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Solvent effects on terpenoid compositions and antioxidant activities of *Cinnamomum camphora* (L.) J. Presl extracts and the main antioxidant agent evaluation through in vitro and in vivo assay

Yingying Wang[1,2†], Siyi Zhang[1,2†], Yuandan Ma[1,2†], Xianmin Du[1,2], Qi Zong[1,2], Die Lin[1,2], Meng Lai[1,2], Tianyu Huang[1,2], Qingyun Luo[1,2], Lin Yang[3], Zhanyong Li[4] and Zhaojiang Zuo[1,2*]

Abstract

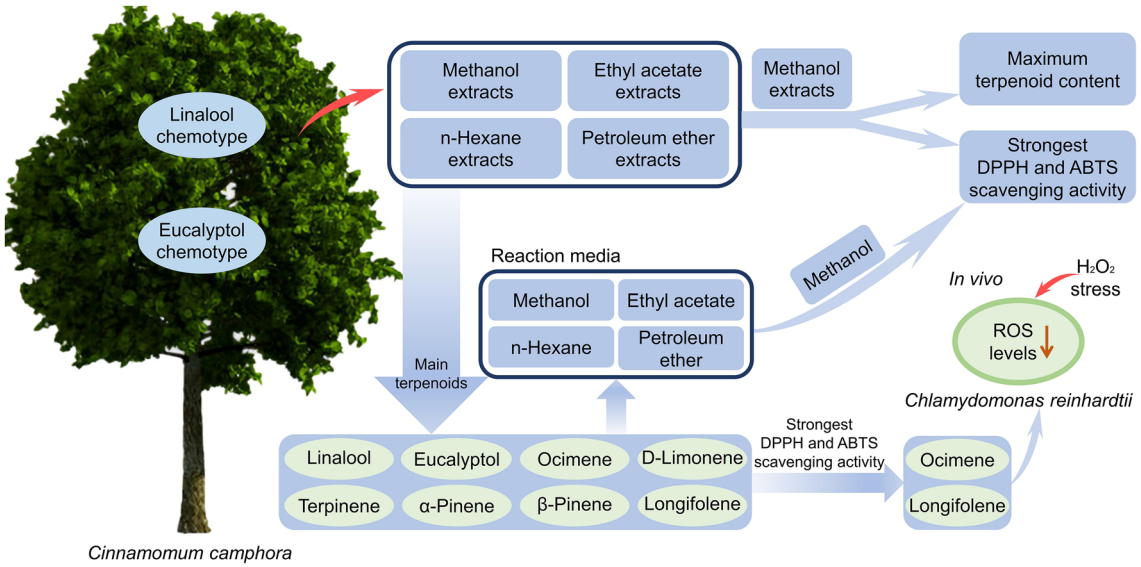
Plant secondary metabolites can protect organisms against oxidative stress caused by adverse environmental conditions. *Cinnamomum camphora* (L.) J. Presl contains plentiful terpenoids and is subdivided into 5 chemotypes. To develop natural antioxidants using the plant terpenoids, the terpenoid composition and antioxidant abilities of methanol, ethyl acetate, n-hexane and petroleum ether extracts from linalool and eucalyptol chemotypes were investigated, and the solvent effects on in vitro antioxidant activity of 8 main terpenoids were analyzed. Meanwhile, the in vivo effects of two strong antioxidant terpenoids were evaluated. For the two chemotypes, the 4 solvents exhibited the same extracting effects on the terpenoid types, but methanol extracts contained the highest content of terpenoids, which should contribute to their strongest scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radicals. In linalool and eucalyptol chemotype extracts, linalool, eucalyptol, ocimene, α-pinene, D-limonene, terpinene, β-pinene and longifolene were the 8 main terpenoids. Among the 4 solvents, the 8 terpenoids showed the strongest free radical-scavenging activity with methanol as the reaction medium, which might result from strong-polarity methanol easily activating C=C unsaturated bonds in these compounds. This might also contribute to the strongest scavenging activity of methanol extracts against free radicals. Among these main components, ocimene and longifolene separately showed the strongest activity in scavenging DPPH and ABTS free radical. In in vivo assay, the two compounds significantly lowered the reactive oxygen species (ROS) levels in *Chlamydomonas reinhardtii*, a single-celled model organism, under H_2O_2 stress, and the lowering effects gradually enhanced with increasing the compound concentration, resulting in the corresponding promoting effect on the algal growth. At the same concentration, longifolene showed the strongest effect on protecting the algal cells against oxidative stress. Therefore, methanol was suitable for extracting terpenoids in natural antioxidant development, and ocimene and longifolene were two strong antioxidant terpenoids without cell toxicity, with the latter having stronger in vivo antioxidant activity.

Keywords Antioxidant, *Cinnamomum camphora*, Oxidative stress, Solvent effect, Terpenoid

†Yingying Wang, Siyi Zhang and Yuandan Ma contributed equally to this work.

*Correspondence:
Zhaojiang Zuo
zuozhaojiang@126.com
Full list of author information is available at the end of the article

Graphical Abstract



Introduction

In organisms, reactive oxygen species (ROS) and free radicals are produced during the normal metabolic processes, with an equilibrium in the production and scavenging [1]. However, the exposure to some environmental factors such as pollution, O_3, industrial chemicals and stress conditions promotes the accumulation of massive ROS, leading to secondary oxidative stress [2, 3]. Oxidative stress not only damages protein, lipid and DNA biomolecules [3, 4], but could be connected with several diseases in human bodies, such as aging, diabetes, cancer, inflammation, cardiovascular diseases, Alzheimer's and Parkinson's diseases [5, 6]. Therefore, the effective reduction of ROS and free radicals is crucial to maintaining human health and promoting other organisms tolerating adverse environmental conditions.

Plant secondary metabolites can be considered natural antioxidants, and are being utilized in the production of medicines, cosmetics, foods, etc. [7, 8]. Among plentiful plant sources, medicinal plants should have the maximum developing potential for their abundant secondary metabolites. Based on this reason, the antioxidant abilities of essential oils and extracts from some medicinal plants have been detected, such as *Hertia cheirifolia* (L.) Kuntze [9], *Berberis calliobotry* Bien ex. Koehne [10], *Pistacia lentiscus* L. [11], *Scutellaria baicalensis* Geor. [12], *Bacopa monnieri* (L.) Pennell [13] and *Mimosa acutistipula* (Mart.) Benth [14]. Meanwhile, different solvents led to various extraction effects and antioxidant abilities, e.g., methanol (MeOH) extracts had stronger antioxidant abilities for containing more secondary metabolites in contrast to water extracts [15, 16]. When *Amygdalus communis* L. was extracted with water, MeOH and ethyl acetate (EA), the maximum phenolic content was found in EA extracts, while the maximum flavonoid content was detected in MeOH extracts [17]. However, the total phenolic content in EA extracts from *Onosma pulchra* Riedl. was lower than that in MeOH extracts [18]. For 7 solvents (water, MeOH, acetone, ethanol, EA, chloroform, and n-hexane (Hex)), the maximum flavonoids and total phenolics were extracted by MeOH and water from *Erigeron annuus* (L.) Desf. flower, respectively, and MeOH extracts showed the strongest antioxidant ability [19].

Terpenoids are the most diverse group of plant secondary metabolites, with more than 40,000 compounds [20]. Plant essential oils and extracts with terpenoids, as the main chemicals, showed strong scavenging abilities against ROS and free radicals, e.g., the essential oils from *Juniperus macrocarpa* Sm. [21], lavandin (*Lavandula*×*intermedia* Emeric ex. Loisel) and lavender (*Lavandula angustifolia* Miller) [22], *Citrus medica* L. [23] and *Hedychium coronarium* J. Koening [24] can scavenge $O_2^{-}\cdot$, ·OH, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radicals, and the extracts from *Jasminum multiflorum* Burm. F. and *Jasminum Sambac* Aiton [25], *Citrus aurantium* L. flowers [26]

and *Gaultheria leucocarpa* Blume [27] can scavenge H_2O_2, DPPH and ABTS free radicals. Among the diverse terpenoids, astaxanthin that belongs to carotenoids (tetraterpenes, C40) is considered as the strongest natural antioxidant and has been commercially produced. However, it is only synthesized in very limited algae and yeasts, leading to its very low yield and extremely high price [28, 29]. Except for that, there are no other terpenoids as commercial antioxidant agents, although some of them such as limonene, linalool, α-pinene, eucalyptol, γ-terpinene, β-pinene, terpinene-4-ol, myrcene and α-phellandrene have exhibited antioxidant activities through in vitro assay [8, 30, 31]. In that case, more potential antioxidant terpenoids should be identified, especially through in vivo evaluation, which is essential for their development and utilization as natural antioxidant agents.

Cinnamomum camphora (L.) J. Presl belongs to the genus *Cinnamomum* in Lauraceae. It is an excellent tree species with producing a wide spectrum of terpenoids to repel insects and is also used for treating diseases as an important Chinese herbal medicine [32, 33]. According to the typical terpenoid, 5 main chemotypes are distinguished, including linalool, eucalyptol, borneol, camphor and iso-nerolidol chemotype [34, 35]. In previous study, more terpenoids were extracted by ethanol from the fresh and fallen *C. camphora* leaves in contrast to water [36, 37]. The ethanol extracts from the former 4 chemotypes exhibited in vitro antioxidant abilities. Among a year, the extracts in summer showed the strongest antioxidant ability, and linalool and eucalyptol chemotype extracts had the strongest antioxidant ability among the 4 chemotype extracts [38].

In this study, the terpenoid composition and antioxidant abilities in MeOH, EA, Hex and petroleum ether (PE) extracts from linalool and eucalyptol chemotypes in summer were investigated, and the solvent effects on in vitro antioxidant activity of 8 main terpenoids were analyzed. Meanwhile, the in vivo effects of two strong antioxidant terpenoids were evaluated. These findings uncovered the suitable solvent for terpenoid extraction from *C. camphora* and identified two strong antioxidant agents, which were beneficial to development and utilization of the plant terpenoids as natural antioxidants.

Materials and methods

Preparation of extracts from *C. camphora*

In July, the healthy mature leaves were randomly collected from linalool and eucalyptol chemotypes of *C. camphora* with height of 10–12 m, and the growth conditions of the trees have been described in previous study [35]. In each chemotype, a random selection of 4 plants was carried out for leaf collection, with each as a repeat. These leaves were dried with a freeze dryer and smashed to powder using a pulverizer. The powder of 10 g was separately added into 100 mL MeOH, EA, Hex and PE to extract terpenoids at room temperature. After 48 h, the mixture was centrifuged at 7000 g, and the supernatant (100 $mg{\cdot}mL^{-1}$) was collected and used for terpenoid composition analysis and antioxidant activity measurement.

Analysis of terpenoid composition in the extracts

The terpenoid compositions in MeOH, EA, Hex and PE extracts were analyzed with a gas chromatography–mass spectrometry (GC–MS), and the operating conditions were described in a previous study [39]. Briefly, the GC (7890B, Agilent Technologies, Santa Clara, CA, USA) was run with the column temperature raising from 50 to 180 °C at a rate of 20 $°C{\cdot}min^{-1}$, and from 180 to 220 °C at a rate of 10 $°C{\cdot}min^{-1}$. For MS (5977B, Agilent Technologies), the ionization energy was set at 70 eV, and quadrupole temperature and source temperature were set at 150 °C and 230 °C, respectively. The qualitative analysis of the GC–MS data was performed by searching NIST Library (NIST 14, Gaithersburg, USA). The quantitative analysis of the corresponding terpenoids in the extracts was performed using the standards, including linalool, eucalyptol, ocimene, α-pinene, D-limonene, terpinene, β-pinene and longifolene (Aladdin, Shanghai, China). For other monoterpenoids and sesquiterpenoids, their content was calculated referring to D-limonene and longifolene, respectively.

Measurement of free radical-scavenging activities of the extracts

MeOH, EA, Hex and PE extracts were diluted to 0.25, 0.5, 1, 1.5 and 2 $mg{\cdot}mL^{-1}$ using the same solvent. DPPH free radical solution of 0.2 mM was also correspondingly prepared with MeOH, EA, Hex and PE, respectively. According to the description of Burits and Bucar [40], the diluted extracts of 2 mL were added into DPPH free radical solution of 2 mL following a rule of the same solvent, and the optical density at 517 nm (OD_{517}) was recorded after 30 min at 25 °C. For the scavenging activity, it was calculated following the formula, scavenging activity (%) = (Ac − As)/ Ac × 100%, where Ac and As represented the OD value of the control and sample, respectively. The extract concentration with 50% scavenging ability (IC_{50}) was evaluated according to the plot of percentage scavenging ratio against extract concentration.

Following the procedure of Petretto et al. [41], ABTS free radical solution was prepared by using a mixture of potassium persulfate (2.45 mM) and ABTS (7 mM) in MeOH, EA, Hex and PE, respectively. The diluted extracts of 150 μL were mixed with the ABTS free radical

solution of 3 mL following the rule of the same solvent, and the OD_{734} was recorded after 30 min at 25 °C. The ABTS free radical-scavenging activity was evaluated following the above formula, and the IC_{50} was also calculated.

Determination of free radical-scavenging activities of the main terpenoids

The two free radical-scavenging activities of 8 main terpenoids such as linalool, eucalyptol, ocimene, α-pinene, D-limonene, terpinene, β-pinene and longifolene in the two chemotype extracts were determined. These terpenoid solutions of 500 mM were separately prepared with the 4 solvents, including MeOH, EA, Hex and PE. For assaying the DPPH free radical-scavenging activity, a certain amount of terpenoids was added into 2 mL DPPH free radical solution following the rule of the same solvent and supplemented the same solvent to 4 mL. For assaying the ABTS free radical-scavenging activity, a similar operation was performed in a 3.15 mL reaction system, with the ABTS free radical solution of 3 mL. The content of the terpenoids was from 0.015 to 1.5 mmol and 0.015 to 0.3 mmol, respectively, in scavenging DPPH and ABTS free radicals (detailed content of each terpenoid in Additional file 2: Tables S1, S2).

In vivo antioxidant activity assay of ocimene and longifolene

Chlamydomonas reinhardtii, a single-celled model organism, was used to assay the in vivo antioxidant activities of ocimene and longifolene. *C. reinhardtii* cells were kept in tris–acetate-phosphate (TAP) medium under the condition of 16-h light (30 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 8-h dark at 25 °C. The algal cell cultures of 25 mL in a conical flask (about 5×10^6 $cells \cdot mL^{-1}$) were pretreated with 1, 5 and 10 μM ocimene and longifolene for 1 h, respectively, and then they were treated with 4.8 mM H_2O_2. After 12 and 24 h, the cell density was determined with a hemocytometer (25 × 16), and the ROS levels were determined during the 24-h treatment.

For measuring the ROS levels, *C. reinhardtii* pellets were collected by centrifugation at 4000 g, and incubated with 2′,7′-dichlorofluorescein diacetate (H_2DCF-DA) of 100 μM for 40 min. The non-fluorescent probe of H_2DCF-DA entering the cells was hydrolyzed to generate 2′,7′-dichlorodihydrofluorescein (DCFH) without fluorescence. However, the DCFH was oxidized by ROS to form fluorescent 2′,7′-dichlorofluorescein (DCF) in the cells. Then, the fluorescence was observed using a fluorescence microscope (Olympus BX51, Japan), and the fluorescence (about 530 nm) intensity was recorded with a flow cytometry (BD Accuri™ C6 Plus, USA) [42].

Statistical analysis

There were 4 replicates in each measurement, and the statistical analyses were carried out with Origin 8.0 following the Tukey test in one-way ANOVA.

Results

Free radical-scavenging activities of *C. camphora* extracts

For linalool chemotype of *C. camphora*, the DPPH and ABTS free radical-scavenging activities gradually enhanced with raising the concentration of MeOH, EA, Hex and PE extracts. Among the 4 extracts, MeOH extracts showed the strongest scavenging activity against DPPH and ABTS free radicals (Fig. 1A and C), with the lowest IC_{50} (0.17 and 0.49 $mg \cdot mL^{-1}$) (Fig. 1B and D).

For eucalyptol chemotype of *C. camphora*, MeOH extracts also exhibited the strongest scavenging activity against DPPH and ABTS free radicals, with the IC_{50} of 0.12 and 0.11 $mg \cdot mL^{-1}$, respectively (Fig. 2).

Terpenoid composition in *C. camphora* extracts

Among MeOH, EA, Hex and PE extracts from linalool chemotype, there were remarkable differences in the total ion chromatograms, with the high chromatographic peaks in MeOH extracts (Additional file 1: Fig. S1). In these extracts, 25 terpenoids were detected, including 11 monoterpenoids and 14 sesquiterpenoids. Among these compounds, D-limonene, ocimene, linalool, camphor, elixene, longifolene, humulene, cis-β-copaene, bicyclogermacrene, elemol and viridiflorol were the main components, and linalool was the typical component, with the highest content (Table 1).

For the most of terpenoids, their higher content was always detected in the MeOH extracts, while their lower content in the PE extracts (Table 1). For the MeOH extracts, the total content of terpenoids was 16.0% ($P < 0.05$), 21.4% ($P < 0.05$) and 28.7% ($P < 0.05$) higher than that in EA, Hex and PE extracts, respectively (Fig. 3A).

There were 34 terpenoids in the 4 extracts from eucalyptol chemotype, including 19 monoterpenoids and 15 sesquiterpenoids. Among these compounds, α-pinene, β-phellandrene, β-pinene, myrcene, D-limonene, eucalyptol, β-terpineol, borneol, myrcenol, α-terpineol, elixene, longifolene, humulene and bicyclogermacrene were the main components, and eucalyptol was the typical component, with the highest content (Table 2). Similar to the linalool chemotype extracts, MeOH extracts from eucalyptol chemotype also contained the highest content of terpenoids (Fig. 3B, Additional file 1: Fig. S2).

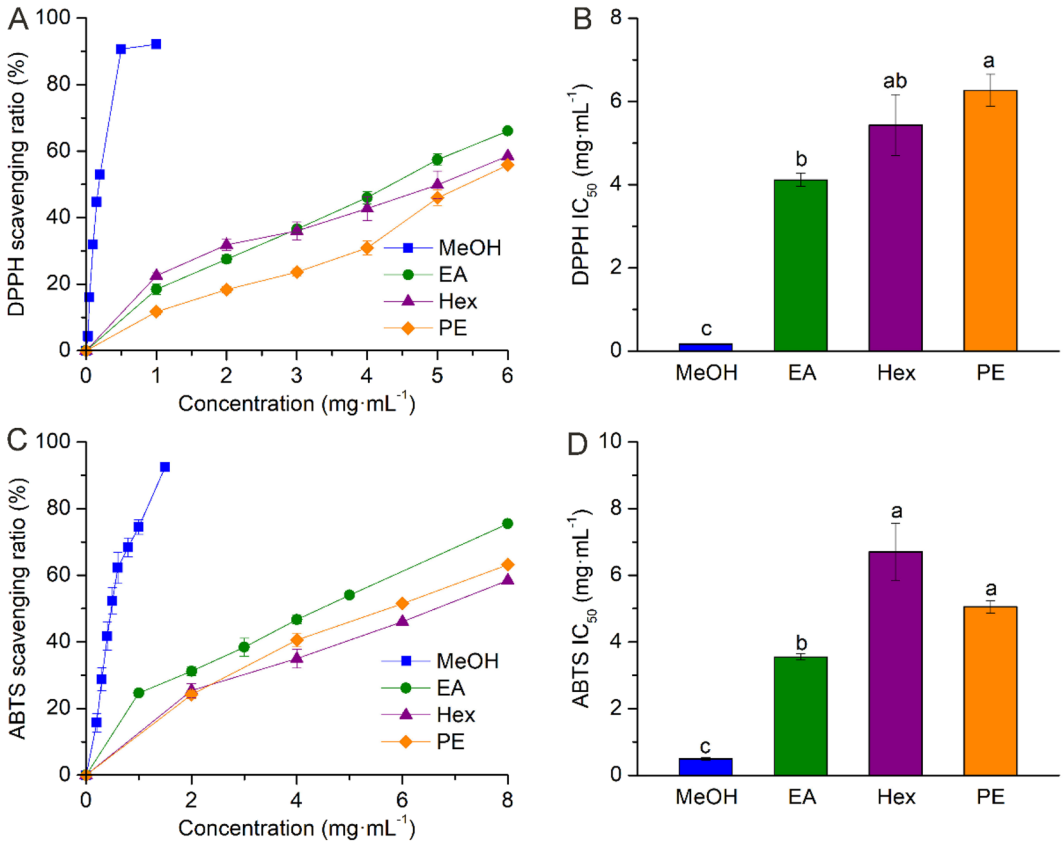


Fig. 1 Scavenging activities of different extracts from linalool chemotype of *C. camphora* against DPPH (**A** and **B**) and ABTS (**C** and **D**) free radicals. *MeOH* Methanol, *EA* Ethyl acetate, *Hex* n-Hexane, *PE* Petroleum ether. Different lowercase letters indicate the significant difference at $P < 0.05$. Means ± SE ($n = 4$)

Free radical-scavenging activities of the main terpenoids in different solvents

When MeOH, EA, Hex and PE were used as the reaction media, the DPPH free radical-scavenging activity gradually enhanced with increasing the concentration of the 8 main terpenoids, including linalool, eucalyptol, ocimene, α-pinene, D-limonene, terpinene, β-pinene and longifolene. All terpenoids exhibited the optimum scavenging activity in MeOH among the 4 reaction media (Fig. 4), even if in ocimene this scavenging activity was slightly stronger than that in others reaction media only at concentration ≥ 0.225 mM. Also the ABTS free radical-scavenging activity was highest in MeOH compared to the other media (Fig. 5).

Free radical-scavenging activity differences of the main terpenoids

In MeOH reaction medium, ocimene showed the strongest scavenging activity against DPPH free radical, and the scavenging ratio reached to 86.5% when ocimene content was 0.375 mmol. For longifolene, its scavenging activity was second to ocimene, but was stronger than other 6 terpenoids (Fig. 6A). Similarly, ocimene and longifolene in EA, Hex and PE also exhibited strong scavenging activities against DPPH free radical (Fig. 6B–D).

For ABTS free radical, longifolene showed the strongest scavenging activity in the 4 reaction media, while the scavenging activity of ocimene was second to longifolene. For ABTS free radical, also α-pinene showed a good scavenging activity. For the other terpenoids, the activities were similar and much lower than that of aforementioned terpenoids (Fig. 7).

Effects of ocimene and longifolene on *C. reinhardtii* growth under H_2O_2 stress

H_2O_2 stress significantly ($P < 0.05$) inhibited *C. reinhardtii* growth, and the cell density was reduced by 23.0% and 50.0%, respectively, after 12 and 24 h. In the pretreatments with 1, 5 and 10 μM ocimene (O1 + H_2O_2, O5 + H_2O_2, and O10 + H_2O_2), *C. reinhardtii* improved the tolerance to the H_2O_2 stress, and the cell density gradually increased with raising the ocimene concentration (Fig. 8A). The same result but more pronounced was

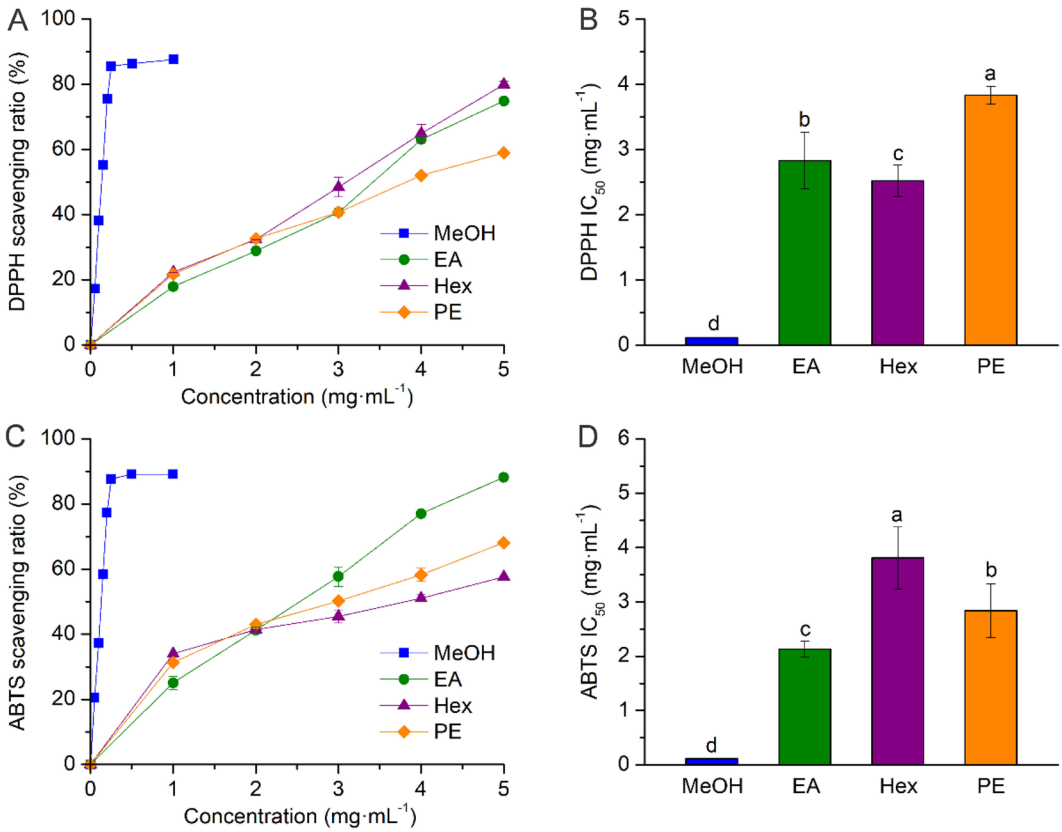


Fig. 2 Scavenging activities of different extracts from eucalyptol chemotype of *C. camphora* on DPPH (**A** and **B**) and ABTS (**C** and **D**) free radicals. *MeOH* Methanol, *EA* Ethyl acetate, *Hex* n-Hexane, *PE* Petroleum ether. Different lowercase letters indicate the significant difference at $P < 0.05$. Means ± SE ($n = 4$)

observed with the addition of 1, 5 and 10 μM longifolene (L1 + H_2O_2, L5 + H_2O_2, and L10 + H_2O_2) (Fig. 8B).

Effects of ocimene and longifolene on the ROS levels in *C. reinhardtii* under H_2O_2 stress

Under H_2O_2 stress, the ROS in *C. reinhardtii* cells were accumulated to the highest level after 1 h, and the cells showed strongest fluorescence intensity. However, the pretreatments with ocimene and longifolene remarkably lowered the ROS levels, and the lowering effects gradually enhanced with raising the compound concentration. At the same concentration, longifolene showed stronger lowering effect with respect to ocimene (Fig. 9).

Discussion

Plants synthesize a wide spectrum of secondary metabolites, and different solvents showed various extracting effects on these compounds. Compared with water extracts, MeOH extracts from *Gynostemma pentaphyllum* Thunb contained more phenolic compounds and showed higher antioxidant ability [15]. For grapevine leaves, the types of secondary metabolites in MeOH extracts were 2 folds of those in water extracts, and the terpenoid content in MeOH extracts was 3.9 folds of that in water extracts [39]. Among MeOH, water and EA, MeOH exhibited the maximum effect on extracting secondary metabolites from *Amygdalus Communis* L. hulls [17]. Among water, MeOH, acetone, ethanol, EA, chloroform and Hex, the maximum flavonoids were extracted by MeOH from *Helianthus annuus* L. flower, and the maximum phenolics were extracted by water [19]. Compared with ethanol, EA can extract more flavonoids from *Amomum compactum* Sol. ex Maton fruits [43]. In the present study, the 4 solvents also showed different extracting effects on the terpenoids from linalool and eucalyptol chemotypes of *C. camphora*, and the maximum terpenoids content was detected in MeOH extracts (Fig. 3). For each terpenoid compound, MeOH always exhibited the maximum extracting effect among the 4 solvents (Tables 1, 2). These results indicate that MeOH is an optimum solvent for extracting plant secondary metabolites from the aspect of extraction efficacy.

For the two chemotypes of *C. camphora*, the monoterpenoid content in MeOH, EA, Hex and PE extracts was

Table 1 The main terpenoids in different extracts from the linalool chemotype of *C. camphora*

Retention time (min)	Terpenoids	Formula	MeOH extracts (μM)	EA extracts (μM)	Hex extracts (μM)	PE extracts (μM)
6.536	α-Pinene	$C_{10}H_{16}$	1.04 ± 0.34	0.87 ± 0.11	0.99 ± 0.27	0.91 ± 0.33
7.006	β-Pinene	$C_{10}H_{16}$	0.72 ± 0.20	0.56 ± 0.15	0.59 ± 0.04	0.50 ± 0.12
7.114	Myrcene	$C_{10}H_{16}$	10.32 ± 1.04	6.59 ± 0.29	6.38 ± 2.49	4.50 ± 0.43
7.526	D-Limonene	$C_{10}H_{16}$	322.84 ± 7.75	263.62 ± 5.98	296.43 ± 2.01	248.31 ± 9.12
7.573	Eucalyptol	$C_{10}H_{18}O$	16.33 ± 1.08	15.27 ± 3.08	13.44 ± 2.65	11.45 ± 2.31
7.663	Ocimene	$C_{10}H_{16}$	164.98 ± 7.10	136.28 ± 2.81	121.98 ± 10.76	115.08 ± 3.17
8.065	2-Carene	$C_{10}H_{16}$	11.24 ± 3.25	15.35 ± 0.56	7.68 ± 0.28	5.97 ± 0.39
8.133	Linalool	$C_{10}H_{18}O$	3393.55 ± 169.11	3053.71 ± 154.99	2874.94 ± 37.44	2723.97 ± 126.97
8.599	Camphor	$C_{10}H_{18}O$	31.56 ± 6.44	25.05 ± 0.86	24.97 ± 1.01	22.98 ± 3.08
8.768	Borneol	$C_{10}H_{18}O$	12.21 ± 1.72	12.02 ± 1.69	8.94 ± 1.53	8.18 ± 1.17
8.951	α-Terpineol	$C_{10}H_{18}O$	15.01 ± 0.34	9.40 ± 0.21	13.47 ± 0.85	8.55 ± 1.02
9.974	δ-Elemene	$C_{15}H_{24}$	8.00 ± 2.27	5.88 ± 0.25	4.25 ± 0.27	3.62 ± 0.39
10.042	Elixene	$C_{15}H_{24}$	50.00 ± 6.65	47.46 ± 1.51	36.83 ± 1.64	32.80 ± 3.69
10.347	α-Copaene	$C_{15}H_{24}$	1.80 ± 0.16	0.93 ± 0.21	1.71 ± 0.04	1.35 ± 0.28
10.444	β-Elemene	$C_{15}H_{24}$	31.67 ± 5.38	13.56 ± 4.44	19.16 ± 1.14	16.47 ± 0.72
10.652	Longifolene	$C_{15}H_{24}$	200.05 ± 24.07	127.25 ± 6.01	137.48 ± 3.56	122.02 ± 15.26
10.760	γ-Elemene	$C_{15}H_{24}$	24.62 ± 1.65	21.15 ± 0.86	17.80 ± 2.36	13.43 ± 1.53
10.957	α-Gurjunene	$C_{15}H_{24}$	2.98 ± 1.49	2.04 ± 0.26	2.31 ± 0.21	2.07 ± 0.50
11.011	Humulene	$C_{15}H_{24}$	61.32 ± 7.93	57.05 ± 5.53	52.80 ± 1.33	48.70 ± 2.64
11.237	cis-β-Copaene	$C_{15}H_{24}$	43.18 ± 4.12	42.69 ± 1.56	39.03 ± 1.20	39.17 ± 5.04
11.301	β-Eudesmen	$C_{15}H_{24}$	25.11 ± 2.23	18.47 ± 2.28	20.95 ± 0.69	20.01 ± 1.27
11.380	Bicyclogermacrene	$C_{15}H_{24}$	77.06 ± 10.98	68.27 ± 7.74	64.28 ± 2.01	63.65 ± 4.93
11.578	δ-Cadinene	$C_{15}H_{24}$	6.91 ± 0.54	6.25 ± 0.82	6.84 ± 0.82	5.61 ± 0.71
11.887	Elemol	$C_{15}H_{26}O$	492.28 ± 11.52	368.54 ± 19.52	338.27 ± 9.33	356.81 ± 16.88
13.261	Viridiflorol	$C_{15}H_{26}O$	97.10 ± 0.73	89.42 ± 13.57	86.67 ± 2.91	83.28 ± 13.64

MeOH Methanol, *EA* Ethyl acetate, *Hex* n-Hexane, *PE* Petroleum ether. Means ± SE ($n = 4$)

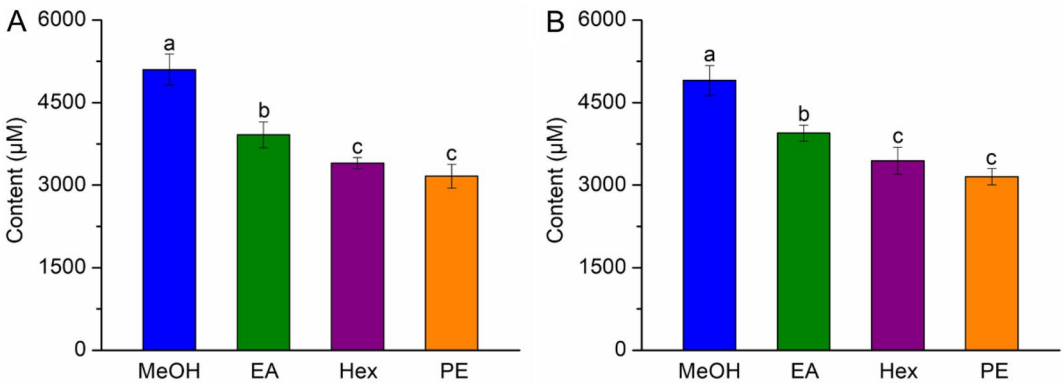


Fig. 3 The total content of terpenoids in the extracts from linalool (**A**) and eucalyptol (**B**) chemotypes. *MeOH* Methanol, *EA* Ethyl acetate, *Hex* n-Hexane, *PE* Petroleum ether. Different lowercase letters indicate the significant difference at $P < 0.05$. Means ± SE ($n = 4$)

much higher than sesquiterpenoid content (Tables 1, 2). These results were coincident with previous findings in the ethanol extracts [38] and volatile organic compounds (VOCs) [35] from *C. camphora*. Among the terpenoids, linalool and eucalyptol were typical monoterpenes in the corresponding chemotype [38] and exhibited the highest content in the extracts from corresponding chemotype (Tables 1, 2). For the two chemotypes, there were

Table 2 The main terpenoids in different extracts from the eucalyptol chemotype of *C. camphora*

Retention time (min)	Terpenoids	Formula	MeOH extracts (μM)	EA extracts (μM)	Hex extracts (μM)	PE extracts (μM)
6.450	β-Thujene	$C_{10}H_{16}$	21.34 ± 6.25	15.12 ± 1.58	17.18 ± 2.34	16.48 ± 2.85
6.536	α-Pinene	$C_{10}H_{16}$	42.07 ± 2.70	29.83 ± 2.45	27.27 ± 2.41	22.68 ± 5.99
6.712	Camphene	$C_{10}H_{16}$	3.31 ± 0.64	1.72 ± 0.41	1.52 ± 0.15	1.33 ± 0.46
6.970	β-Phellandrene	$C_{10}H_{16}$	146.09 ± 19.91	131.55 ± 13.34	126.05 ± 23.97	106.50 ± 4.59
7.006	β-Pinene	$C_{10}H_{16}$	45.63 ± 0.94	28.94 ± 3.09	37.94 ± 6.65	29.95 ± 3.55
7.114	Myrcene	$C_{10}H_{16}$	23.46 ± 1.86	19.75 ± 4.34	20.73 ± 3.59	20.43 ± 1.72
7.282	α-Phellandrene	$C_{10}H_{16}$	1.82 ± 0.72	0.74 ± 0.05	1.25 ± 0.19	1.02 ± 0.39
7.401	α-Terpinene	$C_{10}H_{16}$	2.64 ± 0.36	2.27 ± 0.11	2.50 ± 0.19	2.31 ± 0.23
7.526	D-Limonene	$C_{10}H_{16}$	130.45 ± 15.57	82.22 ± 4.39	90.55 ± 12.14	108.42 ± 8.22
7.573	Eucalyptol	$C_{10}H_{18}O$	3143.39 ± 157.36	2232.34 ± 65.61	2125.91 ± 102.8	1820.53 ± 55.99
7.663	Ocimene	$C_{10}H_{16}$	6.69 ± 0.25	4.91 ± 0.23	5.83 ± 1.29	4.98 ± 0.45
7.792	γ-Terpinene	$C_{10}H_{16}$	24.07 ± 2.23	16.04 ± 3.66	13.31 ± 2.34	11.49 ± 0.71
7.889	β-Terpineol	$C_{10}H_{18}O$	122.92 ± 14.65	118.91 ± 15.99	101.71 ± 16.65	88.19 ± 1.03
8.065	2-Carene	$C_{10}H_{16}$	10.14 ± 0.87	8.72 ± 0.34	7.69 ± 0.66	5.32 ± 1.61
8.133	Linalool	$C_{10}H_{18}O$	3.39 ± 0.26	2.35 ± 0.25	1.81 ± 0.35	2.16 ± 0.18
8.377	Fenchene	$C_{10}H_{16}$	2.82 ± 0.28	2.58 ± 0.32	2.11 ± 0.54	2.44 ± 0.41
8.768	Borneol	$C_{10}H_{18}O$	59.15 ± 3.85	47.03 ± 3.19	42.49 ± 3.18	37.39 ± 3.03
8.843	Myrcenol	$C_{10}H_{18}O$	30.87 ± 4.44	27.60 ± 4.77	26.88 ± 4.92	26.52 ± 2.13
8.951	α-Terpineol	$C_{10}H_{18}O$	687.20 ± 2.27	567.37 ± 8.98	486.50 ± 35.91	537.25 ± 26.79
9.974	δ-Elemene	$C_{15}H_{24}$	3.43 ± 0.46	2.03 ± 0.15	2.72 ± 0.24	2.47 ± 0.59
10.042	Elixene	$C_{15}H_{24}$	29.20 ± 4.69	19.28 ± 0.56	20.65 ± 4.78	22.59 ± 0.72
10.347	α-Copaene	$C_{15}H_{24}$	1.75 ± 0.09	1.73 ± 0.22	1.67 ± 0.59	1.48 ± 0.12
10.444	β-Elemene	$C_{15}H_{24}$	8.01 ± 0.24	7.96 ± 0.37	7.65 ± 2.34	6.55 ± 0.76
10.652	Longifolene	$C_{15}H_{24}$	71.06 ± 9.35	66.09 ± 3.34	55.35 ± 1.74	57.29 ± 5.28
10.760	γ-Elemene	$C_{15}H_{24}$	4.19 ± 0.56	3.42 ± 0.52	4.06 ± 0.39	3.76 ± 0.33
11.011	Humulene	$C_{15}H_{24}$	39.10 ± 2.41	29.26 ± 2.96	31.42 ± 0.90	30.47 ± 8.02
11.197	Germacrene	$C_{15}H_{24}$	8.52 ± 0.59	2.14 ± 0.07	3.43 ± 0.67	3.40 ± 0.05
11.237	cis-β-Copaene	$C_{15}H_{24}$	12.38 ± 0.87	11.08 ± 0.17	11.54 ± 2.43	11.48 ± 0.92
11.301	β-Eudesmen	$C_{15}H_{24}$	8.98 ± 1.45	6.16 ± 0.45	7.81 ± 2.11	7.11 ± 0.62
11.380	Bicyclogermacrene	$C_{15}H_{24}$	44.02 ± 7.11	41.01 ± 1.93	29.12 ± 1.48	38.29 ± 0.86
11.578	δ-Cadinene	$C_{15}H_{24}$	2.79 ± 0.33	3.15 ± 0.59	2.60 ± 0.24	2.42 ± 0.04
11.887	Elemol	$C_{15}H_{26}O$	1.59 ± 0.17	1.07 ± 0.09	1.45 ± 0.12	1.24 ± 0.08
12.034	γ-Patchoulene	$C_{15}H_{24}$	1.51 ± 0.16	1.22 ± 0.31	0.91 ± 0.02	1.39 ± 0.13
13.261	Viridiflorol	$C_{15}H_{26}O$	4.31 ± 0.48	2.40 ± 0.17	1.70 ± 0.05	2.05 ± 0.36

MeOH Methanol, *EA* Ethyl acetate, *Hex* n-Hexane, *PE* Petroleum ether. Means ± SE ($n = 4$)

obvious differences in the types and content of terpenoids (Tables 1, 2). For the monoterpenoids, their differences were caused by different expression of the genes in methylerythritol-4-phosphate (MEP) pathway and monoterpene synthases, while the sesquiterpenoid differences were caused by different expression of the genes in mevalonate (MVA) pathway and sesquiterpene synthases [35].

For plant secondary metabolites, they can be extracted with different solvents, which result in various antioxidant abilities. In contrast to water extracts, the MeOH extracts from *Gynostemma pentaphyllum* (Thunb) Makino [15] and *Stachys cretica* L. [44] showed stronger antioxidant activity, due to their higher content of phenolic and flavonoid compounds. The similar stronger antioxidant activity was also found in ethanol extracts from *Sida linifolia* Juss ex. Cav. compared with water extracts, due to higher content of secondary metabolites, including terpenoids, flavonoids, phenolics, steroids, alkaloids, tannins and saponins [45]. When *E. annuus* flower was extracted with 7 solvents, the MeOH extracts showed the strongest activity in quenching DPPH free radical and reducing Cu^{2+} and Fe^{3+}, as well as the strongest protective effect on TM3 mouse Leydig cells against

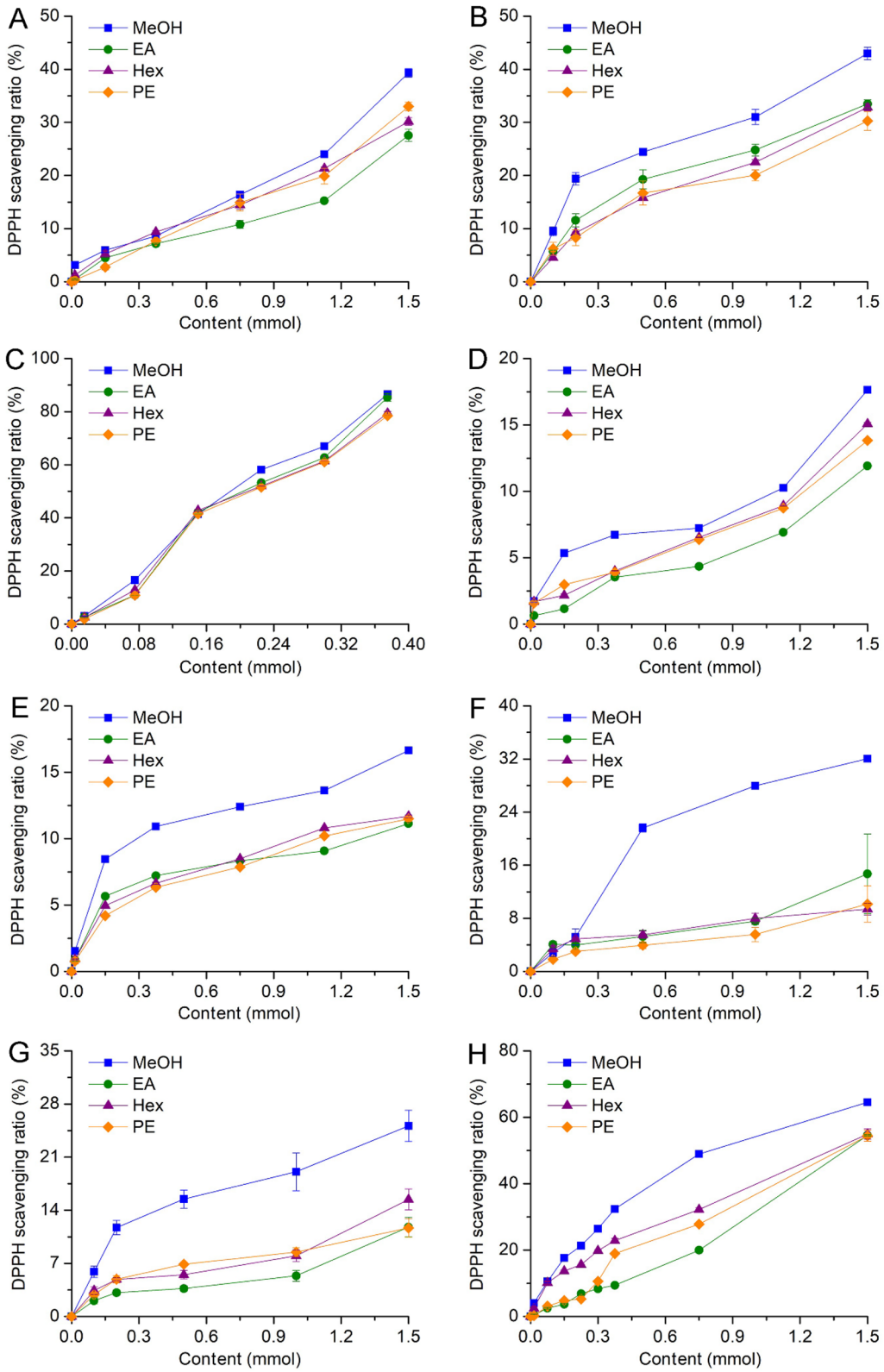


Fig. 4 Scavenging activities of linalool (**A**), eucalyptol (**B**), ocimene (**C**), D-limonene (**D**), terpinene (**E**), α-pinene (**F**), β-pinene (**G**) and longifolene (**H**) against DPPH free radical. *MeOH* Methanol, *EA* Ethyl acetate, *Hex* n-Hexane, *PE* Petroleum ether. Means ± SE ($n = 4$)

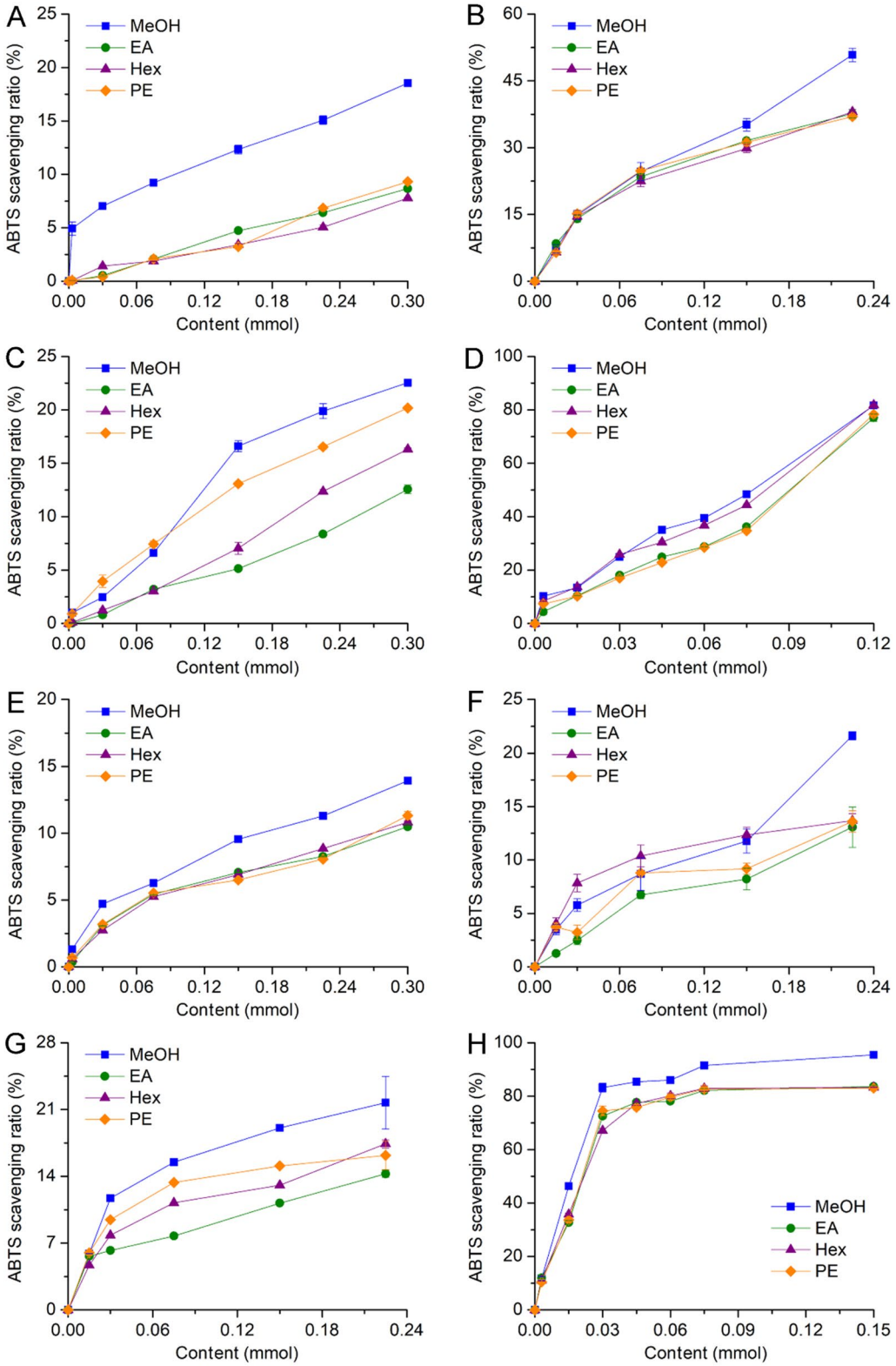


Fig. 5 Scavenging activities of linalool (**A**), eucalyptol (**B**), ocimene (**C**), D-limonene (**D**), terpinene (**E**), α-pinene (**F**), β-pinene (**G**) and longifolene (**H**) against ABTS free radical. *MeOH* Methanol, *EA* Ethyl acetate, *Hex* n-Hexane, *PE*: Petroleum ether. Means ± SE ($n = 4$)

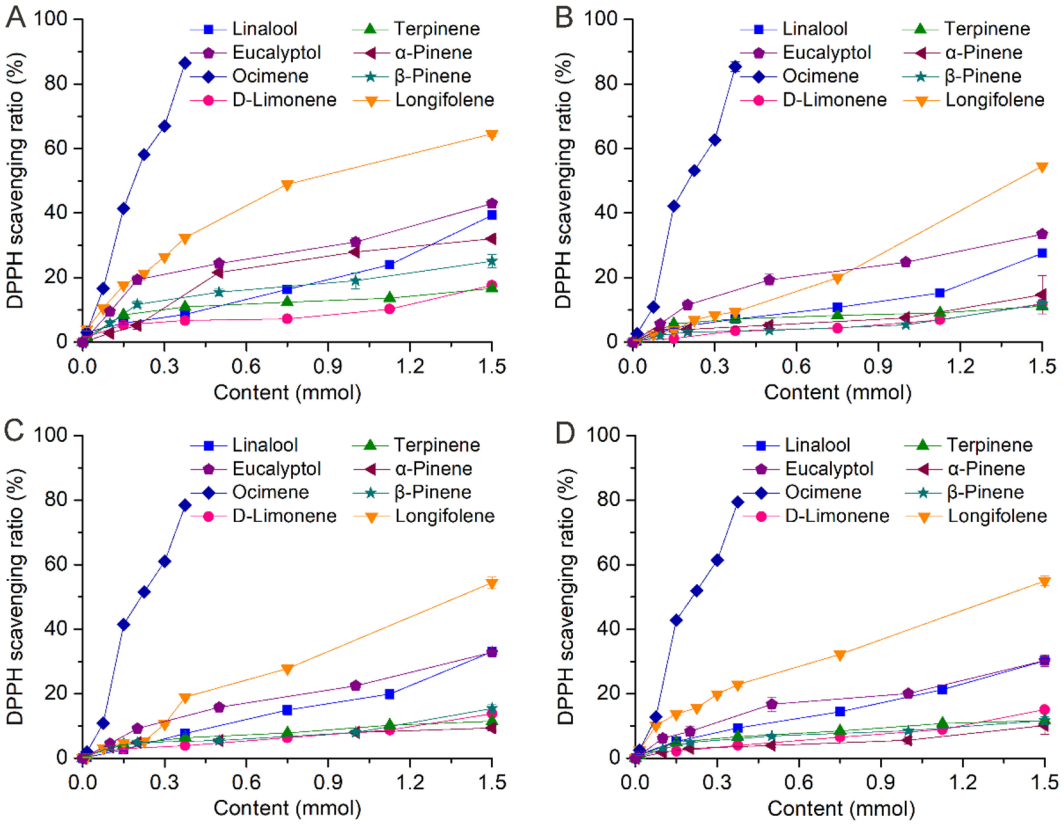


Fig. 6 Scavenging activities of 8 main terpenoids against DPPH free radical with methanol (**A**), ethyl acetate (**B**), n-hexane (**C**) and petroleum ether (**D**) as the reaction media. Means ± SE ($n = 4$)

oxidative damage, due to their highest level of total flavonoids [19]. For *C. camphora*, MeOH extracts exhibited the strongest antioxidant ability among the 4 extracts (Figs. 1, 2), and showed stronger antioxidant ability compared with previous ethanol extracts [38], which was associated with the higher terpenoids content in MeOH extracts (Fig. 3).

In the 4 extracts from *C. camphora*, the terpenoids content was disproportionate with the antioxidant ability (Figs. 1, 2and3). For 8 main terpenoids, they always showed the strongest antioxidant ability with MeOH as the reaction medium (Figs. 4, 5), which might be caused by the strongest polarity of MeOH among the 4 reaction media [30]. These results demonstrate that MeOH can provide an appropriate reaction condition for activating terpenoid unsaturated bonds to quench free radicals and ROS, which might also contribute to MeOH extracts exhibiting the strongest antioxidant ability. This might also lead to the stronger antioxidant ability of MeOH extracts compared with previous ethanol extracts from *C. camphora* [38].

Terpenoids, phenolics and flavonoids are the main compounds in plant secondary metabolites and have strong antioxidant abilities. Among them, terpenoids have the most diverse constitution and maximum content. They were regarded as the main antioxidant compounds in lots of plant extracts, such as lavandin and lavender [22], *C. medica* [23], *H. coronarium* [24], *C. aurantium* flower [26], *Gardenia jasminoides* J. Ellis flower [46] and *G. leucocarpa* [27]. Phenolics and flavonoids were also considered as the main antioxidant compounds in some plant extracts, such as *A. communis* [17], *O. pulchra* [18], *E. annuus* flower [19] and *Mimosa acutistipula* (Mart.) Benth [14].

Although terpenoids, phenolics and flavonoids have the potential for developing as natural antioxidants, limited specific compounds are identified as the functional agents. In terpenoids, astaxanthin, belongs to tetraterpenes, is considered as the strongest natural antioxidant, and has been commercially produced but with very limited yield [28, 29]. Limonene, linalool, geraniol and camphor were the main monoterpenoids in essential oils from coriander seeds, which were regarded as the main antioxidant compounds without antioxidation evaluation [47]. In citrus, limonene was the typical monoterpene, and was identified as the main

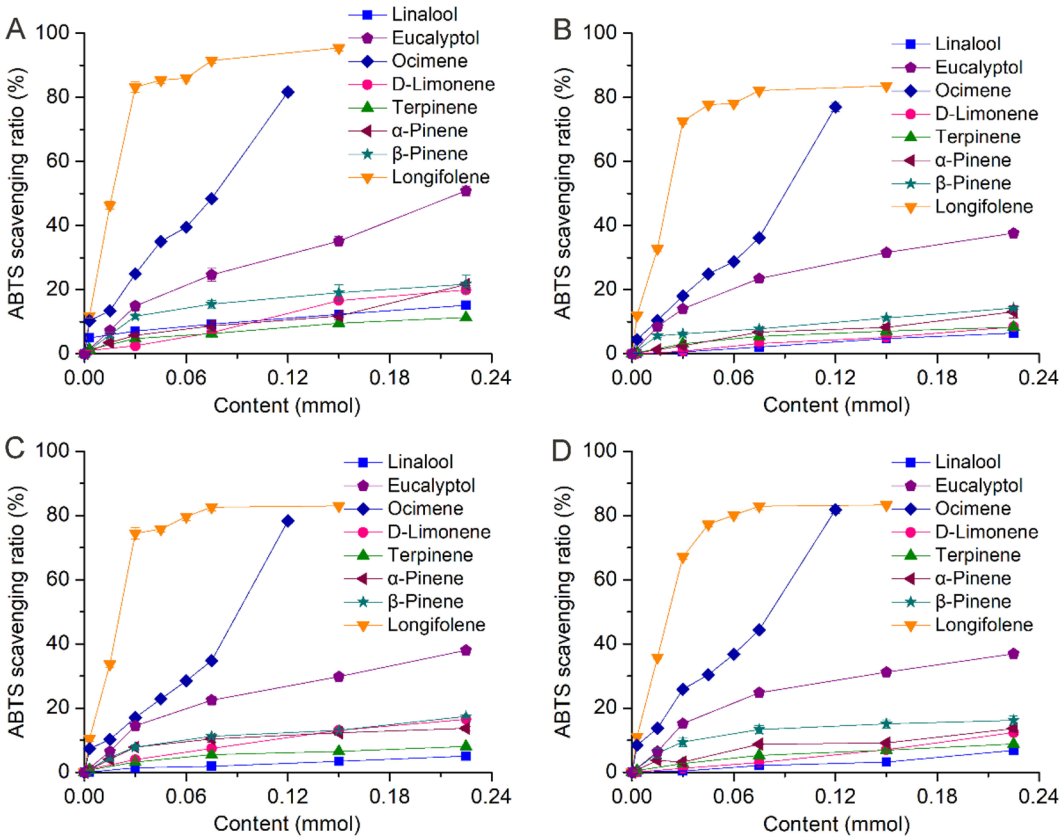


Fig. 7 Scavenging activities of 8 main terpenoids against ABTS free radical with methanol (**A**), ethyl acetate (**B**), n-hexane (**C**) and petroleum ether (**D**) as the reaction media. Means ± SE ($n = 4$)

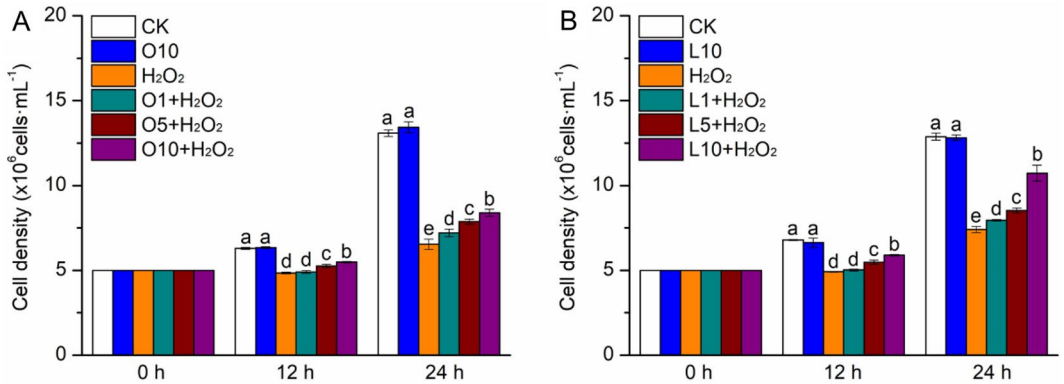


Fig. 8 Effects of ocimene (**A**) and longifolene (**B**) on the growth of *C. reinhardtii* treated with H_2O_2. O10 and L10: *C. reinhardtii* cells were treated with 10 μM ocimene and longifolene, respectively. O1 + H_2O_2, O5 + H_2O_2 and O10 + H_2O_2: *C. reinhardtii* cells were pretreated with 1, 5 and 10 μM ocimene and then stressed by H_2O_2. L1 + H_2O_2, L5 + H_2O_2 and L10 + H_2O_2: *C. reinhardtii* cells were pretreated with 1, 5 and 10 μM longifolene and then stressed by H_2O_2. Different lowercase letters indicate the significant difference at $P < 0.05$. Means ± SE ($n = 4$)

antioxidant agent by scavenging DPPH free radical [48]. In addition, eucalyptol, linalool, α-pinene, γ-terpinene, β-pinene, terpinene-4-ol, myrcene and α-phellandrene have been identified as the antioxidant agents through in vitro assay by quenching DPPH and/or ABTS free radicals [8, 30, 31]. There are different numbers of C = C unsaturated bonds in terpenoids, which are response for the antioxidant activities of these compounds [30].

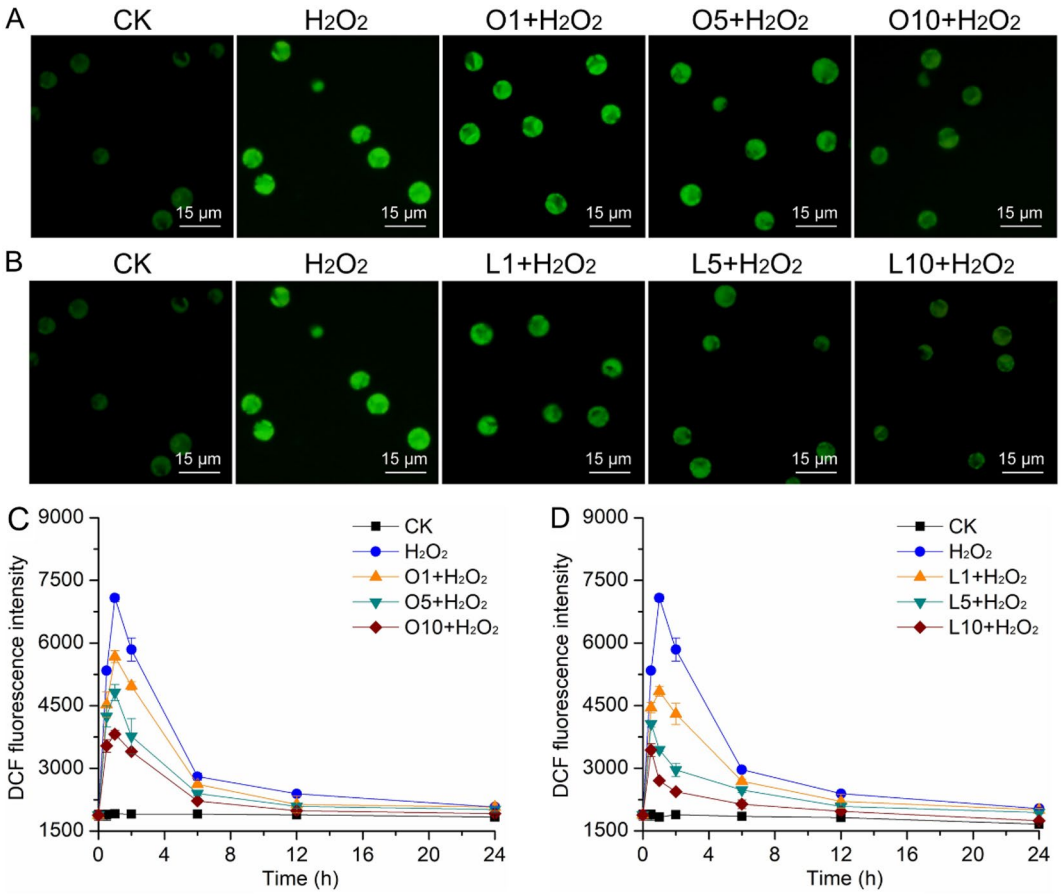


Fig. 9 Effects of ocimene (**A** and **C**) and longifolene (**B** and **D**) on the ROS levels in *C. reinhardtii* treated with H_2O_2. **A** and **B**: Images of the fluorescence from 2′,7′-dichlorofluorescein (DCF) generated from ROS oxidating fluorescence probe after H_2O_2 treatment for 1 h. **C** and **D**: Fluorescence intensity during 24-h H_2O_2 treatment. O1 + H_2O_2, O5 + H_2O_2 and O10 + H_2O_2: *C. reinhardtii* cells were pretreated with 1, 5 and 10 μM ocimene and then stressed by H_2O_2. L1 + H_2O_2, L5 + H_2O_2 and L10 + H_2O_2: *C. reinhardtii* cells were pretreated with 1, 5 and 10 μM longifolene and then stressed by H_2O_2. Means ± SE (n = 4)

For example, γ-terpinene exhibits strong antioxidant activity for easily losing the H at the 3- and 6-positions in the two C = C unsaturated bonds, and α-terpinene loses the H at the 5- and 6-positions [49].

In this study, linalool, eucalyptol, ocimene, α-pinene, D-limonene, terpinene, β-pinene and longifolene exhibited different scavenging effects on DPPH and ABTS free radicals, with the strongest antioxidant ability with MeOH as the reaction medium (Figs. 4, 5). This result might be due to the strong polarity of MeOH, that easily causing the activation of C = C unsaturated bonds in terpenoids. Among the 8 main terpenoids, ocimene and longifolene showed strong antioxidant abilities, with the strongest scavenging effect on DPPH and ABTS, respectively (Figs. 6, 7). There are 3 C = C unsaturated bonds in ocimene with one conjugated double bond [30], which might lead to its strong antioxidant activity. Longifolene is a tricyclic sesquiterpene with 1 C = C unsaturated bond, of which 1,4-cyclohexadiene moiety might enhance the scavenging activity against ABTS free radical [30].

For in vivo evaluating the antioxidant effects of plant extracts, several organisms and cells have been used as testing materials, e.g., mice fed with the extracts from linden bee pollen [50], *Atractylis gummifera* L. (Less.) [51], *Ganoderma lucidum* L. [52] and *Artemisia brevifolia* L. [53] can improve antioxidant enzyme activities and decline ROS content under stress conditions, and eucalyptus extracts showed the similar effects on chicken [54]. When *Caenorhabditis elegans* and *Escherichia coli* pretreated with *Rosa roxburghii* Plena extracts were exposed to paraquat, a decrease was detected in the ROS levels [55]. The similar decrease was also found in *C. elegans* treated with *Warburgia* salutaris (Bertol.f.) Chiov. bark extracts under H_2O_2 stress [56]. Under H_2O_2 stress, *Schinus terebinthifolius* Raddi fruit [57] and *Plantago australis* Lam. [58] extracts showed obvious effects on reducing ROS levels in yeast cells. However, there are

scarce in vivo assays about the specific compounds from plant extracts quenching ROS.

Stress conditions can cause ROS accumulation in organisms and burst in short time. For example, ROS burst was detected in *C. reinhardtii* cells exposed to NaCl and Na_2CO_3 [59] as well as cyanobacterial VOCs β-cyclocitral [60] for 0.5 h. In this study, ROS burst was also detected in *C. reinhardtii* treated with H_2O_2 for 1 h, and ocimene and longifolene effectively declined the ROS accumulation with dose-dependent (Fig. 9), which resulted in the corresponding protective effects on the cell growth (Fig. 8). Meanwhile, the single treatment with 10 μM ocimene or longifolene did not impact the algal growth (Fig. 8). Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) are essential antioxidant enzymes in scavenging ROS, whose activities can be induced to improve by ROS accumulation [3, 61]. In this study, the activities of the three antioxidant enzymes significantly ($P<0.05$) improved after 24-h H_2O_2 treatment, but gradually decreased with raising the concentration of ocimene and longifolene, due to the gradual decrease of ROS levels (Additional file 1: Fig. S3). In cells, ROS can attack biomacromolecules, leading to oxidative damage to nucleic acids, membrane lipids and proteins [3, 4], and the oxidation of membrane lipids leads to the generation of malondialdehyde which is indicated by thiobarbituric acid reactive substance (TBARS). In the treatment with H_2O_2 for 24 h, the TBARS content was remarkably higher than the control, but it gradually declined with raising the concentration of ocimene and longifolene, due to the gradual decrease of ROS levels (Additional file 1: Fig. S4). These results indicate that ocimene and longifolene have the potential for developing as natural antioxidant agents without cell toxicity.

Conclusion

For linalool and eucalyptol chemotypes of *C. camphora*, MeOH extracts exhibited the strongest antioxidant ability among the 4 extracts, due to their highest terpenoid content, indicating that MeOH was the optimum solvent for extracting terpenoids from *C. camphora*. Meanwhile, MeOH might activate terpenoid unsaturated bonds to improve free radical-scavenging activity, which also contributed to the strongest antioxidant ability of MeOH extracts. Among the 8 main terpenoids in the plant extracts, ocimene and longifolene exhibited strong antioxidant abilities, and can effectively decline ROS levels in in vivo assay without cell toxicity, demonstrating that the two monoterpenoids have the potential for developing as natural antioxidant agents.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40538-023-00524-2.

Additional file 1: Results of ion chromatograms of *C. camphora* extracts, antioxidant enzyme activities and TBARS content in in vivo assay.

Additional file 2: Gradients of terpenoid content in scavenging free radicals.

Author contributions

YW analyzed the terpenoid composition and took part in the in vivo antioxidant evaluation. SZ performed the in vivo antioxidant evaluation. YM performed the in vitro antioxidant assay. XD took part in the in vivo antioxidant evaluation. QZ and DL took part in the in vitro antioxidant assay. ML took part in the in vivo antioxidant evaluation. TH and QL took part in terpenoid composition analysis. LY and ZL provide helps in the in vivo antioxidant evaluation. ZZ formulated and directed the experiment, as well as wrote and modified the manuscript.

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Availability of data and materials

The data presented in this study are available upon request from the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Hangzhou 311300, China. [2]Zhejiang Provincial Key Laboratory of Forest Aromatic Plants-Based Healthcare Functions, Zhejiang A&F University, Hangzhou 311300, China. [3]Tianjin Key Laboratory of Animal and Plant Resistance, College of Life Sciences, Tianjin Normal University, Tianjin 300387, China. [4]College of Life Sciences, Langfang Normal University, Langfang 065000, China.

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References

1. Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol. 2014;24:R453–62. https://doi.org/10.1016/j.cub.2014.03.034.
2. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4:118–26. https://doi.org/10.4103/0973-7847.70902.
3. Ma Y, Wang B, Zhang R, Gao Y, Zhang X, Li Y, Zuo Z. Initial simulated acid rain impacts reactive oxygen species metabolism and photosynthetic abilities in *Cinnamonum camphora* undergoing high temperature. Ind Crop Prod. 2019;135:352–61. https://doi.org/10.1016/j.indcrop.2019.04.050.

4. Petrov V, Hille J, Mueller-Roeber B, Gechev TS. ROS-mediated a biotic stress-induced programmed cell death in plants. Front Plant Sci. 2015;6:1–16. https://doi.org/10.3389/fpls.2015.00069.
5. Gandhi S, Abramov AY. Mechanism of oxidative stress in neurodegeneration. Oxid Med Cell Longev. 2012;2012: 428010. https://doi.org/10.1155/2012/428010.
6. Khaper N, Bailey CDC, Ghugre NR, Reitz C, Awosanmi Z, Waines R, Martino TA. Implications of disturbances in circadian rhythms for cardiovascular health: a new frontier in free radical biology. Free Radical Bio Med. 2018;119:85–92. https://doi.org/10.1016/j.freeradbiomed.2017.11.006.
7. Niki E. Do antioxidants impair signaling by reactive oxygen species and lipid oxidation products? FEBS Lett. 2012;586:3767–70. https://doi.org/10.1016/j.febslet.2012.09.025.
8. Masyita A, Sari RM, Astuti AD, Yasir B, Rumata NR, Emran TB, Nainu F, Simal-Gandara J. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chem X. 2022;13: 100217. https://doi.org/10.1016/j.fochx.2022.100217.
9. Majouli K, Hlila MB, Hamdi A, Flamini G, Jannet HB, Kenani A. Antioxidant activity and α-glucosidase inhibition by essential oils from *Hertia cheirifolia* (L.). Ind Crop Prod. 2016;82:23–8. https://doi.org/10.1016/j.indcrop.2015.12.015.
10. Khan S, Nazir M, Saleem H, Raiz N, Saleem M, Anjum SMM, Zengin G, Mukhtar M, Tousif MI, Mahomoodally FM, Ahemad N. Valorization of the antioxidant, enzyme inhibition and phytochemical propensities of *Berberis calliobotrys* Bien. Ex Koehne: a multifunctional approach to probe for bioactive natural products. Ind Crop Prod. 2019;141:111693. https://doi.org/10.1016/j.indcrop.2019.111693.
11. Yosr Z, Imen BHY, Rym J, Chokri M, Mohamed B. Sex-related differences in essential oil composition, phenol contents and antioxidant activity of aerial parts in *Pistacia lentiscus* L. during seasons. Ind Crop Prod. 2018;121:151–9.
12. Miao N, Yun C, Shi Y, Gao Y, Wu S, Zhang Z, Han S, Wang H, Wang W. Enhancement of flavonoid synthesis and antioxidant activity in *Scutellaria baicalensis* aerial parts by UV-A radiation. Ind Crop Prod. 2022;187: 115532. https://doi.org/10.1016/j.indcrop.2022.115532.
13. Patel A, Jaiswal N, Srivastava PK, Patra DD. Enhancing secondary metabolite production and antioxidants in *Bacopa monnieri* grown on tannery sludge contaminated soil. Ind Crop Prod. 2022;187: 115365. https://doi.org/10.1016/j.indcrop.2022.115365.
14. Bezerra LFG, da Silva APS, da Cunha RX, de Oliveira JRS, de Barros MD, da Silva Vycttor MMA, de Menezes Lima VL. Antioxidant, anti-inflammatory and analgesic activity of *Mimosa acutistipula* (Mart.) Benth. J Ethnopharmacol. 2023;303:115964. https://doi.org/10.1016/j.jep.2022.115964.
15. Šamec D, Valek-Žulj L, Martinez S, Grúz J, Piljac A, Piljac-Žegarac J. Phenolic acids significantly contribute to antioxidant potency of *Gynostemma pentaphyllum* aqueous and methanol extracts. Ind Crop Prod. 2016;84:104–7. https://doi.org/10.1016/j.indcrop.2016.01.035.
16. Sarikurkcu C, Jeszka-Skowron M, Ozerc MS. *Valeriana dioscoridis* aerial parts'extracts - A new source of phytochemicals with antioxidant and enzyme inhibitory activities. Ind Crop Prod. 2020;148: 112273. https://doi.org/10.1016/j.indcrop.2020.112273.
17. Tlili N, Kirkan B, Sarikurkcu C. LC–ESI–MS/MS characterization, antioxidant power and inhibitory effects on α-amylase and tyrosinase of bioactive compounds from hulls of *Amygdalus communis*: The influence of the extracting solvents. Ind Crop Prod. 2019;128:147–52. https://doi.org/10.1016/j.indcrop.2018.11.014.
18. Sarikurkcu C, Sahinler SS, Ceylan O, Tepe B. *Onosma pulchra*: Phytochemical composition, antioxidant, skin-whitening and anti-diabetic activity. Ind Crop Prod. 2020;154: 112632. https://doi.org/10.1016/j.indcrop.2020.112632.
19. Zhang L, Xu Q, Li L, Lin L, Yu J, Zhu J, Zhang H, Xia G, Zang H. Antioxidant and enzyme-inhibitory activity of extracts from *Erigeron annuus* flower. Ind Crop Prod. 2020;148: 112283. https://doi.org/10.1016/j.indcrop.2020.112283.
20. Morrone D, Hillwig ML, Mead ME, Lowry L, Fulton DB, Peters RJ. Evident and latent plasticity across the rice diterpene synthase family with potential implications for the evolution of diterpenoid metabolism in the cereals. Biochem J. 2011;435:589–95. https://doi.org/10.1042/BJ20101429.
21. Lesjak MM, Beara IN, Orčić DZ, Petar KN, Simin ND, Emilija SD, Mimica-Dukić NM. Phytochemical composition and antioxidant, anti-inflammatory and antimicrobial activities of *Juniperus macrocarpa* Sibth. et Sm. J Funct Foods 2014; 7: 257–268. https://doi.org/10.1016/j.jff.2014.02.003.
22. Kıvrak Ş. Essential oil composition and antioxidant activities of eight cultivars of lavender and lavandin from western Anatolia. Ind Crop Prod. 2018;117:88–96. https://doi.org/10.1016/j.indcrop.2018.02.089.
23. Guo J, Gao Z, Xia J, Ritenour MA, Li G, Shan Y. Comparative analysis of chemical composition, antimicrobial and antioxidant activity of citrus essential oils from the main cultivated varieties in China. LWT Food Sci Technol. 2018;97:825–39.
24. Ray A, Jena S, Dash B, Kar B, Halder T, Chatterjee T, Ghosh B, Panda PC, Nayak S, Mahapatra N. Chemical diversity, antioxidant and antimicrobial activities of the essential oils from Indian populations of *Hedychium coronarium* Koen. Ind Crop Prod. 2018;112:353–62. https://doi.org/10.1016/j.indcrop.2017.12.033.
25. Khidzir KM, Cheng SF, Chuah CH. Interspecies variation of chemical constituents and antioxidant capacity of extracts from *Jasminum sambac* and *Jasminum multiflorum* grown in Malaysia. Ind Crop Prod. 2015;74:635–41. https://doi.org/10.1016/j.indcrop.2015.05.053.
26. Değirmenci H, Erkurt H. Relationship between volatile components, antimicrobial and antioxidant properties of the essential oil, hydrosol and extracts of *Citrus aurantium* L. flowers. J Infect Public Heal. 2020;13:58–67. https://doi.org/10.1016/j.jiph.2019.06.017.
27. Hu YJ, Chen ML, Liang D. Lignans and terpenoids from *Gaultheria leucocarpa* var. yunnanensis and their anti-inflammatory and antioxidant activities. Fitoterapia. 2022;162:105293. https://doi.org/10.1016/j.fitote.2022.105293.
28. Lin B, Cui Y, Yan M, Wang Y, Gao Z, Meng C, Qin S. Construction of astaxanthin metabolic pathway in the green microalga *Dunaliella viridis*. Algal Res. 2019;44: 101697. https://doi.org/10.1016/j.algal.2019.101697.
29. Zhou X, Zheng T, Xie Y, Zou S, Xu S, Lai M, Zuo Z. Astaxanthin accumulation in *Microcystis aeruginosa* under different light quality. Bioresource Technol. 2022;346: 126629. https://doi.org/10.1016/j.biortech.2021.126629.
30. Wojtunik KA, Ciesla LM, Waksmundzka-Hajnos M. Model studies on the antioxidant activity of common terpenoid constituents of essential oils by means of the 2,2-diphenyl-1-picrylhydrazyl method. J Agr Food Chem. 2014;62:9088–94. https://doi.org/10.1021/jf502857s.
31. Wang CY, Chen YW, Hou CY. Antioxidant and antibacterial activity of seven predominant terpenoids. Int J Food Prop. 2019;22:230–8. https://doi.org/10.1080/10942912.2019.1582541.
32. Hamidpour R, Hamidpour S, Hamidpour M, Shahlari M. Camphor (*Cinnamomum camphora*), a traditional remedy with the history of treating several diseases. Int J Case Rep Image. 2013;4:86–9. https://doi.org/10.5348/ijcri-2013-02-267-RA-1.
33. Yang F, Long E, Wen J, Cao L, Zhu C, Hu H, Wei XX, Yang XY, Wang CF, Zhang LM, Wang XY, Ji PY, Zheng HQ, Wu ZD, Lv ZY. Linalool, derived from *Cinnamomum camphora* (L.) Presl leaf extracts, possesses molluscicidal activity against *Oncomelania hupensis* and inhibits infection of *Schistosoma japonicum*. Parasite Vector. 2014;7:407.
34. Luo Q, Xu C, Zheng T, Ma Y, Li Y, Zuo Z. Leaf morphological and photosynthetic differences among four chemotypes of *Cinnamomum camphora* in different seasons. Ind Crop Prod. 2021;169: 113651. https://doi.org/10.1016/j.indcrop.2021.113651.
35. Tian Z, Luo Q, Zuo Z. Seasonal emission of monoterpenes from four chemotypes of *Cinnamomum camphora*. Ind Crop Prod. 2021;163: 113327. https://doi.org/10.1016/j.indcrop.2021.113327.
36. Chen S, Zheng T, Ye C, Huannixi W, Yakefu Z, Meng Y, Peng X, Tian Z, Wang J, Ma Y, Yang Y, Ma Z, Zuo Z. Algicidal properties of extracts from *Cinnamomum camphora* fresh leaves and their main compounds. Ecotox Environ Safe. 2018;163:594–603.
37. Yakefu Z, Huannixi W, Ye C, Zheng T, Chen S, Peng X, Tian Z, Wang J, Yang Y, Ma Z, Zuo Z. Inhibitory effects of extracts from *Cinnamomum camphora* fallen leaves on algae. Water Sci Technol. 2018;77:2545–54. https://doi.org/10.2166/wst.2018.199.
38. Luo Q, Tian Z, Zheng T, Xu S, Ma Y, Zou S, Zuo Z. Terpenoid composition and antioxidant activity of extracts from four chemotypes of *Cinnamomum camphora* and their main antioxidant agents. Biofuel Bioprod Bior. 2022;16:510–22. https://doi.org/10.1002/bbb.2320.

39. Zuo Z, Chen Z, Zhang R, Gao Y, Wang Y. A potential algaecide from the pruning wastes of grape (*Vitis vinifera*) stems and leaves. Environ Eng Manag J. 2018;17:2895–904. https://doi.org/10.30638/EEMJ.2018.290.
40. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. Phytother Res. 2000;14:323–8. https://doi.org/10.1002/1099-1573(200008)14:5%3c323::aid-ptr621%3e3.0.co;2-q.
41. Petretto GL, Maldini M, Addis R, Chessa M, Foddai M, Rourke JP, Pintore G. Variability of chemical composition and antioxidant activity of essential oils between *Myrtus communis* var. Leucocarpa DC and var Melanocarpa DC. Food Chem. 2016;197:124–31. https://doi.org/10.1016/j.foodchem.2015.10.056.
42. Zheng T, Zhou M, Yang L, Wang Y, Wang Y, Meng Y, Liu J, Zuo Z. Effects of high light and temperature on *Microcystis aeruginosa* cell growth and β-cyclocitral emission. Ecotoxicol Environ Safe. 2020;192: 110313. https://doi.org/10.1016/j.ecoenv.2020.110313.
43. Nurcholis W, Putri DNS, Husnawati H, Aisyah SI, Priosoeryanto BP. Total flavonoid content and antioxidant activity of ethanol and ethyl acetate extracts from accessions of *Amomum compactum* fruits. Ann Agr Sci. 2021;66:58–62. https://doi.org/10.1016/j.aoas.2021.04.001.
44. Kirkan B. Antioxidant potential, enzyme inhibition activity, and phenolic profile of extracts from *Stachys cretica* subsp. Vacillans Ind Crop Prod. 2019;140:111639.
45. Nwankwo NE, Okeke ES, Nworah FN, Ezeako EC. Phytochemical composition and potential anti-inflammatory and antioxidant mechanisms of leaf extracts of *Sida linifolia* L (Malvaceae). J Herb Med. 2023;38:100630. https://doi.org/10.1016/j.hermed.2023.100630.
46. Zhang X, Luo Q, Ma Y, Zuo Z. Chemical composition and antioxidant activities of the extracts from the flowers of two *Gardenia jasminoides* variants. Curr Top Nutraceut Res. 2021;19:228–34. https://doi.org/10.37290/ctnr2641-452X.19,228-234.
47. Zeković Z, Pavlić B, Cvetanović A, Đurović S. Supercritical fluid extraction of coriander seeds: Process optimization, chemical profile and antioxidant activity of lipid extracts. Ind Crop Prod. 2016;94:353–62. https://doi.org/10.1016/j.indcrop.2016.09.008.
48. Bier MCJ, Medeiros ABP, De Kimpe N, Soccol CR. Evaluation of antioxidant activity of the fermented product from the biotransformation of R-(+)-limonene in solid-state fermentation of orange waste by *Diaporthe* sp. Biotechnol Res Innov. 2019;3:168–76. https://doi.org/10.1016/j.biori.2019.01.002.
49. Öztürk M. Anticholinesterase and antioxidant activities of savoury (*Satureja thymbra* L.) with identified major terpenes of the essential oil. Food Chem. 2012;134:48–54. https://doi.org/10.1016/j.foodchem.2012.02.054.
50. Jin TY, Saravanakumar K, Wang MH. *In vitro* and *in vivo* antioxidant properties of water and methanol extracts of linden bee pollen. Biocatal Agr Biotechnol. 2018;13:186–9. https://doi.org/10.1016/j.bcab.2017.12.010.
51. Bouabid K, Lamchouri F, Toufik H, Faouzi MEA. Phytochemical investigation, *in vitro* and *in vivo* antioxidant properties of aqueous and organic extracts of toxic plant: *Atractylis gummifera* L. J Ethnopharmacol. 2020;253: 112640. https://doi.org/10.1016/j.jep.2020.112640.
52. Adeyi AO, Awosanya SA, Adeyi OE, James AS, Adenipekun CO. *Ganoderma lucidum* ethanol extract abrogates metabolic syndrome in rats: *In vivo* evaluation of hypoglycemic, hypolipidemic, hypotensive and antioxidant properties. Obes Med. 2021;22: 100320. https://doi.org/10.1016/j.obmed.2021.100320.
53. Ijaz MU, Iqbal M, Iqbal MA, Ashraf A, Al-Ghanim KA, Al-Misned F, Ahmed Z, Karataş D, Atique U, Mahboob S. *In vivo* antioxidant efficacy and therapeutic potential of *Artemisia brevifolia* leaves extract against CCl_4-induced reproductive damages in male albino rats. J King Saud Univ Sci. 2022;34:101816. https://doi.org/10.1016/j.jksus.2021.101816.
54. Li W, Zhang X, He Z, Chen Y, Li Z, Meng T, Li Y, Cao Y. *In vitro* and *in vivo* antioxidant activity of eucalyptus leaf polyphenols extract and its effect on chicken meat quality and cecum microbiota. Food Res Int. 2020;136:109302. https://doi.org/10.1016/j.foodres.2020.109302.
55. Huang D, Li C, Chen Q, Xie X, Fu X, Chen C, Huang Q, Huang Z, Dong H. Identification of polyphenols from *Rosa roxburghii* Tratt pomace and evaluation of *in vitro* and *in vivo* antioxidant activity. Food Chem. 2022;377: 131922. https://doi.org/10.1016/j.foodchem.2021.131922.
56. Abdelfattah MAO, Dmirieh M, Bakrim WB, Mouhtady O, Ghareeb MA, Wink M, Sobeh M. Antioxidant and anti-aging effects of *Warburgia salutaris* bark aqueous extract: Evidences from *in silico*, *in vitro* and *in vivo* studies. J Ethnopharmacol. 2022;292: 115187. https://doi.org/10.1016/j.jep.2022.115187.
57. de Oliveira VS, Augusta IM, da Conceição Braz MV, Riger CJ, Prudêncio ER, Sawaya ACHF, Sampaio GR, da Silva Torres EAF, Saldanha T. Aroeira fruit (*Schinus terebinthifolius* Raddi) as a natural antioxidant: Chemical constituents, bioactive compounds and *in vitro* and *in vivo* antioxidant capacity. Food Chem. 2020;315: 126274. https://doi.org/10.1016/j.foodchem.2020.126274.
58. Gonzales M, Villena GK, Kitazono AA. Evaluation of the antioxidant activities of aqueous extracts from seven wild plants from the Andes using an *in vivo* yeast assay. Results Chem. 2021;3: 100098. https://doi.org/10.1016/j.rechem.2021.100098.
59. Zuo Z, Chen Z, Zhu Y, Bai Y, Wang Y. Effects of NaCl and Na_2CO_3 stresses on photosynthetic ability of *Chlamydomonas reinhardtii*. Biologia. 2014;69:1314–22. https://doi.org/10.2478/s11756-014-0437-x.
60. Sun Q, Zhou M, Zuo Z. Toxic mechanism of eucalyptol and β-cyclocitral on *Chlamydomonas reinhardtii* by inducing programmed cell death. J Hazard Mater. 2020;389: 121910. https://doi.org/10.1016/j.jhazmat.2019.121910.
61. Xu C, Wang B, Luo Q, Ma Y, Zheng T, Wang Y, Cai Y, Zuo Z. The uppermost monoterpenes improving *Cinnamomum camphora* thermotolerance by serving signaling functions. Front Plant Sci. 2022;13:1072931. https://doi.org/10.3389/fpls.2022.1072931.

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