



Research Article

Chemical Composition, Antioxidant Activity, and the Predicted Anti-Aging Mechanisms of Lotus Seed Coat: Evidence From the *Caenorhabditis elegans* Model and Network Pharmacology

Chunshuai Li^{1,2}, Xianwen Ye^{1,2,*} , Yujie Wang², Chongjun Zhao², Xinfang Xu², Xiangri Li^{2,*} 

¹School of Pharmacy, Jiangxi University of Chinese Medicine, 330004 Nanchang, Jiangxi, China

²Research Center of Traditional Chinese Medicine Processing/Beijing Key Laboratory for Quality Evaluation of Chinese Medicine, Beijing University of Chinese Medicine, 102400 Beijing, China

*Correspondence: yexianwen@jxutcm.edu.cn (Xianwen Ye); lixiangri@sina.com (Xiangri Li)

Academic Editor: Mehmet Ozaslan

Submitted: 10 September 2025 Revised: 18 December 2025 Accepted: 31 December 2025 Published: 5 February 2026

Abstract

Background: Aging is a critical global public health challenge, and natural products represent a key area in anti-aging research. Therefore, this study aimed to explore the chemical composition and anti-aging efficacy of the lotus seed coat. **Methods:** Ultra-high-performance liquid chromatography-Orbitrap mass spectrometry (UHPLC/Orbitrap-MS) was used to analyze chemical components. The lifespan of *Caenorhabditis elegans* (*C. elegans*) treated with lotus seed coat extract was measured, along with oxidative stress markers and antioxidant enzyme activity. Network pharmacology and molecular dynamics simulations were employed to predict any associated anti-aging mechanisms. **Results:** A total of 67 compounds were tentatively identified in the lotus seed coat, including 14 alkaloids, 19 flavonoids, and 6 phenolic acids. Lotus seed coat extract prolonged the lifespan of *C. elegans* ($p < 0.05$), reduced lipofuscin and reactive oxygen species (ROS) levels ($p < 0.001$), increased superoxide dismutase (SOD) and catalase (CAT) activity, and decreased malondialdehyde (MDA) content. Network pharmacology linked the anti-aging effects of the lotus seed coat extract to the regulation of *SRC*, *PIK3R1*, *AKT1*, *IL6*, and *TNF* genes expression and implicated the phosphatidylinositol 3-kinase/protein kinase B pathway (PI3K–Akt), mitogen-activated protein kinase (MAPK), hypoxia-inducible factor 1 (HIF-1), forkhead box O (FOXO), tumor necrosis factor (TNF), and mammalian target of rapamycin (mTOR) signaling pathways. Molecular simulations confirmed that neferine strongly binds to phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), histone acetyltransferase p300 (EP300), and RAC-alpha serine/threonine-protein kinase (AKT1). **Conclusion:** Lotus seed coat is rich in bioactive compounds and exhibits significant anti-aging potential.

Keywords: lotus seed coat; anti-aging; *Caenorhabditis elegans*; antioxidant; network pharmacology

1. Introduction

Aging is characterized by a gradual decline in bodily functions, leading to increased susceptibility to cardiovascular and neurodegenerative diseases, which can have profound impacts on the overall health of elderly individuals [1,2]. Research on anti-aging has been ongoing throughout history, with various theories proposed to explain the mechanisms of aging. These include theories on free radicals, oxidative stress, immune aging, DNA damage, mitochondrial damage, and cell autophagy [3,4]. While existing anti-aging agents have demonstrated certain efficacy with specific targets, they face challenges such as the limited efficacy of single-component agents, risks of side effects with long-term use, and low bioavailability [5]. Moreover, they are unable to cover the multi-dimensional mechanisms of aging. Against this backdrop, the exploration of highly effective, low-toxicity, and multi-target synergistic anti-aging drugs from natural products has emerged as a key research trend that also aligns with the health needs of “holistic intervention and long-term safety [6,7]”.

Lotus seeds are dried, fully matured seeds from the aquatic plant *Nelumbo nucifera* Gaertn. They are widely acknowledged as having purported longevity-enhancing bioactivities [8,9]. Lotus seed coat is a by-product of lotus seed processing, accounting for approximately 15% of the total weight of lotus seeds [10]. It is typically discarded as waste and casually disposed of in ditches or along roadsides. The lotus seed coat corresponds to the traditional medicinal term Lianyi [11]. It consists of the membranous structure attached to the surface of the lotus seed kernel (endosperm) after the outer hard shell of the lotus seed has been removed. It appears reddish-brown when dried [12,13]. According to *Bencao Qiyuan*, lotus seeds are described as “tonifying when peeled; securing essence and invigorating the spleen when used with the coat”. This suggests the lotus seed coat can prevent the dissipation of qi, blood, and body fluids, thereby exerting an anti-aging effect [14]. Research on the lotus seed coat has gradually increased in recent years, particularly with regard to its chemical composition and antioxidant capabilities. However, the potential



association between the lotus seed coat and longevity still needs further investigation.

Driven by the emerging interest in the anti-aging potential of lotus seed coat, we designed a multi-layered study to systematically investigate its bioactive components, antioxidant capacity, and *in vivo* anti-aging effects, followed by analysis of the predicted mechanism. First, we employed HPLC/Orbitrap-MS to characterize the chemical composition of the lotus seed coat. This step was critical for linking specific compounds to subsequent biological effects. Since excessive accumulation of free radicals is a well-documented driver of cellular senescence and aging, we then evaluated the *in vitro* antioxidant capacity of the extract using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The results of these assays provided preliminary validation of its anti-aging potential.

To further confirm the effects in a living system, *C. elegans* was selected as an *in vivo* model. *C. elegans* is a widely accepted and utilized organism for anti-aging research due to its short lifespan, genetic tractability, and conservation of key aging-related pathways. Core aging-related endpoints were evaluated following exposure of wild-type N2 worms to graded concentrations of lotus seed coat extract. These included organism lifespan, the levels of lipofuscin (a senescence biomarker) and reactive oxygen species (ROS), the activity of antioxidant enzymes (superoxide dismutase [SOD], catalase [CAT]), and the level of malondialdehyde (MDA), a lipid peroxidation marker. Finally, to link the observed phenotypic effects with molecular mechanisms, network pharmacology analysis was performed to predict potential targets and signaling pathways underlying the anti-aging activity of lotus seed coat. This computational approach identified key molecular mediators, while also providing a theoretical foundation for future mechanistic validation studies.

2. Materials and Methods

2.1 Chemical Profiling of Lotus Seed Coat Using LC/MS

2.1.1 Preparation of Reference Standard

The reference standard, including neferine, quercetin, and kaempferol, was dissolved in methanol at a concentration of 0.1 mg/mL. All solutions were stored at -20°C until use.

2.1.2 Sample Preparation

Professor Xiangri Li, an expert in Chinese materia medica from Beijing University of Chinese Medicine, identified the lotus seeds as the dried mature nuts of *Nelumbo nucifera* Gaertn., a plant belonging to the genus *Nelumbo* (family Nymphaeaceae). The lotus seed coat (Fig. 1) has been deposited in the Laboratory of Chinese Materia Medica Processing, Beijing University of Chinese Medicine, and is available for inspection and verification by peers.

For positive ion mode analysis, 1.0 g of the powder was extracted with $0.3\text{ mol}\cdot\text{L}^{-1}$ hydrochloric acid-methanol solution under 60 min ultrasonication, filtered, and 5 mL of the filtrate was evaporated to dryness in a water bath. For negative ion mode analysis, approximately 1.0 g of lotus seed coat powder was mixed with 70% methanol, ultrasonicated for 60 min, filtered through filter paper, and the filtrate was then passed through a $0.22\text{ }\mu\text{m}$ microporous membrane. The residue was reconstituted with methanol, transferred to a 5 mL volumetric flask, made up to volume with methanol, and filtered through a $0.22\text{ }\mu\text{m}$ microporous membrane.

2.1.3 Testing Conditions

A Vanquish UHPLC system (Thermo Fisher Scientific, San Jose, CA, USA) was employed for separation. Samples were separated on an Acquity HSS T3 column ($2.1 \times 100\text{ mm}$, $1.8\text{ }\mu\text{m}$, Waters, MA, USA). The chemical components of the lotus seed coat were analyzed in positive and negative ion modes. Chromatographic and mass spectrometric conditions are provided in **Supplementary Materials (Section LC/MS Testing Conditions)**.

2.1.4 Data Processing

Data were processed with Xcalibur TM 4.1 software (Thermo Fisher). MS/MS data were further analyzed using an online LC/MS database (<http://www.hmdb.ca>; <https://massbank.eu/MassBank/>), and by comparing the MS/MS spectra with reference standards and the literature.

2.2 Assessment of In Vitro Free Radical Scavenging Ability

2.2.1 Preparation of Sample Solutions

One gram of lotus seed coat powder was weighed and extracted with 20 mL of 70% methanol (v/v) for 60 min in an ultrasonic water bath (40 kHz, 500 W). The extracts were passed through filter paper and stored at 4°C . The extraction rate of the lotus seed coat is approximately 22.4%.

2.2.2 DPPH Free Radical Assay

Free radicals (atoms or groups with unpaired electrons) are closely related to aging. Common methods for measuring free radical scavenging capacity include the DPPH and ABTS assays. The DPPH solution employed in the assay was prepared at a concentration of 0.4 mmol/L, with 70% methanol serving as the diluent. For the standard group, 100 μL of ascorbic acid standard solution was added to 100 μL DPPH solution. For the sample group, 100 μL of lotus seed coat sample extract diluent was added to 100 μL of DPPH solution. The reaction system was maintained at room temperature under dark conditions for 30 minutes, after which the absorbance was measured at 517 nm using a microplate reader.



Fig. 1. Processing pathways and morphological differences: Medicinal vs. Edible Lotus Seeds.

2.2.3 ABTS^{·+} Stable Free Radical Assays

The ABTS^{·+} solution was prepared by mixing 2 mL of 7.4 mmol/L ABTS solution and 2 mL of 2.6 mmol/L K₂S₂O₈ solution and storing at room temperature for 12–16 h away from light. This was diluted with methanol. The absorbance of the ABTS solution was within the range of 0.70 ± 0.02 at 734 nm. Sample dilution (100 μL) or ascorbic acid standard solution (25–185 μg/mL) was pipetted into a 96-well cell plate, and 100 μL of ABTS^{·+} solution was added. After mixing well, the reaction was performed at room temperature for 6 min. Clearance was calculated with the following Eqn. 1:

$$\text{Clearance (\%)} = \frac{A_o - A_x}{A_x} \times 100\% \quad (1)$$

A_x: absorbance of the sample solution/standard solution after the reaction.

A₀: absorbance of the blank reaction.

The DPPH/ABTS clearance of the sample was calculated by fitting the equation to the standard curve, and then expressed as ascorbic acid equivalent: mg ascorbic acid eqg DW (mgAAE/g DW).

2.3 Evaluation of Lotus Seed Coat Extract on the Aging of *C. elegans*

2.3.1 Culture and Passage of *C. elegans*

The cultivation and experimental manipulation of *C. elegans* were conducted in accordance with established protocols [15]. Freeze-dried powder of 70% methanol extract from the coat of lotus seeds was dissolved in water and added to the nematode growth medium (NGM) plate. Sim-

ultaneously, *E. coli* OP50 was spread on the NGM plate to serve as nourishment for *C. elegans*. In preliminary toxicological experiments, the lotus seed coat was found to have no toxic effect on *C. elegans* in the concentration range of 0.01–1 mg·mL⁻¹. The aging experiment consisted of four groups: blank (without treatment, BG), low-dose lotus seed coat extract (0.04 mg/mL, LG), medium-dose lotus seed coat extract (0.2 mg/mL, MG), and high-dose lotus seed coat extract (1 mg/mL, HG).

2.3.2 Evaluation of *C. elegans* Lifespan

C. elegans at the L4 stage were chosen and transferred onto NGM plates (3 plates per group, 30 strips per plate). To maintain a consistent drug concentration, *C. elegans* were transferred to new NGM plates the following day, and the number of deceased individuals was recorded. The blank group was used as the control, and survival time was calculated from the transfer time until all *C. elegans* had died, which marked the end of the experiment. The experiment was repeated three times. The lifespan of each group of *C. elegans* was recorded and used to calculate the average, median, and maximum lifespans. Evaluation criteria for the death of *C. elegans* were no movement or swallowing action, and no response after light touch.

2.3.3 Biochemical and Oxidative Stress Biomarker Assays in *C. elegans*

All experiments used synchronized L4-stage *C. elegans* under uniform conditions. For the lipofuscin assay, worms were transferred to fresh NGM plates (3 plates/group, 30 worms/plate) and cultured for 5 days, then rinsed three times with M9 buffer, anesthetized, and

mounted on slides. Blue fluorescence intensity was then recorded via an inverted microscope and analyzed with ImageJ 1.54f (National Institutes of Health, Bethesda, MD, USA). For the ROS assay, worms were cultured as above, then rinsed, centrifuged (supernatant discarded), and incubated with 200 μ L of 100 μ M DCFH-DA (Solarbio, ID31309, Beijing, China) for 120 min. Green fluorescence intensity was then recorded via an inverted microscope and analyzed with ImageJ. For the SOD/CAT activity and MDA content assay, worms were transferred to drug-supplemented NGM plates and cultured for 5 days, then rinsed, centrifuged, repeatedly washed with M9 buffer, and stored at -80 $^{\circ}$ C. For assessment, the samples were ground, centrifuged, and the supernatant analyzed using kits (A001-3-2, A003-1-2, A007-2-1, Nanjing Jiancheng, Nanjing, China) as per the manufacturer's instructions.

2.4 Network Construction and Analysis

2.4.1 Target Prediction and the Construction of Protein-Protein Interaction (PPI) Networks

Targets for the lotus seed coat compounds were predicted using two databases: SEA (<https://sea.bkslab.org/>) and SwissTargetPrediction (<http://www.swisstargetprediction.ch/>). The chemical composition of the lotus seed coat was determined based on in-house experimental results [10]. Candidate targets were standardized to UniProtKB IDs and corresponding gene symbols via the UniProtKB database. Aging-related targets were retrieved from differential genes identified using the Genecards database (<https://www.genecards.org/>). Common targets were defined as the intersection of compound-predicted targets and aging-related targets [16].

To construct the PPI network, UniProt IDs of common targets were uploaded to the STRING database. The species was set as *Homo sapiens*, and the interaction confidence score threshold was adjusted to 0.90 (highest confidence). The resulting PPI network was visualized using Cytoscape 3.9.1 (Cytoscape Consortium, Seattle, WA, USA), followed by topological analysis via the CytoHubba and MCODE plug-ins to identify key nodes and functional modules.

2.4.2 GO and KEGG Pathway Enrichment Analysis

Metascape (<http://metascape.org/>) is a robust platform enabling users to apply currently popular bioinformatics analysis methods to bulk gene and protein analysis for knowledge of gene or protein function. The gene names for the above vital targets were entered into the Metascape website, and species were limited to humans. This enabled Gene Ontology (GO) functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the Metascape website.

2.4.3 Molecular Docking and Molecular Dynamics Simulations

Molecular docking was performed using Autodock Vina 1.2.5 (The Scripps Research Institute, La Jolla, CA,

USA) to assess the binding affinity of small molecules to the key protein. Subsequently, three representative small molecule-protein complexes were selected for molecular dynamics (MD) simulations to evaluate complex stability. The main procedures were carried out as follows:

① The AMBER14 force field was employed to optimize the energy of the protein-ligand system. A periodic boundary simulation box was constructed (expanded by 10 \AA), filled with TIP3P water molecules (density: 0.997 $\text{g}\cdot\text{L}^{-1}$), and supplemented with 0.9% NaCl to mimic the physiological ionic environment.

② The simulation was conducted over a duration of 50,000 ps (50 ns) at a temperature of 310 K (NVT ensemble with Langevin temperature control) to simulate physiological conditions, with a pH of 7.4 (physiological condition). The initial structure was optimized using the default steepest descent method (500 steps).

③ Key output parameters included: Root Mean Square Deviation (RMSD) to assess overall conformational stability; Root Mean Square Fluctuation (RMSF) to analyze local dynamic fluctuations of residues in the active site; Radius of Gyration (Rg) to measure protein compactness; binding energy to evaluate the energy released upon ligand-protein binding; and Free Energy Landscape (FEL, also referred to as free energy surface) to investigate processes such as protein folding and molecular motion, thus helping to understand molecular stability and dynamic behavior across different states.

2.5 Statistical Analysis

Standard curves were analyzed using linear regression. All statistical analyses were performed using GraphPad Prism 8.3.0 (Boston, MA, USA). The ANOVA method was used for statistical comparisons between groups. All data are the mean of three replicate determinations, presented as the mean \pm s.e.m. (error bars).

3. Results

3.1 Chemical Profiling of Lotus Seed Coat

The chemical constituents of the lotus seed coat were analyzed by UHPLC/Orbitrap-MS, with the base peak chromatogram shown in Fig. 2. Data interpretation was performed using online LC/MS databases (HMDB: <https://www.hmdb.ca/>; MassBank: <https://massbank.eu/MassBank/>) and by comparison with literature-reported compounds. A total of 67 compounds were tentatively identified, comprising 14 alkaloids, 19 flavonoids, 6 phenolic acids, 12 fatty acids, 2 lactones, 7 saccharides, and 7 tannins. Among these, quercetin, kaempferol, and neferine were confirmed by comparison with authentic standards. Detailed information is provided in **Supplementary Tables 1,2**.

3.1.1 Inference of Alkaloid Compounds

The inferred compounds are shown in **Supplementary Materials** and were derived from references and on-

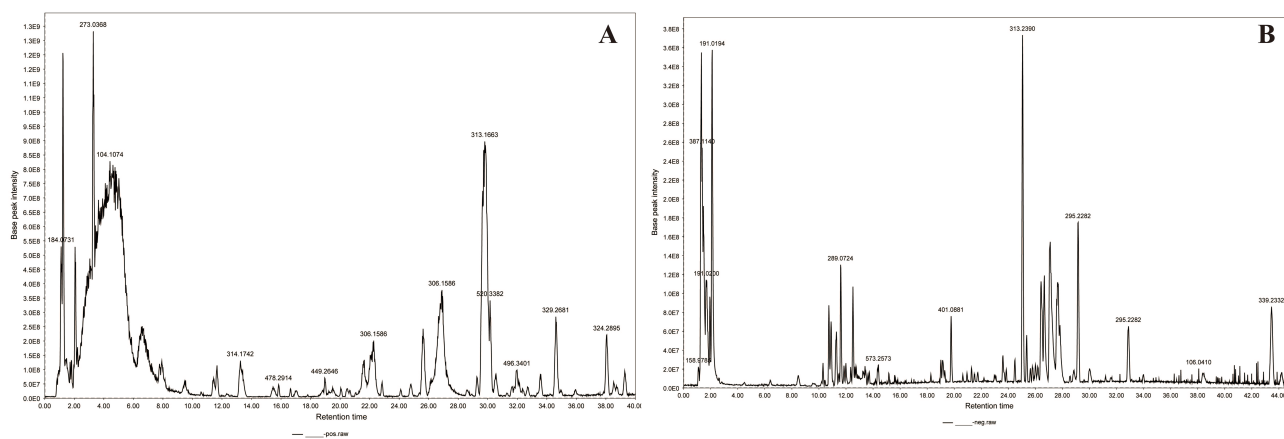


Fig. 2. Base peak chromatograms of lotus seed coat. (A) positive ion modes; (B) negative ion modes

line databases [11,12,17]. The main alkaloid components in lotus seed coat are classified into aporphine-type, mono-benzylisoquinoline-type, and bis-benzylisoquinoline-type alkaloids. Cleavage of bis-benzylisoquinoline alkaloids primarily involves the fragmentation of two benzyl groups [12,18]. Typically, α -cleavage occurs at the N-atom, leading to the loss of neutral fragments of $\text{CH}_2=\text{N}-\text{CH}_3$ (m/z 43) or NH_2CH_3 (m/z 31). The isoquinoline moiety fragments undergo further cleavage and rearrangement to form biphenyl compounds [19]. Taking neferine as an example, compound 14 (Supplementary Table 1) has a retention time of 29.78 min. In positive ion mode, its quasi-molecular ion is m/z 625.3265 $[\text{M}+\text{H}]^+$. The secondary mass spectrometry fragment ions include m/z 594.2845 $[\text{M}+\text{H}-\text{NH}_2\text{CH}_3]^+$, 489.2375 $[\text{M}+\text{H}-\text{C}_6\text{H}_4(\text{OCH}_3)\text{CH}_2-\text{CH}_3]^+$, and 206.1174 $[\text{M}+\text{H}-\text{C}_6\text{H}_4(\text{OCH}_3)\text{CH}_2-\text{CH}_3-\text{C}_{10}\text{H}_{11}\text{NO}-\text{C}_6\text{H}_4\text{O}_2\text{CH}_2]^+$. Combined with the reference standard, this compound was identified as neferine, with the molecular formula $\text{C}_{38}\text{H}_{44}\text{N}_2\text{O}_6$.

3.1.2 Inference of Flavonoids and Their Glycosides

In general, flavonoid glycosides, with the exception of carbon glycosides, undergo initial cleavage at the glycosidic bond to generate fragment ions of flavone aglycones that have lost their glycosyl groups [20]. Subsequently, these fragment ions undergo cleavage like that of flavonoids. The most prominent cleavage pathway for flavonoids is Retro-Diels-Alder (RDA) cleavage of the C ring, with positions 1 and 3 of the C ring being commonly cleaved to generate an ion containing the m/z 151 of the A ring. Upon comparison with a reference standard, compounds 38 and 41 (Supplementary Table 2) were identified as quercetin and kaempferol [21].

3.1.3 Inference of Proanthocyanidins

Proanthocyanidins are a class of polymers consisting of flavan-3-ols condensed through C-C bonds. They typically have catechins or epicatechins as the basic unit, with the primary mode of cleavage being rupture of the co-

valent bonds between the constituent units [22,23]. Like flavonoids, the primary mode of cleavage of flavan-3-ols is also RDA cleavage of the C ring. The molecular ion peak is m/z 577.1351, and secondary fragment ions include m/z 425.0881, m/z 407.0772, m/z 289.0718, and m/z 161.0243.

3.1.4 Inference of Phenolic Acid

Most phenolic acids contain carboxyl or phenolic hydroxyl groups, readily losing neutral molecules such as H_2O , COOH , and CO_2 under high-energy collisions [24]. Ferulic and caffeic acids readily lose acyl groups. In the case of ferulic acid, for example, the molecular ion peak is m/z 193.0504 in negative ion mode, and the secondary fragment ions are m/z 178.0272, m/z 149.0608, and m/z 134.0373.

3.1.5 Inference of Fatty Acids

The fatty acids contained in the lotus seed coat are mostly straight-chain unsaturated fatty acids. Hydroxy-octadecenoic acid was the most abundant among the fatty acids identified in this experiment. The cleavage pathway of the fatty acids is generally straightforward, with easy loss of H_2O or CO fragments, and firm molecular ionic peaks [11].

3.2 Assay for *In Vitro* Free Radical Scavenging Ability

Linear regression analysis was performed using clearance as the vertical coordinate and the ascorbic acid control mass concentration ($\mu\text{g}/\text{mL}$) as the horizontal coordinate (Fig. 3). In the DPPH method, ascorbic acid was linear in the range 1~30 $\mu\text{g}/\text{mL}$, $y = 0.0198x + 0.0983$, $R^2 = 0.9995$. Similarly, in the ABTS method, ascorbic acid was linear in the range 2~12 $\mu\text{g}/\text{mL}$, $y = 0.0624x + 0.0802$, $R^2 = 0.9997$ (Fig. 3A).

A 70% methanolic extract of lotus seed coat exhibited potent *in vitro* free radical scavenging ability, expressed as ascorbic acid equivalents. With the DPPH and the ABTS methods, the ascorbic acid equivalents of lotus seed bark were 78.25 mg/g and 53.59 mg/g, respectively (Fig. 3B).

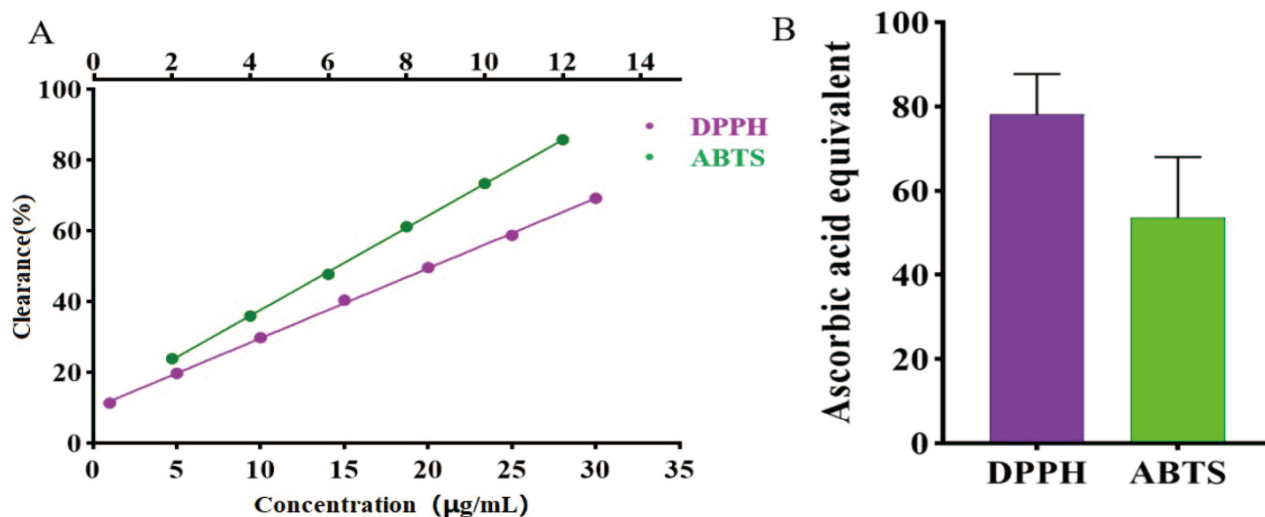


Fig. 3. Assay results for *in vitro* free radical scavenging ability of the lotus seed coat. (A) Ascorbic acid standard curve. (B) Statistical analysis of the ascorbic acid equivalent content of the lotus seed coat. DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

The results showed that the lotus seed coat had good free radical scavenging ability *in vitro*, indicating that it also had good anti-aging activity [25]. Due to the limitations of *in vitro* experiments, we also carried out *in vivo* anti-aging experiments.

3.3 Effect of Lotus Seed Coat Extract on the Aging of *C. elegans*

3.3.1 The Effect of Lotus Seed Coat Extract on the Lifespan of *C. elegans*

Based on experimental data, we calculated the average, median, and maximum lifespan of *C. elegans*. The *C. elegans* lifespan curve is shown in Fig. 4A. Compared with the blank group, various doses of lotus seed coat extract were found to increase the average lifespan of *C. elegans*. The average lifespan in the blank group was 14.31 days, compared to 15.11, 15.97, and 15.82 days in the low-, medium-, and high-dose groups of lotus seed coat extract, respectively (Fig. 4A). The results were significantly different ($p < 0.05$), indicating that lotus seed coat can effectively prolong the healthy lifespan of *C. elegans*.

3.3.2 Lipofuscin Content in *C. elegans*

The lipofuscin content of *C. elegans* accumulates with age, with its autofluorescence properties used as a sign of aging [26]. Lotus seed coat extract significantly reduced the lipofuscin level in the nematode body (Fig. 4B and Fig. 5). The level was 49.99%, 59.58% and 70.12% lower in the low-, medium-, and high-dose groups compared to the blank group, respectively ($p < 0.001$).

3.3.3 ROS Content in *C. elegans*

The ROS content in the body of *C. elegans* increases with the aging process. Excessive accumulation of ROS in

the body can activate aging-related signaling pathways, inducing cell cycle arrest and the acquisition of a senescence-associated secretory phenotype (SASP) in cells, thereby accelerating the aging process [27]. Lotus seed coat extract significantly reduced the ROS level in *C. elegans*, as shown in Fig. 4C and Fig. 6. Lipofuscin levels in *C. elegans* were reduced by 28.92%, 40.87%, and 56.12% in the low-, medium-, and high-dose groups, respectively, compared to the blank group ($p < 0.001$).

3.3.4 SOD and CAT Activity, and MDA Content in *C. elegans*

SOD and CAT are the main antioxidant enzymes in *C. elegans* and can eliminate and reduce excessive free radicals, thereby delaying aging. MDA is one of the essential biomarkers of the oxidative stress response and can also reflect the aging status [28]. The results for SOD and CAT activity in the *C. elegans* body are shown in Fig. 4D. Lotus seed coat extract significantly improved the antioxidant enzyme activity in the *C. elegans* body in a dose-dependent manner ($p < 0.001$). The results for MDA content in the nematode body are shown in Fig. 4E. Both the medium- and high-dose groups significantly reduced MDA content in the *C. elegans* body ($p < 0.05$), with a non-significant decrease observed in the low-dose group.

3.4 Network Pharmacology Analysis Predictions

After screening and removing duplicate targets, the 67 compounds identified by mass spectrometry in lotus seed coats were predicted to have 672 drug targets (Fig. 7). Disease targets were retrieved from Genecard databases using the keyword "aging". Those with a relevance score > 5.00 were selected after screening out duplicate targets, resulting in 1238 aging disease targets. Next, the targets of lotus

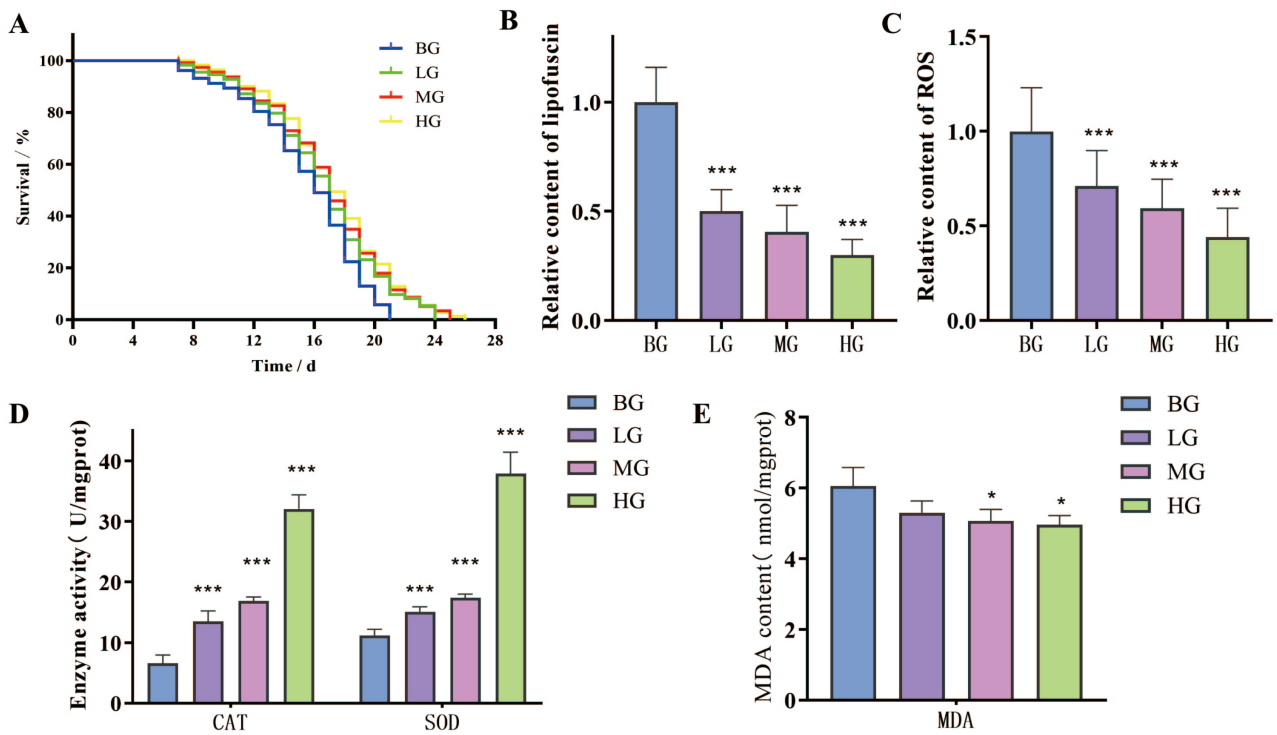


Fig. 4. The effect of lotus seed coat extract on the aging of *C. elegans*. (A–E) The effects of lotus seed coat extract on the lifespan, lipofuscin content, reactive oxygen species (ROS) levels, antioxidant enzyme (SOD/CAT) activities, and Malondialdehyde (MDA) content in *C. elegans*. (* $p < 0.05$, *** $p < 0.001$ vs. BG group.)

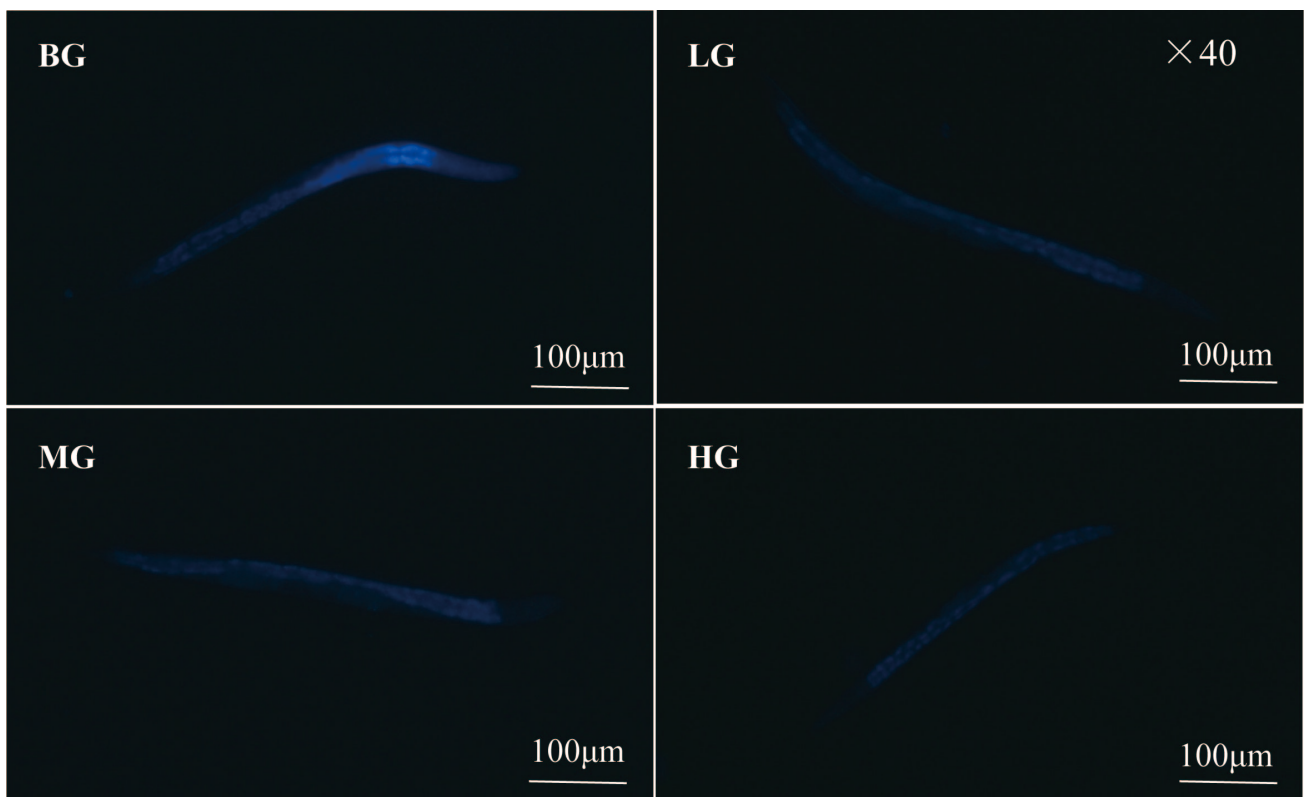


Fig. 5. Lipofuscin fluorescence image of *C. elegans*. Scale bar = 100 µm.

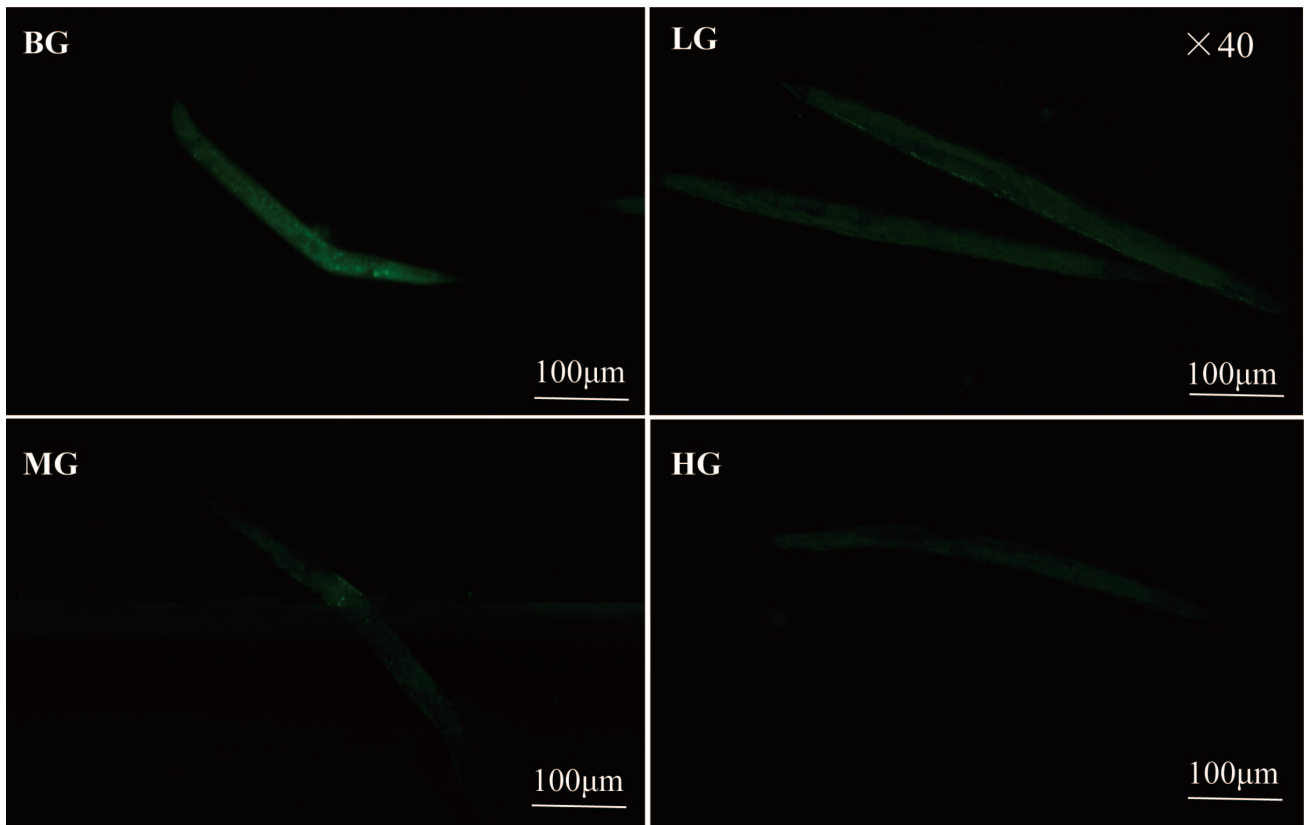


Fig. 6. ROS fluorescence image of *C. elegans*. Scale bar = 100 μm.

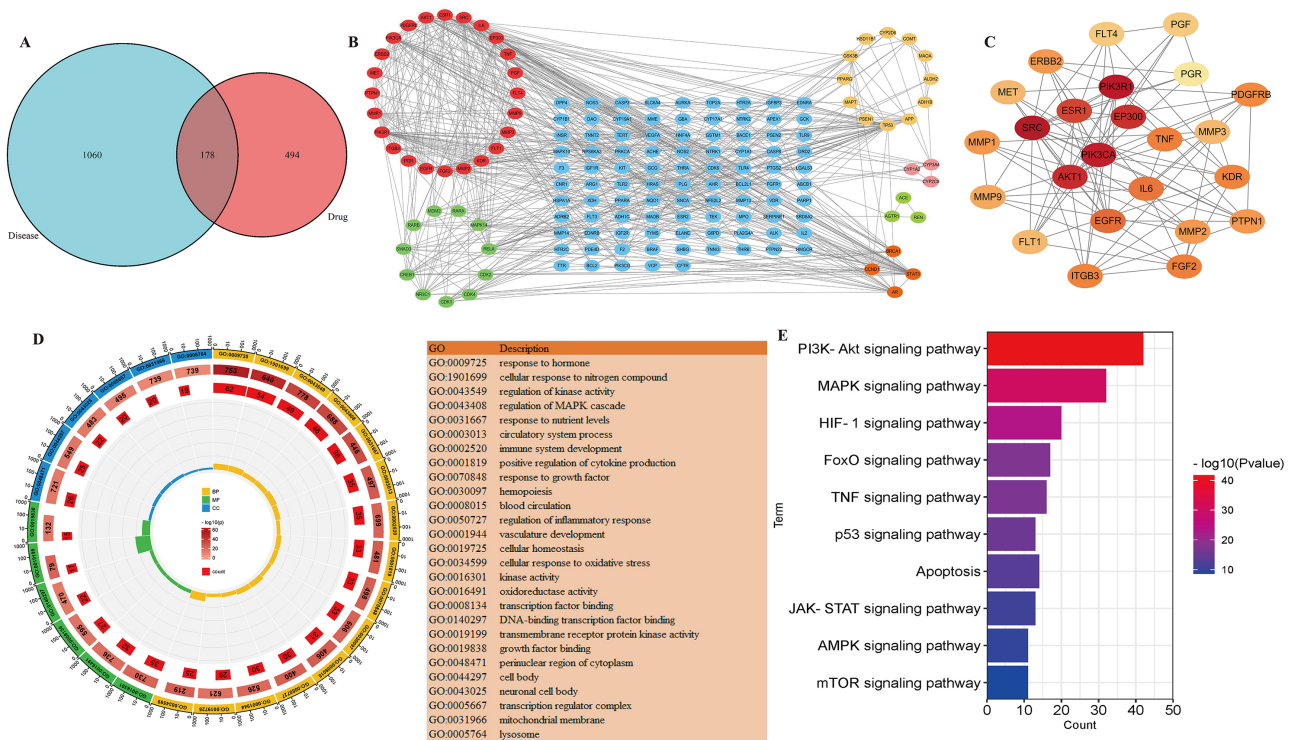


Fig. 7. Results of network pharmacology analysis. (A) Common targets of the compound and of aging. (B) A protein-protein interaction network with a common target. Different colors represent different clustering modules created by the MCODE algorithm. (C) In the first module, the hub targets were grouped. (D) GO enrichment analysis of common targets. (E) KEGG enrichment analysis of common targets. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

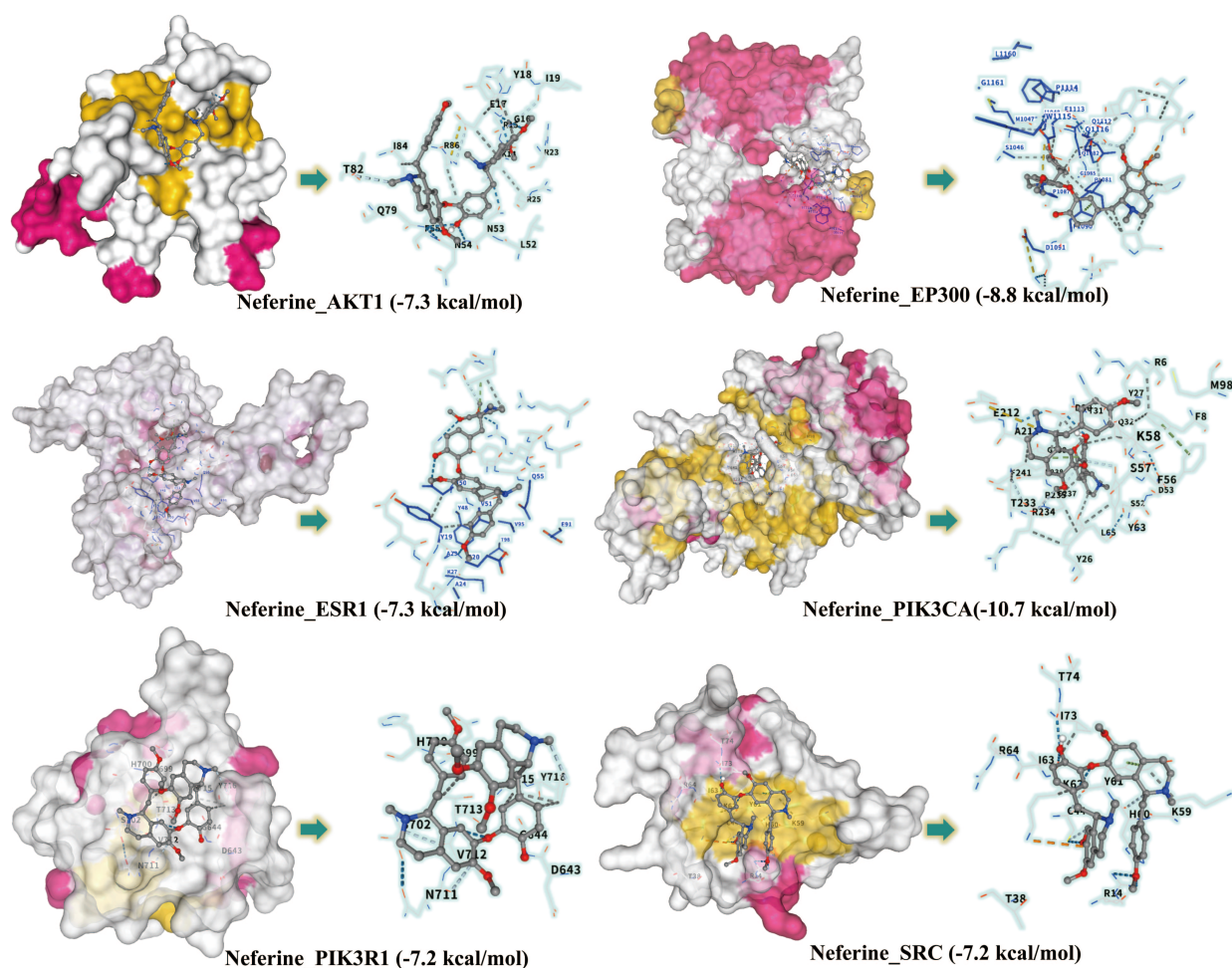


Fig. 8. Binding modes and affinities of Neferine with six core proteins.

seed coat compounds were intersected with the targets of senescence diseases using R language, giving rise to 225 common targets (Fig. 7A).

3.4.1 Construction of Protein-Protein Interaction (PPI) Networks

Common targets were first imported into the STRING database. Those without interactions were removed, and high-confidence targets with a threshold value >0.900 were screened. The results were analyzed topologically using Cytoscape 3.7.2 software (Cytoscape Consortium, Seattle, WA, USA) to obtain an initial PPI network of 152 nodes and 580 edges. The target network was clustered using the MCODE plug-in, and targets with similar functions or closely related nodes were classified into clusters of the same topology. The Cytohubba plug-in was subsequently used for core target selection (Fig. 7B,C).

In the PPI network, the degree of freedom for each node represents the number of interactions with other nodes. The higher the value for the degree of freedom, the higher the importance of that node. The first 10 core genes predicted by this analysis were *SRC*, *PIK3R1*, *PIK3CA*, *AKT1*, *EP300*, *ESR1*, *EGFR*, *IL6*, *TNF*, and *FGF2*.

3.4.2 GO Functional Annotation and KEGG Pathway Enrichment Analysis

GO and KEGG pathway enrichment analyses were performed to analyze the biological functions and pathways involved in the 225 common targets. GO functional annotation enrichment analysis focused on three aspects: biological processes (BP), cellular composition (CC), and molecular functions (MF). The analysis identified 2016 BP, including regulation of hormone levels, regulation of nitrogenous compounds in cells, regulation of enzyme activity, and regulation of the MAPK cascade reaction; 122 CC including the cytoplasmic perinuclear region, cell bodies, transcriptional regulator complexes, mitochondrial membranes, and lysosomes; and 194 MF including oxidoreductase activity, transcription factors, protein kinase activity, and growth factors (Fig. 7D). GO functional annotation enrichment analysis revealed that the anti-aging effect of lotus seed coat is mediated mainly through biological processes related to hormone levels, enzyme activity, oxidative stress, immunity, inflammation, and blood.

KEGG pathway enrichment analysis indicated the anti-aging effect of lotus seed coat was mainly related to autophagy and to the phosphatidylinositol 3-kinase/protein

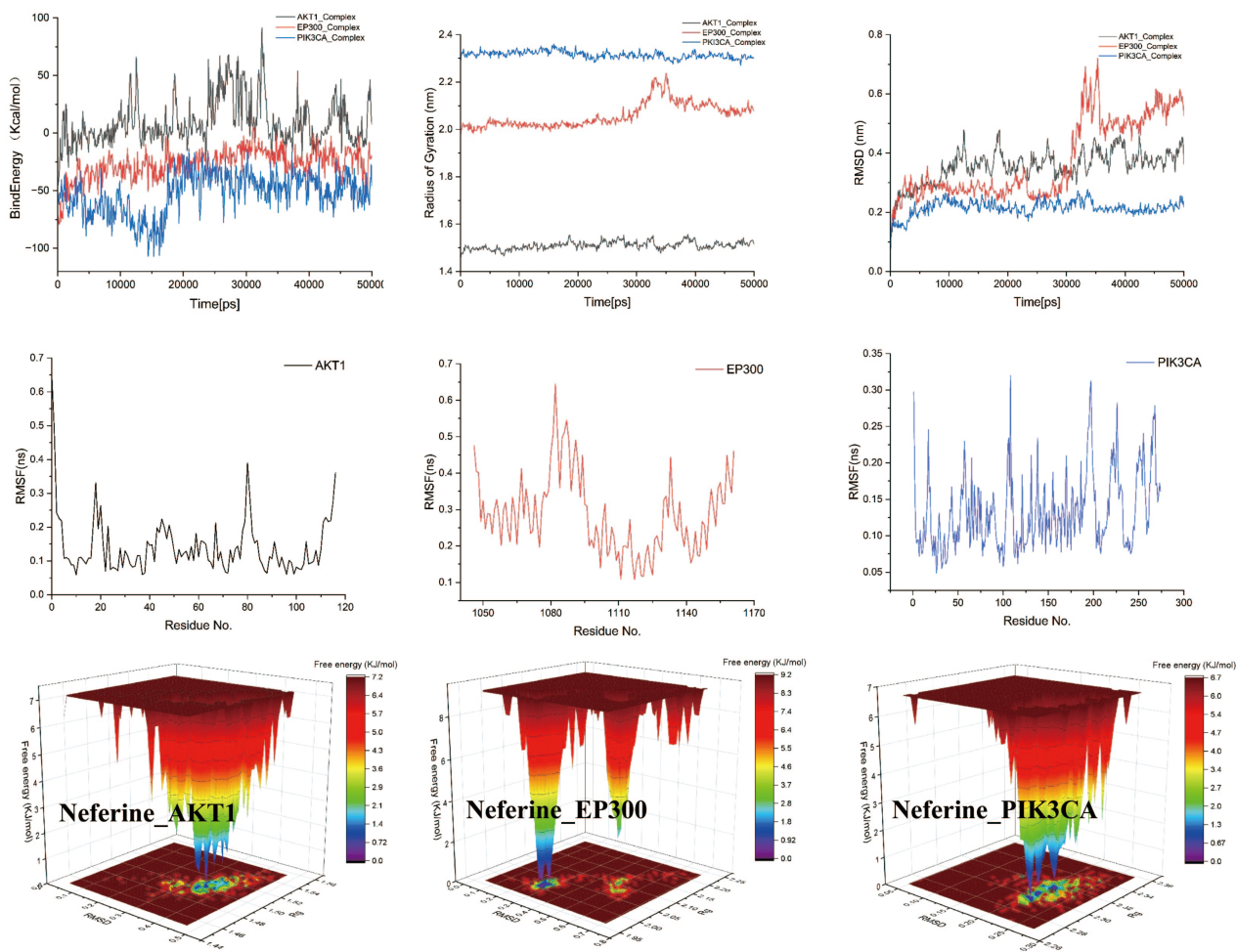


Fig. 9. Molecular dynamics simulation results for Neferine with PIK3CA, EP300, and AKT. PIK3CA, phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha; EP300, histone acetyltransferase p300; AKT1, RAC-alpha serine/threonine-protein kinase.

kinase B pathway (PI3K–Akt), mitogen-activated protein kinase (MAPK), hypoxia-inducible factor 1 (HIF-1), fork-head box O (FOXO), tumor necrosis factor (TNF), and mammalian target of rapamycin (mTOR) signaling pathways (Fig. 7E).

3.4.3 Molecular Docking and Molecular Dynamics Simulation Analysis

Neferine was identified as the primary alkaloid component in the lotus seed coat [29]. Analysis by high-performance liquid chromatography (HPLC) revealed that its content in the lotus seed coat was 0.033 mg/g (Supplementary Fig. 1). We next selected the top six key proteins in terms of degree value for molecular docking analysis with neferine. The binding energy of each protein to neferine was found to be <0 , indicating a strong affinity with each (Fig. 8). Since phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), histone acetyltransferase p300 (EP300), and RAC-alpha serine/threonine-protein kinase (AKT1) ranked as the top three proteins for affinity and are also associated with

the PI3K–Akt signaling pathway, these were selected for molecular dynamics simulations. The results showed that each complex tended to stabilize within 50 ns, with the PIK3CA-neferine complex being the most stable (Fig. 9).

4. Discussion

Aging is a natural physiological process in organisms characterized by the gradual accumulation of cellular damage and metabolic waste, leading to functional decline, increased vulnerability, and ultimately organismal death [30]. To date, significant progress has been made in anti-aging therapeutics, particularly in the development of natural and synthetic compounds. These anti-aging strategies and compounds are primarily focused on the clearance of senescent cells, augmentation of NAD, anti-inflammatory and antioxidant defense, restoration of proteostasis, and activation of telomerase [31].

In the current study, 67 components of the lotus seed coat were inferred by LC-MS, including 14 alkaloids, 19 flavonoids, 6 phenolic acids, 12 fatty acids, and several other compounds. Our results showed that the lotus seed

coat can eliminate free radicals *in vitro*, indicating that it may have robust anti-aging effects. Lotus seed coat extract was found to significantly prolong the lifespan of the experimental model *C. elegans*. Moreover, it reduced the levels of lipofuscin and ROS, increased the activities of SOD and CAT, and reduced the level of MDA in this model. In summary, treatment with lotus seed coat extract resulted in moderate but significant anti-aging-related phenotypes in *C. elegans*, possibly by attenuating oxidative stress regulatory pathways.

To link the observed phenotypic effects of lotus seed coat extract with potential molecular mechanisms, network pharmacology was employed to integrate compound-target-disease interactions. This multi-target prediction approach found that alkaloids and flavonoids may be the primary anti-aging components in the lotus seed coat. These components exert their effects by regulating genes such as *SRC*, *PIK3R1*, *AKT1*, *IL6*, and *TNF* through the PI3K–Akt, MAPK, and mTOR signaling pathways. Previous studies have shown these pathways act as core regulators of the aging process by modulating oxidative stress, inflammatory responses, metabolic homeostasis, and cell survival [32,33]. Natural products and common anti-aging agents can inhibit overactivation of the pathways through multi-target effects, thereby providing potential strategies for delaying aging [34].

5. Strengths and Limitations of This Study

As a natural product, lotus seed coat extract has the advantages of low effective dose, minimal side effects, and stable efficacy, making it suitable for long-term intervention [35]. Its core mechanisms may overlap with existing anti-aging products, including antioxidant activity and the regulation of classical pathways such as PI3K–Akt/mTOR and FOXO. Lotus seed coat may delay aging by scavenging free radicals, activating endogenous antioxidant systems, and modulating cellular metabolism. Notably, its uniqueness lies in multi-component synergy and multi-target network regulation, forming a composite mode of “holistic intervention-precise regulation” that is particularly suited for natural, long-term aging prevention. This addresses the limitations of single-component anti-aging products, including narrow target scope, low bioavailability, or prominent side effects [36]. Furthermore, this study found that by-products of lotus seed processing can be converted into anti-aging resources, thereby providing a practical example for the high-value utilization of agricultural waste and demonstrating both academic innovation and industrial application.

However, this study did not clarify whether the extract activates classic anti-aging pathways, nor did it validate the observed metabolic regulatory effects in mammalian models. Despite these limitations, it also provided initial assessment and prediction, thereby laying the groundwork for future research endeavors. As the focus of this study was

mainly on preliminary evaluation and prediction, further experimental investigations are required.

6. Conclusion

This preliminary study infers that the lotus seed coat contains active antioxidant components, as determined by component analysis and *in vitro* antioxidant experiments. The anti-aging potential of the lotus seed coat was also verified using *Caenorhabditis elegans* and network pharmacology. Our results provide a replicable and practical example for the resource utilization of agricultural waste.

Availability of Data and Materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

XRL: Conceptualization, Methodology, Supervision; XWY: Investigation, Formal analysis, Supervision, Funding acquisition; CSL: Data curation, Validation, Writing–review & editing; XFX and CJZ: Supervision, Data curation; YJW: Data curation, Validation. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

According to Chinese regulations and policies, this study does not involve human or animal subjects; therefore, ethical approval for the *Caenorhabditis elegans* experiment is not necessary. The lotus seeds used in this experiment were collected from Fuzhou City, Jiangxi Province, China. Professor Xiangri Li, an expert in Chinese materia medica from Beijing University of Chinese Medicine, identified the lotus seeds as the dried mature nuts of *Nelumbo nucifera* Gaertn., a plant belonging to the genus *Nelumbo* (family Nymphaeaceae).

Acknowledgment

The authors are thankful to the Jiangxi/Beijing University of Chinese Medicine for the assistance in conducting this study.

Funding

This study was supported by the Science and Technology Program Project of Administration of Traditional Chinese Medicine of Jiangxi Province (2024A0079), Doctoral Startup Fund (2024BSZR052).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/IJP46482>.

References

- [1] Athanasaki A, Melanis K, Tsantzali I, Stefanou MI, Ntymenou S, Paraskevas SG, *et al.* Type 2 Diabetes Mellitus as a Risk Factor for Alzheimer's Disease: Review and Meta-Analysis. *Biomedicines*. 2022; 10: 778. <https://doi.org/10.3390/biomedicines10040778>.
- [2] Twarowski B, Piątkowska-Chmiel I, Herbet M. Mitochondria as a Therapeutic Target in Neurodegeneration: Strategies for Restoring Cellular Homeostasis. *Current Neuropharmacology*. 2025. <https://doi.org/10.2174/011570159X389970250727031306>. (in press)
- [3] de Magalhães JP. An overview of contemporary theories of ageing. *Nature Cell Biology*. 2025; 27: 1074–1082. <https://doi.org/10.1038/s41556-025-01698-7>.
- [4] Wei Y, Xiong B, Song Z, Zhong S, Zheng N, Zhang A, *et al.* Aging and anti-aging strategies: A review of past and future therapeutics. *Bioorganic & Medicinal Chemistry*. 2025; 129: 118340. <https://doi.org/10.1016/j.bmc.2025.118340>.
- [5] Shetty AK, Kodali M, Upadhyaya R, Madhu LN. Emerging Anti-Aging Strategies - Scientific Basis and Efficacy. *Aging and Disease*. 2018; 9: 1165–1184. <https://doi.org/10.14336/AD.2018.1026>.
- [6] Zhang Z, Yang R, Zi Z, Liu B. A new clinical age of aging research. *Trends in Endocrinology and Metabolism: TEM*. 2025; 36: 440–458. <https://doi.org/10.1016/j.tem.2024.08.004>.
- [7] Lai M, Zhang H-j, Wang F, Shao Y-l, Yang M-w, Hong F-f, *et al.* Anti-aging Effects of Ginseng and Ginsenosides on the Nervous System. *IJP*. 2018; 14: 1188–1197. <https://doi.org/10.3923/ijp.2018.1188.1197>.
- [8] Liu M-H, Lin Z-Y, Yang P-F, He D-L. Research progress on the longevity mechanism of *Nelumbo nucifera* seeds. *Plant Science Journal*. 2019; 37: 396–403. <https://doi.org/10.11913/PSJ.2095-0837.2019.30396>.
- [9] Cao X, Lin X, Wu C, Zhang M, Wang M. Green Extraction-Assisted Pseudo-Targeted Profile of Alkaloids in Lotus Seed Epicarp Based on UPLC-QTOF MS with IDA. *Foods (Basel, Switzerland)*. 2022; 11: 1056. <https://doi.org/10.3390/foods11071056>.
- [10] Li CS, Xu WJ, Ye XW, Wang X, Cheng SQ, Wen J, *et al.* Alkaloids in Lotus Seed Epicarp and Their Content Determination Based on Molecular Network Combined with LC-MS Technology. *Modern Chinese Medicine*. 2023; 25: 296–303. <https://doi.org/10.13313/j.issn.1673-4890.20220510004>. (In Chinese)
- [11] Zhu Z, Zhong B, Yang Z, Zhao W, Shi L, Aziz A, *et al.* LC-ESI-QTOF-MS/MS Characterization and Estimation of the Antioxidant Potential of Phenolic Compounds from Different Parts of the Lotus (*Nelumbo nucifera*) Seed and Rhizome. *ACS Omega*. 2022; 7: 14630–14642. <https://doi.org/10.1021/acsomega.1c07018>.
- [12] Punia Bangar S, Dunno K, Kumar M, Mostafa H, Maqsood S. A comprehensive review on lotus seeds (*Nelumbo nucifera* Gaertn.): Nutritional composition, health-related bioactive properties, and industrial applications. *Journal of Functional Foods*. 2022; 89: 104937. <https://doi.org/https://doi.org/10.1016/j.jff.2022.104937>.
- [13] Liu Y, Ma SS, Ibrahim SA, Li EH, Yang H, Huang W. Identification and antioxidant properties of polyphenols in lotus seed epicarp at different ripening stages. *Food Chemistry*. 2015; 185: 159–164. <https://doi.org/10.1016/j.foodchem.2015.03.117>.
- [14] Sun H, Xin J, Ullah A, Song H, Chen L, Yang D, *et al.* Unveiling the secrets of lotus seed longevity: insights into adaptive strategies for extended storage. *Journal of Experimental Botany*. 2025; 76: 1147–1163. <https://doi.org/10.1093/jxb/erae432>.
- [15] Lin Y, Lin C, Cao Y, Chen Y. *Caenorhabditis elegans* as an in vivo model for the identification of natural antioxidants with anti-aging actions. *Biomedicine & Pharmacotherapy = Biomedecine & Pharmacotherapie*. 2023; 167: 115594. <https://doi.org/10.1016/j.biopha.2023.115594>.
- [16] Ye XW, Wang HL, Cheng SQ, Xia LJ, Xu XF, Li XR. Network Pharmacology-Based Strategy to Investigate the Pharmacologic Mechanisms of *Coptidis Rhizoma* for the Treatment of Alzheimer's Disease. *Frontiers in Aging Neuroscience*. 2022; 14: 890046. <https://doi.org/10.3389/fnagi.2022.890046>.
- [17] Cao J, Yu X, Deng Z, Pan Y, Zhang B, Tsao R, *et al.* Chemical Compositions, Antiobesity, and Antioxidant Effects of Proanthocyanidins from Lotus Seed Epicarp and Lotus Seed Pot. *Journal of Agricultural and Food Chemistry*. 2018; 66: 13492–13502. <https://doi.org/10.1021/acs.jafc.8b05137>.
- [18] Hao C, Yang W, Dong G, Yu Y, Liu Y, Zhang J, *et al.* Visualization and identification of benzyloquinoline alkaloids in various *nelumbo nucifera* tissues. *Heliyon*. 2023; 9: e16138. <https://doi.org/10.1016/j.heliyon.2023.e16138>.
- [19] Deng X, Zhu L, Fang T, Vimolmangkang S, Yang D, Ogutu C, *et al.* Analysis of Isoquinoline Alkaloid Composition and Wound-Induced Variation in *Nelumbo* Using HPLC-MS/MS. *Journal of Agricultural and Food Chemistry*. 2016; 64: 1130–1136. <https://doi.org/10.1021/acs.jafc.5b06099>.
- [20] Yang M, Li J, Zhao C, Xiao H, Fang X, Zheng J. LC-Q-TOF-MS/MS detection of food flavonoids: principle, methodology, and applications. *Critical Reviews in Food Science and Nutrition*. 2023; 63: 3750–3770. <https://doi.org/10.1080/10408398.2021.1993128>.
- [21] Zhu MZ, Wu W, Jiao LL, Yang PF, Guo MQ. Analysis of Flavonoids in Lotus (*Nelumbo nucifera*) Leaves and Their Antioxidant Activity Using Macroporous Resin Chromatography Coupled with LC-MS/MS and Antioxidant Biochemical Assays. *Molecules (Basel, Switzerland)*. 2015; 20: 10553–10565. <https://doi.org/10.3390/molecules200610553>.
- [22] Yu Y, Wei X, Liu Y, Dong G, Hao C, Zhang J, *et al.* Identification and quantification of oligomeric proanthocyanidins, alkaloids, and flavonoids in lotus seeds: A potentially rich source of bioactive compounds. *Food Chemistry*. 2022; 379: 132124. <https://doi.org/10.1016/j.foodchem.2022.132124>.
- [23] Yi Y, Li T, Lv C, He W, Li W, Zhou X, *et al.* Proanthocyanidins isolated from lotus seed skin mitigate glycolipid metabolism disorder through the p38/Nrf2/NF- κ B signaling pathway. *Acta Biochimica et Biophysica Sinica*. 2024; 56: 1300–1310. <https://doi.org/10.3724/abbs.2024042>.
- [24] Yu X, Wang Y, Yan X, Leng T, Xie J, Yu Q, *et al.* Metabolomics Combined with Correlation Analysis Revealed the Differences in Antioxidant Activities of Lotus Seeds with Varied Cultivars. *Foods (Basel, Switzerland)*. 2024; 13: 1084. <https://doi.org/10.3390/foods13071084>.
- [25] Ma Z, Huang Y, Huang W, Feng X, Yang F, Li D. Separation, Identification, and Antioxidant Activity of Polyphenols from Lotus Seed Epicarp. *Molecules (Basel, Switzerland)*. 2019; 24: 4007. <https://doi.org/10.3390/molecules24214007>.
- [26] Xiao Y, Zhang L, Liu Y. Protocol for assessing the healthspan of *Caenorhabditis elegans* after potential anti-aging drug treatment. *STAR Protocols*. 2023; 4: 102285. <https://doi.org/10.1016/j.xpro.2023.102285>.
- [27] Hui H, Xin A, Cui H, Jin H, Yang X, Liu H, *et al.* Anti-aging effects on *Caenorhabditis elegans* of a polysaccharide, O-acetyl glucomannan, from roots of *Lilium davidii* var. *unicolor* Cotton. *International Journal of Biological Macromolecules*. 2020; 155: 846–852. <https://doi.org/10.1016/j.ijbiomac.2020.03.206>.

- [28] Zhang J, Chen R, Yu Z, Xue L. Superoxide Dismutase (SOD) and Catalase (CAT) Activity Assay Protocols for *Caenorhabditis elegans*. *Bio-protocol*. 2017; 7: e2505. <https://doi.org/10.21769/BioProtoc.2505>.
- [29] Bharathi Priya L, Huang CY, Hu RM, Balasubramanian B, Baskaran R. An updated review on pharmacological properties of neferine-A bisbenzylisoquinoline alkaloid from *Nelumbo nucifera*. *Journal of Food Biochemistry*. 2021; 45: e13986. <https://doi.org/10.1111/jfbc.13986>.
- [30] Guo J, Huang X, Dou L, Yan M, Shen T, Tang W, *et al*. Aging and aging-related diseases: from molecular mechanisms to interventions and treatments. *Signal Transduction and Targeted Therapy*. 2022; 7: 391. <https://doi.org/10.1038/s41392-022-01251-0>.
- [31] Li Y, Tian X, Luo J, Bao T, Wang S, Wu X. Molecular mechanisms of aging and anti-aging strategies. *Cell Communication and Signaling: CCS*. 2024; 22: 285. <https://doi.org/10.1186/s12964-024-01663-1>.
- [32] Liu Y, Liu Q, Zhang Z, Yang Y, Zhou Y, Yan H, *et al*. The regulatory role of PI3K in ageing-related diseases. *Ageing Research Reviews*. 2023; 88: 101963. <https://doi.org/10.1016/j.arr.2023.101963>.
- [33] Anerillas C, Altés G, Gorospe M. MAPKs in the early steps of senescence implementation. *Frontiers in Cell and Developmental Biology*. 2023; 11: 1083401. <https://doi.org/10.3389/fcell.2023.1083401>.
- [34] Zhang X, Gao Y, Zhang S, Wang Y, Pei X, Chen Y, *et al*. Mitochondrial dysfunction in the regulation of aging and aging-related diseases. *Cell Communication and Signaling: CCS*. 2025; 23: 290. <https://doi.org/10.1186/s12964-025-02308-7>.
- [35] Wang M, Hu WJ, Wang QH, Yang BY, Kuang HX. Extraction, purification, structural characteristics, biological activities, and application of the polysaccharides from *Nelumbo nucifera* Gaertn. (lotus): A review. *International Journal of Biological Macromolecules*. 2023; 226: 562–579. <https://doi.org/10.1016/j.ijbiomac.2022.12.072>.
- [36] Teng H, Xiao H, Li X, Huang J, Zhang B, Zeng M. Recent advances in the anti-aging effects of natural polysaccharides: Sources, structural characterization, action mechanisms and structure-activity relationships. *Trends in Food Science & Technology*. 2025; 160: 105000. <https://doi.org/https://doi.org/10.1016/j.tifs.2025.105000>.