[Short title + Author Name - P&H title] 31 (2023) 101829

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Saudi Pharmaceutical Journal

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Original article

Exploring the chemical composition, medicinal benefits, and antioxidant activity of *Plumula nelumbinis* essential oil from different habitats in China

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ARTICLE INFO

Keywords:
Plumula nelumbinis
Essential oil
GC–MS
Network pharmacology
Antioxidant activities

ABSTRACT

Plumula nelumbinis, a widely used traditional Chinese medicine known for its calming and nerve-soothing properties, contains essential oil as a primary component. However, research on *P. nelumbinis* essential oil (PNEO) is limited. This study aimed to investigate PNEO components, network target analysis, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses, and antioxidant activity of *P. nelumbinis* from ten different habitats. GC–MS analysis identified 14 compounds in the essential oil, with CP12 (β-Sitosterol) having the highest concentration. Five compounds were identified for the first time in *P. nelumbinis*, with three of them reported for the first time in the *Nelumbo*. Network target analysis revealed 185 potential targets for 11 compounds and GO and KEGG enrichment analyses showed that PNEO was mainly located in the plasma membrane and could regulate a variety of molecular functions. KEGG pathway enrichment analysis revealed that the essential oil was primarily enriched in pathways related to cancer and the nervous system. PNEO demonstrated strong antioxidant activity, with N_8 (Fujiannanping) showing the highest ABTS scavenging capacity and N_7 (Hunanxiangtan) showing the highest DPPH radical scavenging capacity. Cell experiments showed that CP4, CP5 and CP10 had protective effects against H_2O_2-induced oxidative damage. The study suggests that *P. nelumbinis* from different regions may have slightly different pharmacological effects due to the presence of unique compounds, and further research is necessary to explore the potential therapeutic benefits of PNEO.

1. Introduction

Plumula nelumbinis (*P. nelumbinis*) is the dried young cotyledon and radicle of the ripe seed of *Nelumbo nucifera* Gaertn, which is a perennial aquatic herb of Nymphaeaceae and extensively cultivated in Eastern Asia, particularly in China (Mukherjee et al., 2009, Yu et al., 2013). It is not only an edible and medicinal resource, but also recorded in ancient China that it has the functions of calming the mind, stopping bleeding, and treating irritability and confusion (Chen et al., 2021). *P. nelumbinis* contains a variety of bioactive components, such as alkaloids(Chen et al., 2021), flavonoids(Zheng et al., 2018), polysaccharides(Jiang et al., 2018) and essential oil(Lin et al., 2009, Zeng et al., 2010, Nguyen et al., 2021), and has a variety of pharmacological activities, such as anti-inflammatory, anti-oxidation and anti-tumor(Chen et al., 2021). However, the current research mainly focuses on alkaloids and flavonoids, and little is known about the *Plumula nelumbinis* essential oil (PNEO).

Recently, the investigations on the essential oils from natural herbs have attracted much attention of researchers, demonstrating that can be employed as food additives (Gourine et al., 2010). *P. nelumbinis* essential oil (PNEO) contains a high amount of unsaturated fatty acids, such as linoleic acid, oleic acid, and palmitic acid, as well as phenolic compounds, which have strong antioxidant properties(Bi et al., 2006, Li et al., 2009). Although *Nelumbo nucifera* is a widely distributed plant in Asia, there is limited research on the chemical composition of its seed embryo (*P. nelumbinis*) essential oil. Therefore, collecting *P. nelumbinis* samples from different regions is necessary for chemical analysis, pharmacological mechanism research, and antioxidant activity

Peer review under responsibility of King Saud University.

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https://doi.org/10.1016/j.jsps.2023.101829
Received 25 May 2023; Accepted 12 October 2023
Available online 18 October 2023

evaluation.

Compared with the traditional steam distillation methods, supercritical carbon dioxide extraction technology has many superiorities, such as being non-toxic, non-combustible, non-explosive, economic, easy to obtain and easy to remove from the extract, etc. (Chiu et al., 2002). In this study, the PNEO from 10 different regions of China was extracted by supercritical carbon dioxide extraction. Gas chromatography-mass spectrometry (GC–MS) was used to analyze the chemical composition, the network pharmacology method was used to analyze the potential molecular mechanism of the drug (Wu et al., 2021), and the ABTS and DPPH methods were used to evaluate the antioxidant activity, and the protective effect of standard compounds on HepG2 cells was studied by constructing a cell injury model induced by H_2O_2. The research provided more data for their possible medicinal, pharmaceutical and commercial utilization.

2. Materials and methods

2.1. Plant materials

Ten different habitats in China were sampled for *P. nelumbinis* during the summer season, with their corresponding coordinates, altitude and specimens name listed in Table 1. The samples were identified by Associate Professor Ying Zhang from the College of Pharmacy at Jinan University and confirmed as *P. nelumbinis*. Subsequently, all the samples were dried in the shade to ensure their quality.

2.2. Chemical materials

L-ascorbic acid (VC), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were procured from Sigma-Aldrich (Saint Louis, MO, USA). Oleic acid (CAS: 112–80-1), Linoleic acid (CAS: 60–33-3), and β-Sitosterol (CAS: 83–46-5) with a purity exceeding 98 % were sourced from Alfabiotech (Chengdu, China). Ethyl linoleate (CAS: 544–35-4) was obtained from TargetMol (Shanghai, China), while 4-Pyranone (CAS: 108–97-4) was acquired from Kaiwei (Shanghai, China). Absolute ethanol was purchased from Kermel Chemical Reagent Co., Ltd. (Tianjin, China), and Dimethyl sulfoxide (DMSO, >99.8 %) was sourced from Macklin (Shanghai, China). All other chemicals and reagents utilized in this study were of analytical grade and procured from Sinopharm (Shanghai, China).

2.3. Extraction of essential oil

The extraction procedure for obtaining *P. nelumbinis* essential oil was designed and slightly adapted based on the method outlined by Lin and colleagues (Lin et al., 2009). A precise amount of 200 g of *P. nelumbinis* from each habitat was carefully weighed and placed into extraction vessels containing 300 mL of 95 % ethanol for the extraction process. Extraction was conducted at a temperature of 45 °C under a pressure of 25 MPa. The separation process was carried out at a pressure of 12 MPa and a temperature of 35 °C. Once the conditions of the CO_2 extraction apparatus reached the preset parameters, the extraction process was initiated and cycled for a duration of 2 h.

Upon completion of the extraction, approximately 300 mL of a dark green oil-like liquid was obtained. This collected liquid was then concentrated into extracts using a rotary evaporator and subsequently subjected to a 50 mL diethyl ether extraction, with the addition of an appropriate quantity of anhydrous sodium sulfate. The final samples were stored in a refrigerator at −4°C to ensure the integrity of the samples for subsequent analytical experiments.

2.4. GC–MS analysis

The method of GC–MS Analysis was designed with modifications according to previous method (Lin et al., 2009, Zeng et al., 2010). The essential oil components were identified through GC–MS analysis, with the optimized GC conditions utilizing an HP-5MS 5 % phenylmethylsiloxane capillary (30.0 m × 250 μm × 0.25 μm) column and a column temperature ranging from 150 °C to 280 °C. The temperature was held at 150 °C for 3 min before being programmed to increase by 4 °C/min. The temperature was then held at 280 °C for 7 min, resulting in a total separation time of 28 min. The instrument was set to an electron energy of 70 eV and an ion source temperature of 230 °C in the electronic collision mode. The relative proportion of each compound was calculated using the peak area normalization method. The chemical composition of the essential oil was determined by mass spectrometry and retention index, with comparison to the reference mass spectrum reported in the NIST05 library.

2.5. Network pharmacological analysis

To further understand the potential mechanisms of the antioxidant effects of the active components in the PNEO, a network pharmacology approach was used to predict the relevant targets of the compounds. First of all, the compounds regulate SMILES information from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and used to search the SwissTargetPrediction web server (https://swisstargetprediction.ch/) for human target prediction (Probability*>0.1)(Xu et al., 2022).

For the analysis of the PNEO in antioxidant pharmacological mechanism, using DAVID 6.7 database (https://david.ncifcrf.gov/) has carried on the enrichment of Gene Ontology (GO), including cell composition, molecular function and biological processes (Jiao et al., 2022). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using the Metascape database (https://metascape.org) (Xu et al., 2022). Finally, R Studio was used for drawing.

2.6. Antioxidant activity

2.6.1. ABTS assay

The ABTS radical cation-based assays are widely used for measuring antioxidant capacity(Araceli et al., 2012, Ilyasov et al., 2020). 7 mM

Table 1
Coordinates, altitude and Specimens name of the collected *P. nelumbinis* from different habitats in this study.

Sample	Province	Locality	Longitude	Latitude	Altitude [m]	Specimens name
N_1	Anhui	Bozhou	115°78″	33°85″	32	QHXY-PN-E-AHBZ
N_2	Shanxi	Weinan	109°50″	34°50″	355	QHXY-PN-E-SXWN
N_3	Shandong	Weihu	117°13″	34°82″	91	QHXY-PN-E-SDWH
N_4	Zhejiang	Jinhua	119°65″	29°08″	43	QHXY-PN-E-ZJJH
N_5	Jiangxi	Shicheng	116°33″	26°33″	259	QHXY-PN-E-JXSC
N_6	Anhui	Chuzhou	117°09″	31°851″	27	QHXY-PN-E-AHCZ
N_7	Hunan	Xiangtan	112°93″	27°83″	68	QHXY-PN-E-HNXT
N_8	Fujian	Nanping	118°17″	26°65″	87	QHXY-PN-E-FJNP
N_9	Hubei	Honghu	113°45″	29°80″	28	QHXY-PN-E-HBHH
N_{10}	Fujian	Fuzhou	119°30″	26°08″	461	QHXY-PN-E-FJFZ

ABTS aqueous solution was mixed with 2.45 mM K2S2O8 aqueous solution and left in the dark for 24 h at room temperature. The mixture was diluted with absolute ethanol until the absorbance was 0.70 ± 0.005, and this solution was used as the working solution for ABTS (Kotora et al., 2016). For different concentrations of PNEO, 100 μL of different concentrations of PNEO (0.3, 0.4, 0.5, 0.75, 1 mg/mL) and 900 μLABTS working solution were added to the cuvette, thoroughly mixed, incubated in the dark for 30 min, the absorbance was measured at 734 nm (Kong et al., 2012, Zeljković et al., 2015), and the percentage of antioxidant capacity was calculated using the following formula:

$$ABTS_{\%\ of\ scavenging\ activity} = \left[\left(A_{Control} - A_{Sample}\right)/A_{Control}\right] \times 100$$

2.6.2. DPPH assay

The DPPH assay is a widely used, convenient, and cost-effective method for evaluating antioxidant properties by measuring the ability of test substances to act as hydrogen donors or free-radical scavengers (FRS) (Baliyan et al., 2022). 1 mg DPPH was precisely weighed and dissolved in 25 mL absolute ethanol as the DPPH working solution for this experiment. To perform the assay, PNEO sample were mixed with DPPH solution in five tubes at concentrations of 0.3, 0.4, 0.5, 0.75, and 1 mg/mL. The mixture was incubated for 30 min, and the absorbance at 517 nm was measured in triplicate. Each concentration was tested in triplicate, and the average value was used to calculate the corresponding DPPH clearance rate. The percentage of DPPH scavenging activity was calculated as follows:

$$DPPH_{\%\ of\ scavenging\ activity} = \left[\left(D_{Control} - D_{Sample}\right)/D_{Control}\right] \times 100$$

2.6.3. Antioxidant activity of standard compounds

Oleic acid, Ethyl linoleate, Linoleic acid, 4-Pyranone, and β-Sitosterol were selected from the results of the analysis in different habitats of PNEO and dissolved in absolute ethanol for activity analysis, while Vc was selected as a positive control. Then, 20 μL standard compound and positive drug (0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 4, 8, 16 mg/mL, respectively) were added to a 96-well plate, and 180 μL DPPH or ABTS working solution was added. After shaking in the dark, the absorbance value was measured at the wavelength of 517 nm and 734 nm. The calculation method is described in Sections 2.6.1 and 2.6.2.

2.6.4. Cell culture and viability assay

HepG2 cells were purchased from Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). The method of cell culture was formulated according to Xiao's method with a slight modification(Xiao et al., 2022); HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, USA) medium containing 10 % (V/V) fetal bovine serum (FBS, VivaCell, Shanghai) in a humidified incubator containing 5 % CO_2 at 37 °C.

N-acetylcysteine (NAC), a thiol-containing antioxidant, is often used as a direct scavenger of reactive oxygen species, especially hydrogen peroxide, and has shown substantial renoprotective activity (Kalyanaraman 2022, Wang et al., 2022). Therefore, NAC was selected as the positive control drug in this study. HepG2 cells were collected by trypsin digestion and seeded in 96-well plate at a concentration of 1×10^4 cells/well. Different concentrations (0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 mg/mL) of standard compounds (Oleic acid, Ethyl linoleate, Linoleic acid and 4-Pyranone) and NAC (ChemFaces, China) were mixed thoroughly with DMEM, respectively. Because β-Sitosterol could not be directly dissolved in DMEM, it was dissolved in DMSO first, and then mixed with DMEM thoroughly (the concentration was 0.03125, 0.0625, 0.125, 0.25, 0.5, 1, 2, 4 mg/mL). The cells were incubated with DMEM containing drugs, and blank groups without drugs and containing 0.1 %DMSO were set as controls. After 24 h of incubation, 100 μL Methylthiazolyldiphenyl-tetrazolium bromide (MTT, 1 mg/mL, Solarbio, China) was added to each well and incubated for 4 h. The medium was removed and replaced with 150 μL of DMSO, which was shaken thoroughly. The absorbance of each well was measured at 570 nm by microplate reader, and the cell survival rate was calculated as follows (Xu et al., 2023):

$$Cell\ survival\ rate(\%) = \left[1 - \left(OD_{experimental\ group} - OD_{blank\ group}\right)/OD_{experimental\ group}\right]$$

The method described above was used to investigate the optimal experimental concentration of H_2O_2 for constructing the oxidative stress model(Zhao et al., 2023).

2.6.5. Evaluation of HepG2 cells viability

After incubation with 1×10^4 cells/well for 24 h, each well was washed twice with PBS, HepG2 cells were pretreated with different concentrations of drugs for 1 h, then treated with 25 μM H_2O_2 for 4 h, and finally changed to different concentrations of drugs for another 24 h. Cell viability was obtained according to the methods described in Section 2.6.4.

2.7. Statistical analysis

All experiments in this study were repeated three times (n = 3), and data are presented as mean ± standard deviation (SD). Duncan's test of one-way analysis of variance in GraphPad Prism 9.0 software was used for statistical analysis. The significant difference was set as $P < 0.05$.

3. Results

3.1. Essential oil components

The GC–MS analysis revealed a total of 14 compounds present in PNEO extracted from 10 distinct habitats (Table 2 and Figure S1), which consisted of 6 types of terpenoids, 6 types of fatty acids and their derivatives, and 2 other types of compounds (Fig. 1). Notably, CP12 (β-Sitosterol) was found to have the highest concentration, ranging from 40.52 % to 64.93 %, which aligns with previous research(Jia et al., 2009). Additionally, CP12 and CP11 (Campesterol) were identified as the primary components of *P. nelumbinis* essential oil, with the greatest total content of CP12, CP10 (4-Pyranone), and CP8 (2-Linoleoylglycerol). Among the various habitats, the volatile oil types in N_2 (Shanxiweinan) and N_3 (Shandongweihu) were found to be the most diverse, followed by N_8 (Fujiannanping), N_{10} (Fujianfuzhou), N_7 (Hunanxiangtan), and N_5 (Jiangxishicheng). The study identified five compounds (CP1, CP2, CP6, CP10, and CP13) for the first time in *P. nelumbinis*, with three of them (CP1, CP2, and CP10) being reported for the first time in the *Nelumbo*. The differences in essential oil components of *P. nelumbinis* may be attributed to genetic and environmental factors, resulting in varying medicinal properties and antioxidant activity of PNEO from different habitats. Therefore, further research is necessary to explore the pharmacological effects of different compounds within PNEO obtained from various sources.

3.2. Network target analysis

By searching with SwissTargetPrediction, a total of 185 potential targets were identified for 11 compounds, while no potential targets were found for other compounds (Fig. 2 and Table S1). Visualization analysis using Cytoscape software revealed that CP3, C5, CP6, and CP7 had many unique targets, indicating that the pharmacological effects of *P. nelumbinis* from different regions may vary slightly due to the presence of these compounds. From the perspective of target repetition, PTPN1, CYP19A1, HMGCR, CYP17A1, and AR were targets shared by most compounds. Furthermore, the nodes of CP4, CP11, CP12, CP13, and CP14 were closely located, suggesting that they may have similar pharmacological effects.

Table 2
Components analysis of essential oil from *P. nelumbinis* in different habitats.

No.	Compound	RIa	RI[b]	Sample (%)								
					N_2	N_3	N_4	N_5	N_6	N_7	N_8	N_9	N_{10}
/	Tetradecamethylcycloheptasiloxane	1515	1516	—	5.12	3.25	—	—	—	—	—	—	—
CP1	β-sesquiphellandrene	1553	1557	—	—	—	4.47	—	—	—	—	—	—
/	Hexadecamethyl-cyclooctasioxane	1709	1709	—	4.51	2.84	—	—	—	—	—	—	—
CP2	(E) -Atlantone	1821	1785	—	—	—	3.53	—	—	—	—	—	—
/	Octadeamethyl-cyclononasiloxane	1884	1874	—	3.8	1.95	—	—	—	—	—	—	—
CP3	Ethyl palmitate	1965	1996	1.58	5.17	—	—	2.96	—	2.97	1.01	2.01	1.76
/	Eicosamethylcyclodecasiloxane	1974	2023	—	—	2.62	—	—	—	—	—	—	—
CP4	Oleic acid	2207	2175	—	—	—	—	8.56	—	1.7	1.87	—	1.14
CP5	Ethyl linoleate	2221	2193	3.55	—	—	—	—	1.49	6.81	—	—	—
CP6	Ethy oleate	2227	2185	—	1.15	1.26	—	—	—	—	—	—	—
CP7	Linoleic acid	2346	2179	1.31	—	2.76	—	—	—	—	—	—	1.07
/	Bis(2-ethylhexyl) phthalate	2591	2552	3.88	8.73	5.84	13.31	4.11	4.24	3.58	3.38	5.22	3.27
/	9,12-Octadecadienoic acid (Z,Z)-, TMS derivative	2726	2214	—	1.04	—	—	2.39	—	—	3.97	—	4.59
CP8	2-Linoleoylglycerol	2736	2706	—	1.86	4.33	—	10.08	2.93	11.02	10.08	6.81	9.74
CP9	Squalene	2882	2865	—	3.04	4.08	3.99	5.67	8.3	5.51	4.9	7.51	5.49
CP10	4-Pyranone	3104	3074	19.88	8.67	10.21	—	8.04	12.66	12.81	11.63	12.39	12.07
CP11	Campesterol	3271	3165	4.77	4.66	3.66	4.29	4.15	3.91	3.46	4.08	4.46	3.87
CP12	β-Sitosterol	3361	3351	57.82	49.15	49.13	64.93	48.17	60.34	45.94	40.52	56.33	45.34
CP13	a-Amyrin	3384	3376	—	—	—	—	—	—	—	2.68	—	—
CP14	γ-Sitostenone	3485	3483	—	—	—	—	—	—	—	8.29	—	7.31
Total				92.8	96.9	92.49	94.51	94.14	93.87	93.81	92.4	94.73	95.64

RI[a]: Compound listed in the order of elution from methyl silicone capillary column.
RI[b]: Retention indices (RI) relative to n-alkanes (C15-C35) on the same methyl silicone capillary column.
/: Miscellaneous compounds (not subject of subsequent study).

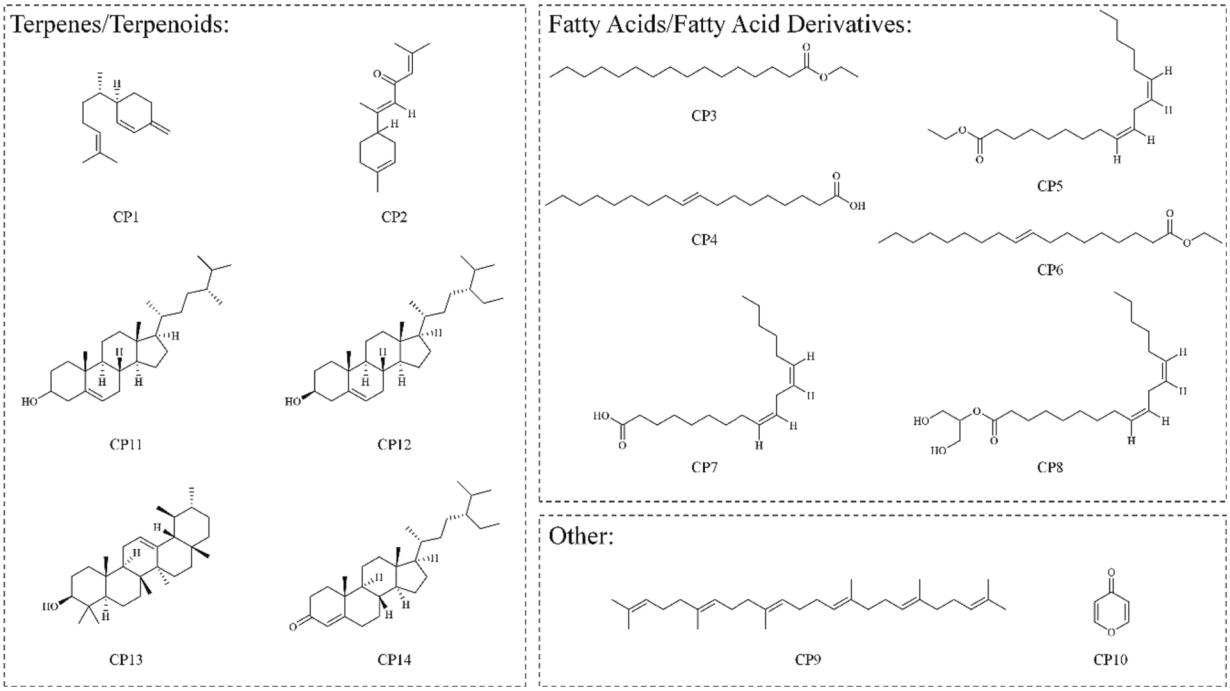

Fig. 1. 14 compounds were identified from PNEO and categorized.

3.3. GO and KEGG enrichment analyses of PNEO

The 185 identified targets were subjected to GO and KEGG enrichment analysis using DAVID and Metascape, as shown in Fig. 2. The PNEO was mainly located in the plasma membrane and could regulate a variety of molecular functions, including cytosol and endoplasmic reticulum, and participate in biological processes such as endoplasmic reticulum membrane and inflammatory response. The KEGG pathway enrichment analysis revealed that the essential oil was primarily enriched in Pathways in cancer, Neuroactive ligand-receptor interaction, Vascular smooth muscle contraction, and Inflammatory mediator regulation of TRP channels.

To visualize the results, a "habitats-compounds-targets-KEGG pathway network" was constructed by integrating the top 4 KEGG pathways with GC–MS results and removing irrelevant targets, resulting in 76 targets that acted as crucial "bridges" between compounds and pathways, as depicted in Fig. 3. The analysis revealed that CP4 (Oleic acid) and CP7 (Linoleic acid) were the primary compounds involved in the KEGG pathways, with a high number of targets pointing to these pathways and a significant predicted probability. It is noteworthy that CP4 was found only in four habitats, namely N_5 (Jiangxishicheng), N_7 (Hunanxiangtan), N_8 (Fujiannanping), and N_{10} (Hunanxiangtan). In the

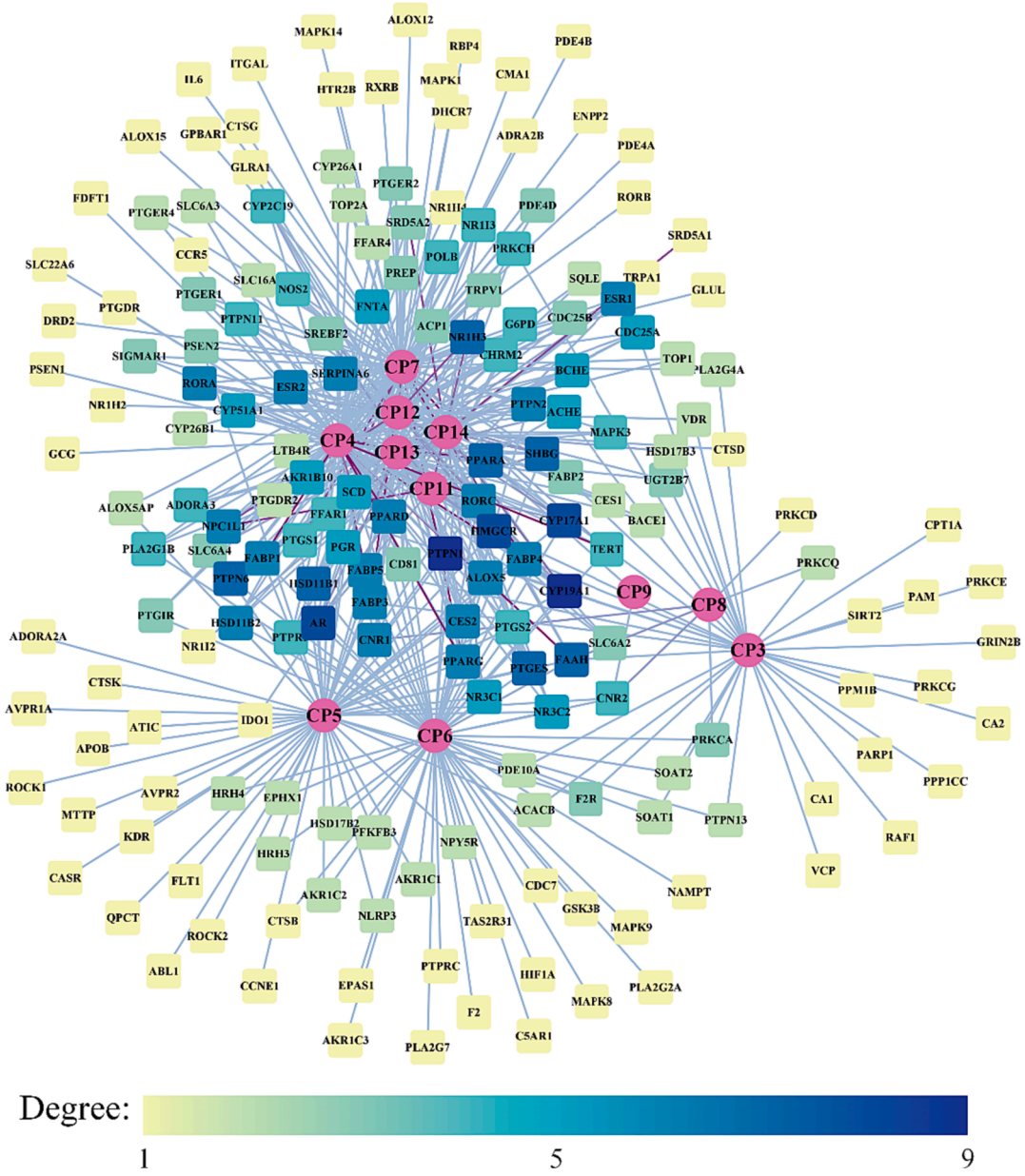

Fig. 2. Compound target network of 11 PNEO compounds and 185 potential targets. (Compounds are represented by pink circles, while targets are represented by squares. The higher the Degree of a target, the greater its cross-repetition. The deeper the Edge, the higher the prediction probability.).

network, PPARA, AR, PPARD, and RORA emerged as the most critical targets, playing a pivotal role in the pharmacological effects of PNEO.

The KEGG pathways identified in this study are primarily involved in cancer, the nervous system, and inflammatory responses. Cancer is a disease that is closely associated with oxidative stress, and antioxidants can prevent the occurrence of cancer by reducing DNA oxidative damage caused by oxidative stress (Pawlowska et al., 2019). Antioxidants can also help reduce neuronal oxidative damage, thereby playing a role in the prevention and treatment of neurological disorders (Banks et al., 2015, Xue et al., 2019). Therefore, to further investigate the potential therapeutic benefits of PNEO, it is necessary to conduct antioxidant studies on PNEO samples from different habitats.

3.4. Antioxidant activity

To comprehensively evaluate the potential therapeutic benefits of PNEO, we conducted a series of experiments to determine its antioxidant activity, including its ability to scavenge ABTS and DPPH radicals. Fig. 4 (A) illustrates the results of the ABTS scavenging capacity of PNEO, revealing that the N_8 (Fujiannanping) sample exhibited the highest activity at a concentration of 1 mg/mL, followed closely by the N_2 (Shanxiweinan) and N_1 (Anhuibozhou) samples. Similarly, the results for DPPH radical scavenging activity (Fig. 4 (B)) indicated that the N_7 (Hunanxiangtan) sample showed the highest activity at a concentration of 1 mg/mL, followed by the N_{10} (Shanxiweinan)and N_5 (Jiangxishicheng) samples. However, despite having the highest content in PNEO, CP12 (β-Sitosterol) does not make a significant contribution to antioxidant activity. These findings suggest that PNEO derived from different habitats exhibit distinct and unique antioxidant activity profiles (Filannino et al., 2020, Luan et al., 2020). Furthermore, the scavenging activity of PNEO increased with concentration, indicating its potential as an antioxidant (As shown in Fig. 5).

To accurately assess the antioxidant activity of compounds in the PNEO, CP4 (Oleic acid), CP5 (Ethyl linoleate), CP7 (Linoleic acid), CP10 (4-Pyranone) and CP12 (β-Sitosterol) as well as the positive drug Vc were selected for comparison. The result is shown in Fig. 6. Obviously,

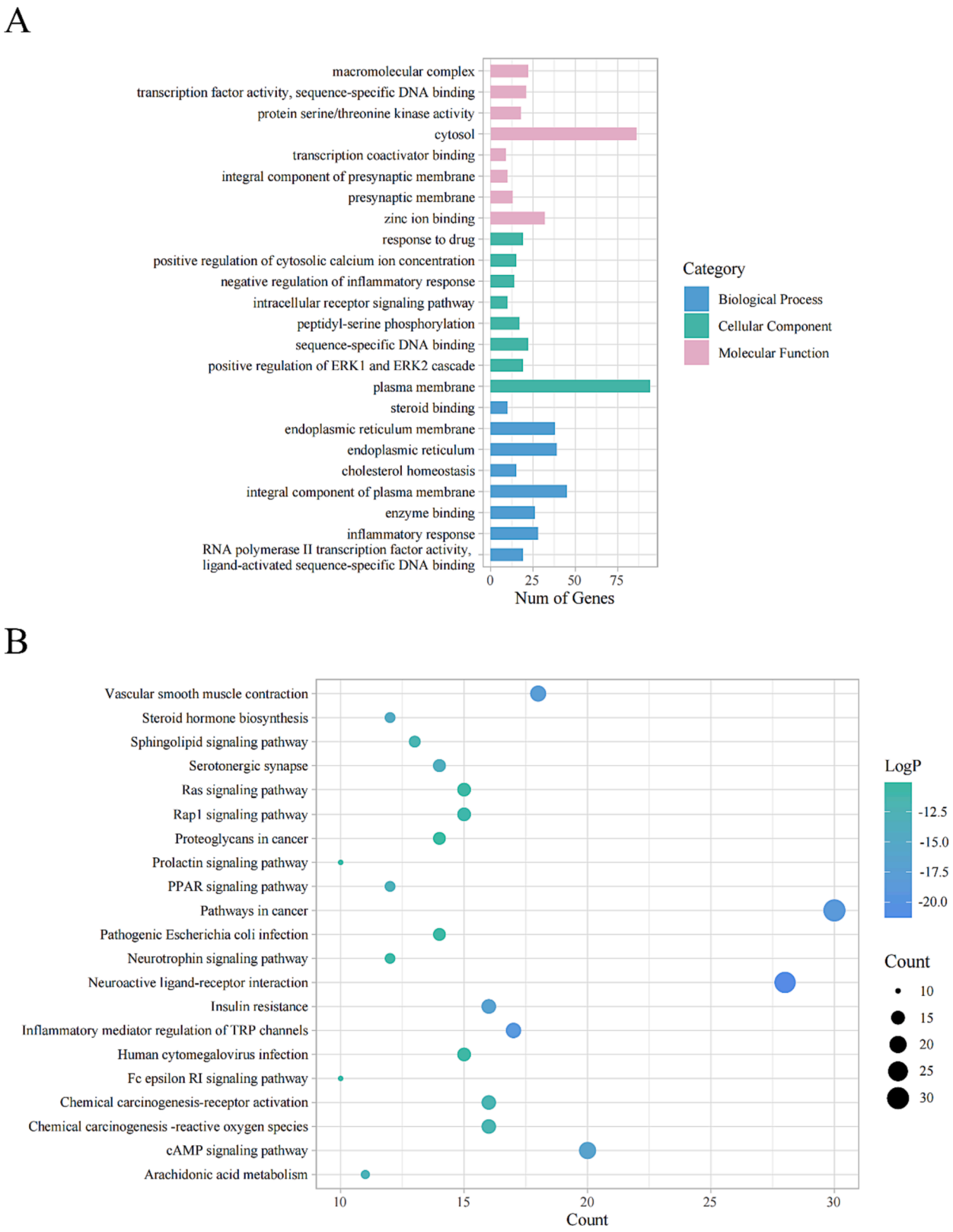

Fig. 3. GO and KEGG enrichment analysis of 185 targets of antioxidant activity in PNEO. (A) Histogram of the top 8 terms in GO enrichment analysis. (B) Bubble chart of 20 signaling pathways in KEGG enrichment analysis.

CP10 exhibited superior antioxidant activity among the five compounds, and its ABTS and DPPH radical scavenging activities were significantly enhanced with increasing concentrations. Although CP12 was abundant in PNEO, its antioxidant activity was significantly weaker. This study provides valuable insights into the antioxidant properties of PNEO and highlights the importance of studying the effects of environmental factors on the antioxidant activity of natural products.

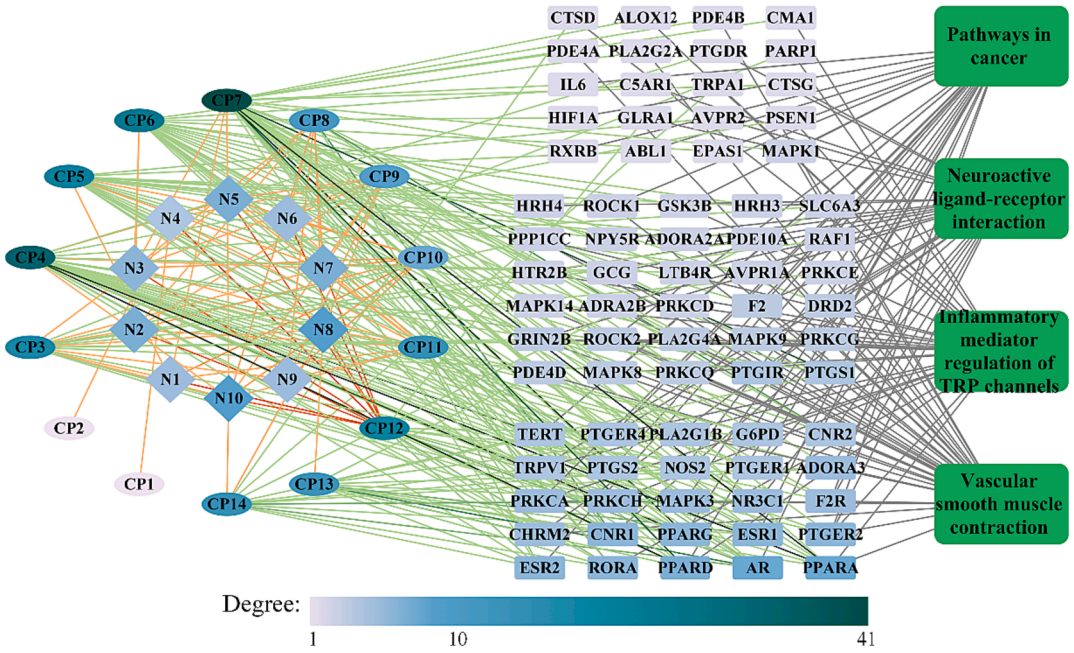

Fig. 4. Habitats - compounds - targets - KEGG pathway network. (Diamonds represent different habitats, ovals represent chemical compounds, and cubes represent targets and KEGG pathways. The size of the Degree value indicates the strength of correlation between the entities.).

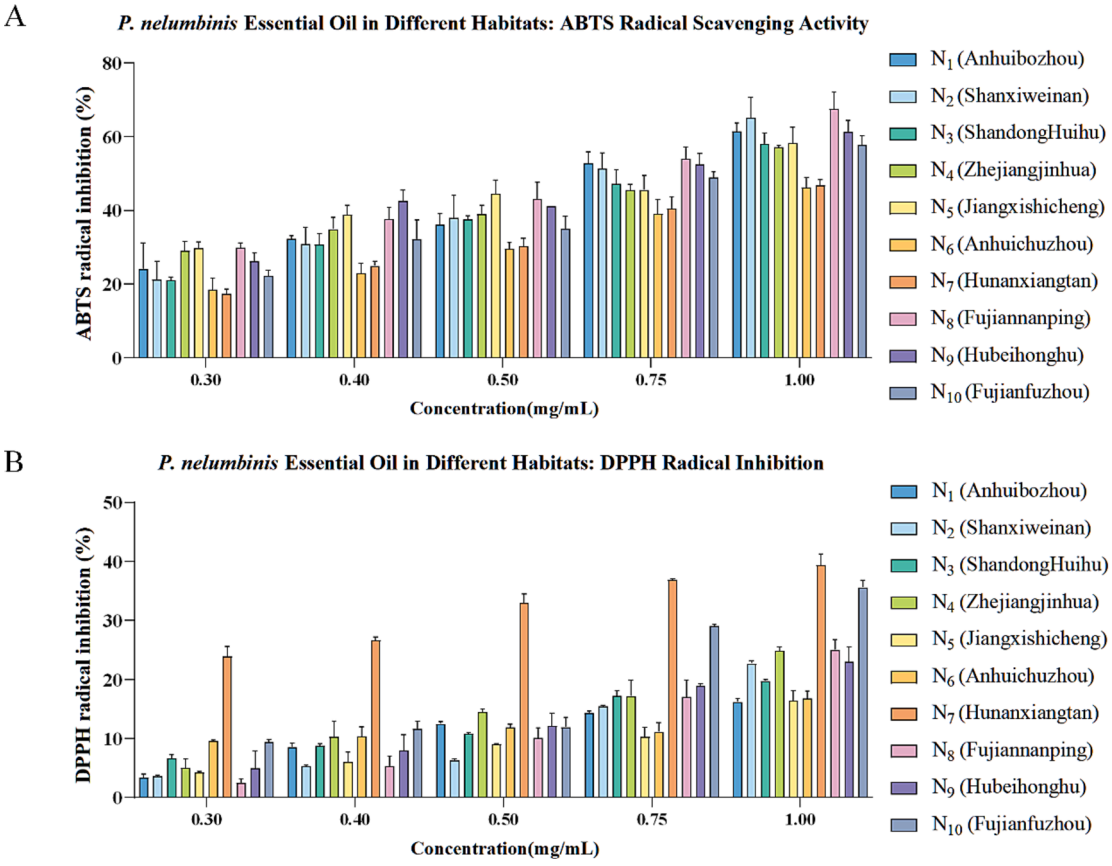

Fig. 5. *P. nelumbinis* Essential Oil in Different Habitats: (A) ABTS Radical Scavenging Activity, (B)DPPH Radical Inhibition.

3.5. Cell viability

To investigate the protective effects of CP4, CP5, CP7, CP10 and CP12 in PNEO against H_2O_2 -induced damage, it was first necessary to perform the toxicity analysis of the compounds on the cells. The viability of HepG2 cells is shown in Fig. 7. CP4 and CP7 showed significant cytotoxicity at concentrations greater than 2 mg/mL. However, CP5 did not show obvious cytotoxicity at higher concentrations (2–16 mg/mL)

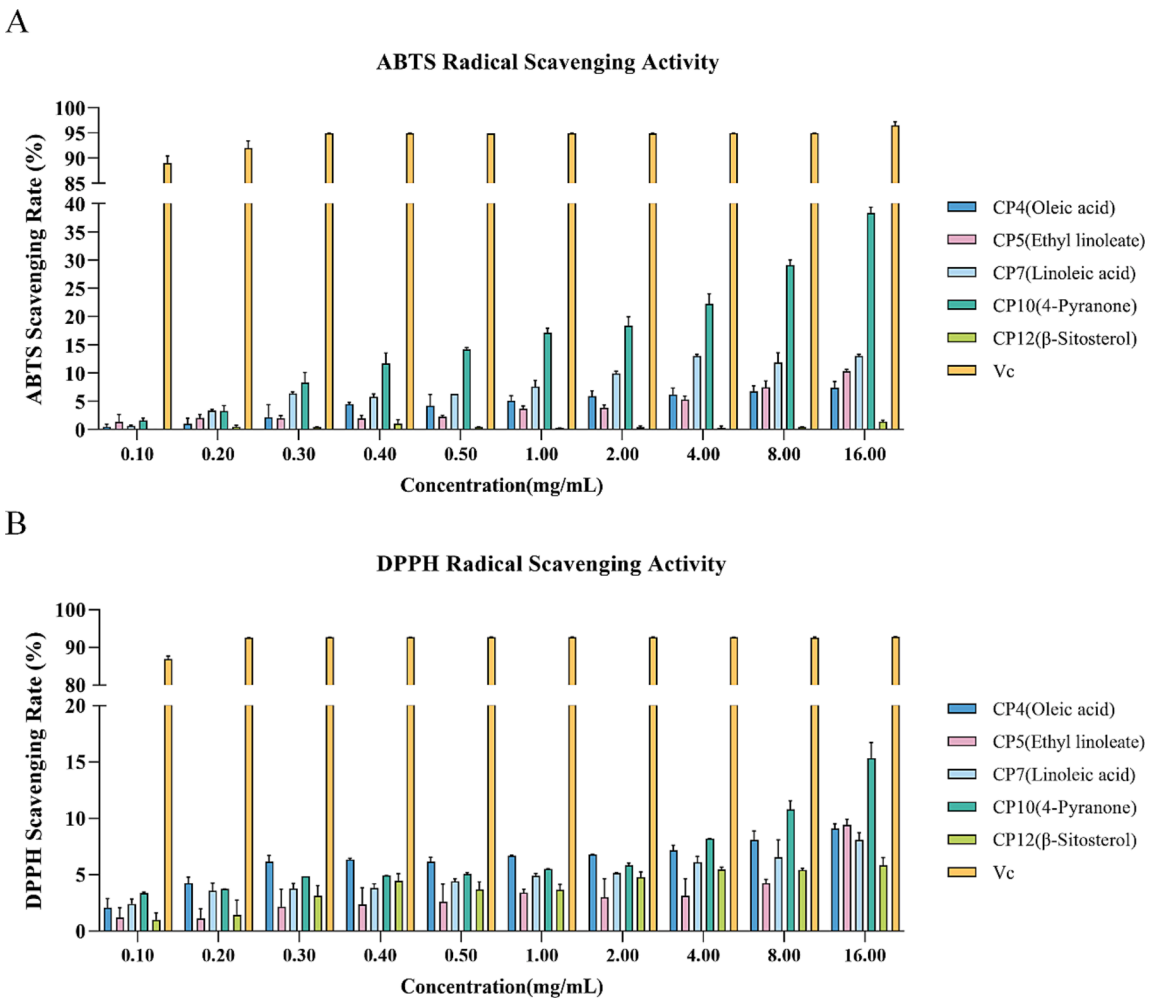

Fig. 6. Antioxidant activity of compounds at different concentrations: (A) ABTS radical scavenging activity, (B) DPPH radical scavenging activity of compounds.

but showed slight cytotoxicity at 0.25 ~ 1 mg/mL. The cytotoxic effect of CP10 was low, but CP12 showed strong cytotoxicity. However, the positive drug NAC had a significant effect on HepG2 proliferation at a high concentration.

Hydrogen peroxide showed proliferation effect on HepG2 cells under the stimulation of low concentration, but with the increase of concentration, it eventually led to cell damage (Stone and Collins 2002). The cell activity of HepG2 drops sharply when the concentration of H_2O_2 is in the range of 75–200 μm and becomes very slow when the concentration is in the range of 6.25–50 μm, as shown in Fig. 8 (A). 50 μM H_2O_2 was set as the model group of cell injury in this experiment.

As shown in Fig. 8 (B), compared with the control group, the viability of HepG2 cells was reduced to 73.97 ~ 78.22 % by incubation with 50 μM H_2O_2 for 4 h, while the viability of cells treated with CP4 and NAC was significantly increased, which means that this drug has a protective effect on H_2O_2-induced oxidative damage of cells. CP5 has obvious protective effect on HepG2 at the concentration of 1 ~ 8 mg/mL, and the cell viability is obviously inhibited in the concentration range of 0.125 ~ 1 mg /mL. CP7 increased the cell viability of HepG2 in the concentration range of 0.125 ~ 1 mg/mL, but in the presence of 50 μM H_2O_2, both of them synergistically inhibited the cell viability. Surprisingly, CP10 has no obvious inhibitory effect on cytotoxicity. When the concentration of CP10 is 4–8 mg/mL, it can synergistically inhibit cell viability with H_2O_2. However, when the concentration of CP10 is in the range of 0.5 ~ 2 mg/mL, CP10 can protect cells from H_2O_2-induced cell damage.

4. Discussion

The current study investigated the essential oil components, network target analysis, and antioxidant activity of *P. nelumbinis* extracted from 10 distinct habitats across China. The results revealed that PNEO contains a wide range of compounds, with CP12 (β-Sitosterol) being the most abundant. Moreover, five compounds (CP1, CP2, CP6, CP10, and CP13) were identified in *P. nelumbinis* for the first time, with three of them (CP1, CP2, and CP10) being reported for the first time in *Nelumbo*. Regarding the network target analysis, PTPN1, CYP19A1, HMGCR, CYP17A1, and AR were commonly targeted by the compounds. Interestingly, CP4 (Oleic acid) and CP7 (Linoleic acid) had unique targets, suggesting that the pharmacological effects of *P. nelumbinis* from different regions may vary slightly due to the presence of these compounds. GO and KEGG pathway enrichment analysis showed that PNEO primarily participates in biological processes such as the endoplasmic reticulum membrane and inflammatory response, and is primarily enriched in cancer, nervous system, among others. To further investigate the potential therapeutic benefits of PNEO, we conducted antioxidant activity experiments, including ABTS and DPPH radical scavenging capacity tests. The results indicated that PNEO exhibits potent antioxidant activity, with the N_8 (Fujiannanping) sample displaying the highest activity in ABTS scavenging capacity, and the N_7 (Hunanxiangtan) sample showing the highest activity in DPPH radical scavenging capacity. The results showed that PNEO possessed strong antioxidant activity, with the highest ABTS scavenging ability in N_8 (Nanping, Fujian province) sample and the highest DPPH scavenging ability in N_7

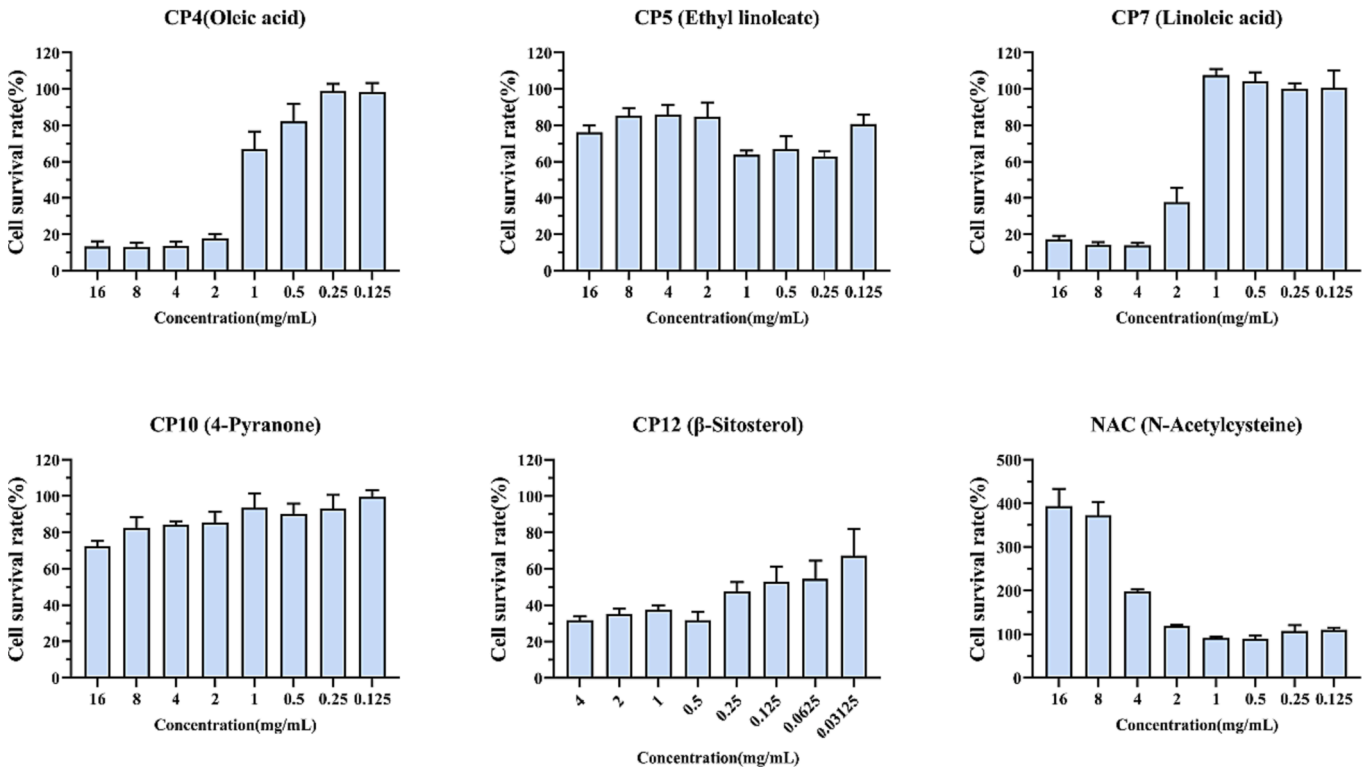

Fig. 7. Effects of CP4, CP5, CP7, CP10, CP12 and NAC on cell viability of HepG2 cells.

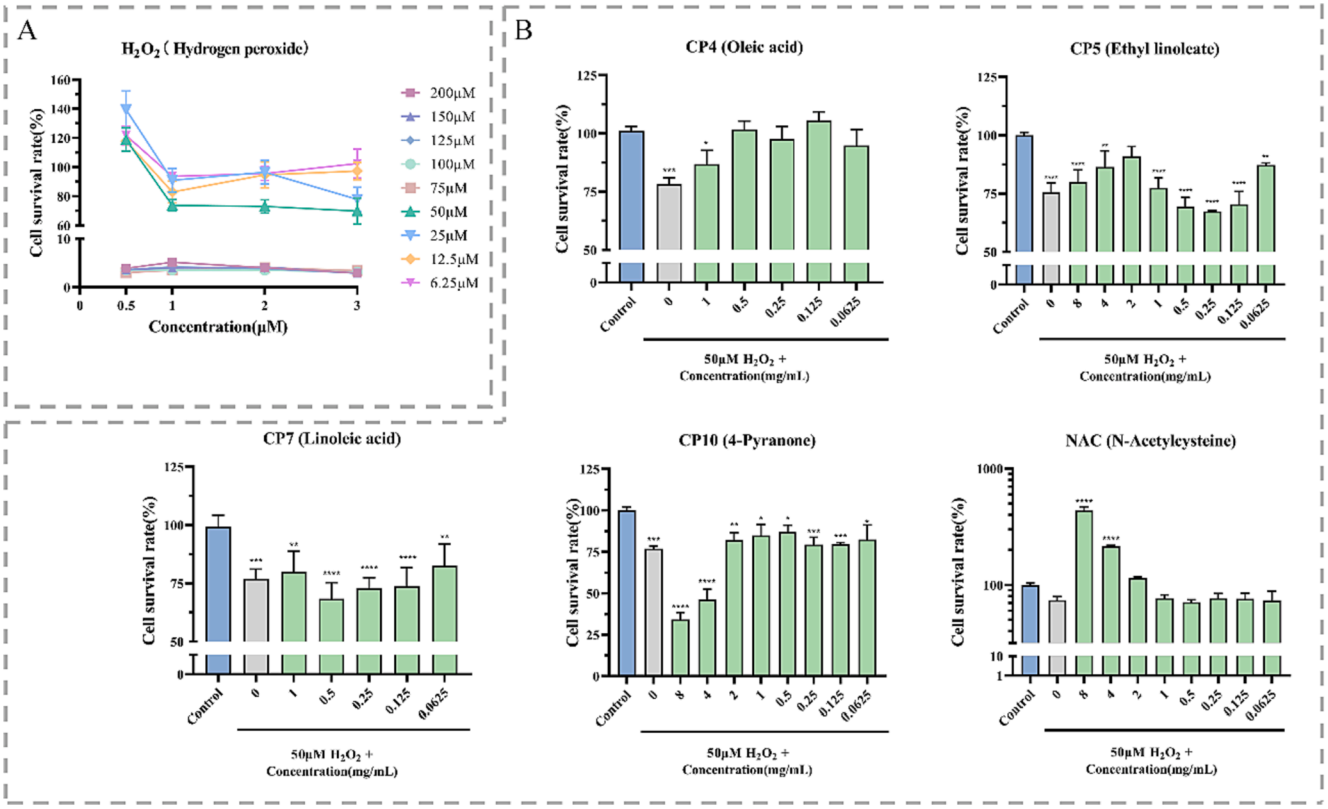

Fig. 8. (A) Effects of different concentrations and durations of H_2O_2 on cell viability, (B) Effects of different drug concentrations on HepG2 cells after H_2O_2-induced oxidative stress (n = 3, $P < 0.05$).

(Xiangtan, Hunan Province) sample. The antioxidant activity of CP4, CP5, CP7 and CP10 was significantly better than that of CP12, and CP4, CP5 and CP10 had certain protective effects against H_2O_2-induced cell damage. For example, N8, the sample with the lowest CP12 content, showed the best activity for ABTS radical scavenging.

However, the efficacy and safety of PNEO for these purposes still require further research to confirm. This study provides valuable insights into the chemical composition, pharmacological effects, and antioxidant activity of *P. nelumbinis*, highlighting the importance of considering habitat source when analyzing PNEO, and enriching the essential oil composition of *P. nelumbinis* and *Nelumbo* plants. The findings of this study have important implications for drug development and the application of PNEO in food and healthcare products, requiring further research to fully understand the potential of this traditional herbal medicine.

5. Conclusions

In this paper, supercritical CO2 extraction method was used to extract PNEO from 10 different habitats. GC–MS analysis showed that the chemical composition of PNEO from different habitats was significantly different. More targets point to the pharmacological effects of cancer on the nervous system. Through the analysis of ABTS and DPPH free radical scavenging results of PENO of ten samples, N_8, N_7 and N_{10} showed obvious antioxidant activity, and through the antioxidant activity analysis of the main active components, it was found that CP4, CP5 and CP10 had certain antioxidant activity and protective effect on oxidative damage of cells. This study is critical for drug development and traditional medicine practice, while highlighting the importance of considering habitat sources when analyzing PNEO, enriched for essential oil components of *P. nelumbinis* and *Nelumbo* plants.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to express our sincere gratitude to Associate Professor Ying Zhang from the College of Pharmacy at Jinan University for her valuable assistance in the identification of the *P. nelumbinis* samples used in this study.

We also wish to thank the National Natural Science Foundation of China (grant number 82073977) and the National Natural Science Foundation of Guangdong Province (grant number 2023A1515011445) for their financial support that enabled the successful completion of this research.

Author Contributions

All authors have contributed significantly to this work. Yujing Huang, Junxia Zheng and Hongxia Fan conceived and designed the study. Guanrong Ou, Liya Zeng, Yexin Li and Weizhen Li performed the experiments and analyzed the data. Haoming Chen contributed to data analysis and interpretation. Likang Wang and Juntao Xie provided critical input on the manuscript and helped with manuscript editing. Junxia Zheng supervised the project and provided overall guidance. All authors have read and approved the final version of the manuscript.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.101829.

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