitoTracker Red in harvesting 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-((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,
| 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 | [31] |
| Amplex red | Oxidation of 10-acetyl-3,7-dihydroxypenoxazine 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 | - Causes autooxidation and a self-amplification of the fluorescent signal | [154, 155] |
| MitoSOX | Oxidation of dihydroethidium by O$_2$•$^-$$^-$ to fluorescent ethidium | Mainly O$_2$•$^-$$^-$ | - Targets the mitochondria | - 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 | [33] |
| Nuclear Peroxy Emerald I | H$_2$O$_2$-mediated conversion of arylboronates to phenol | H$_2$O$_2$ | Measuring nuclear H$_2$O$_2$ fluxes | - Background signal generated from nonspecific lipid uptake of dye | [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$_2$•$^-$$^-$ and singlet oxygen | O$_2$•$^-$$^-$ and singlet oxygen | Does not undergo redox cycling | - Low specificity | [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 | - High background noise | [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$_2$O$_2$-specific probe constructed by combining the H$_2$O$_2$-sensitive regulatory domain of the E. coli transcription factor OxyR and circularly permuted yellow fluorescent protein [52]. When exposed to H$_2$O$_2$, 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$_2$O$_2$ 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$_2$O$_2$, 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$_2$O$_2$ 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 Comniphora 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
| Category              | Natural products                          | Main finding                                      | Molecular pathway                                      | References |
|-----------------------|-------------------------------------------|---------------------------------------------------|-------------------------------------------------------|------------|
| Anti-aging            | *Hibiscus sabdariffa* L. extract          | ↑ Lifespan by 24%                                 | DAF-16 and SKN-1                                       | [54]       |
|                       |                                           | ↓ Lipofuscin                                      |                                                       |            |
|                       |                                           | ↑ Intracellular ROS levels                        |                                                       |            |
| Ginsenosides          |                                           | ↑ Lifespan by 14.02%                              | SKN-1, SIR 2.1, and DAF-16                            | [55]       |
| Glochidion zeylanicum leaf extracts |              | ↓ Mortality by juglone by 42.23%                  | SOD-3, and GST-4                                       | [56]       |
|                       |                                           | ↓ Intracellular ROS by 41.33%                      | HSP-16.2                                              |            |
|                       |                                           |                                                    | DAF-16 and SKN-1                                       |            |
| *Corynebacterium durum* metabolites |          | ↑ Lifespan up to 21.6%                            | SIR-2.1                                               | [64]       |
| Protocatechuic, gallic, and vanillic acid |              |                                                    |                                                       |            |
| Myricetin             |                                           | ↑ Lifespan and body bending in age worms           |                                                       |            |
| 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]       |
| trans-Trismethoxy resveratrol |              | ↓ Triglyceride accumulation by 14%                  | Downregulated stearoyl-CoA desaturase genes, fat-6 and fat-7 | [84]       |
| Bitter melon polysaccharides |              | ↓ Triglyceride content                             | daf-2, fat-5, fat-6 and fat-7 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]       |
|                       | *Mucuna pruriens* 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 |                                                       | [156]      |
|                       |                                           | ↓ α-Synuclein by 17%                               |                                                       |            |
| Gut health improvement | 3,3′-Diindolylmethane                     | ↓ C. elegans intestinal permeability triggered by *P. aeruginosa* infection by 14.81% |                                                       | [100, 101] |
|                       | *L. casei*                                 | ↑ Egg laying and pharyngeal pumping in sick worms | TLR, RACK-1 and p38 MAPK                               | [105]      |
|                       |                                           | ↓ *K. pneumoniae* colonization in the intestine    |                                                       |            |
|                       | *E. coli* strain Nissle 1917              | ↓ Relative permeability of a pathogenic *E. coli* infected gut | ZOO-1                                                | [106]      |
|                       |                                           | ↓ Body-cavity leakage by approximately 20%        |                                                       |            |
| Anti-infective effect | *Diplocyclos palmatus*                    | ↑ Survival time by 50%                             | daf-16 and immune-related genes (clec-60, clec-87, lys-7 and bec-1) | [117]      |
|                       | 5-Hydroxymethyl-2-furaldehyde             | ↑ Survival time by 24 h                            | Multidrug resistance gene expression                  | [118]      |
| *Bifidobacterium* spp. |                                           | ↑ Survival rate against enterohemorrhagic *E. coli* | Transcription of the genes encoding virulence factors | [121]      |
|                       | Broccoli extract                          | ↑ Survival rates of *C. elegans* increase by 28.5% | luxS and pfs genes                                   | [122]      |
|                       | Honokiol and magnolol                     | ↓ Lethality rate in *C. elegans* 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]      |
|                       | *Tripterygium wilfordii* extract          | ↑ Survivability of *S. pyogenes* infection         |                                                       | [126]      |
lifespan-extending effects mentioned were modulated by age-1 and skn-1 [67]. A study demonstrated that fullerol 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′-enol, 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, chrysirin, scutellarein, 6-hydroxyflavone, apigenin, chrysirin, 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 μM, 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 sapo

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 (Aβ) 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 Aβ-dependent paralysis phenotype due to the expression of the human Aβ1–42 peptide in muscle cells. Supplementation with magnolol (2.5–10 μM) delayed the onset of the paralyzed phenotype, in which the time to paralyze 50% of worms treated with 5 μM magnolol was 10 h, 20% longer than that in worms treated with accepted anti-AD drug [90]. Caffeic acid (300 μM)
prolonged the mean lifespan by 15.57%, and daf-16 expression was significantly upregulated in caffeic acid-treated CL4176 worms [91]. Similarly, using another Aβ-expressing worm, CL2006, *Cleistocalyx nervosum* var. *paniala* extract (10 μg/mL) reduced Aβ 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 μg/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 poly-saccharide 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, ZO-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 sesamolin) 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 palma* 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 ≥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, oxendazole, 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 nutra-ceuticals 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; AB: Amyloid beta peptide (Aβ); BODIPY: Boron dipyrromethene; BTD-1-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-performace 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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Declarations

Competing interests

The authors declare that they have no conflicts of interest.

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