

Chemical Composition, Antioxidant, Acetylcholinesterase and β -Lactamase Inhibitory Activities of Essential Oils from *Clerodendrum cyrtophyllum* Turcz. and *Clerodendrum fortunatum* L.

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Abstract: *Clerodendrum cyrtophyllum* Turcz. and *Clerodendrum fortunatum* L. are traditional medicinal plants used for clearing heat, reducing fire, reducing inflammation and detoxification, and relieving cough and analgesia in China. In the current study, the essential oils (EOs) of *C. cyrtophyllum* and *C. fortunatum* were obtained by hydrodistillation. The chemical compositions and the contents of the EOs were measured by GC-MS and GC-FID methods. The antioxidant capacity was measured by 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH), 2'-azinobis(3-ethylbenzthiazoline-6-sulphonate) (ABTS) free radical scavenging capacity and ferric reducing antioxidant power (FRAP). The GC and GC-MS analysis results identified 89 and 63 components in the EOs of *C. cyrtophyllum* and *C. fortunatum*, accounting for 94.9% and 96.2%, respectively. 1-octen-3-ol, linalool, and hexahydrofarnesyl acetone were reported as the same primary components. The inhibition rates of the DPPH radical scavenger assay were $43.49\% \pm 0.95\%$ and $27.23\% \pm 0.40\%$ at 20 mg/mL, while the ABTS radical scavenging assay showed capacities with the IC_{50} values 2.31 ± 0.05 mg/mL and 7.43 ± 1.80 mg/mL, respectively. The FRAP values (Trolox equivalent antioxidant concentration) were 55.61 ± 2.56 μ mol/g and 23.39 ± 1.58 μ mol/g, respectively. The *C. cyrtophyllum* and *C. fortunatum* EOs showed anti-acetylcholinesterase activity with the IC_{50} values 289.10 ± 0.43 μ g/mL and 1060.00 ± 0.82 μ g/mL, and β -lactamase inhibitory activity with the IC_{50} values 41.34 ± 0.84 μ g/mL and 673.50 ± 1.27 μ g/mL, respectively. This study makes a substantial contribution to the chemical and biological knowledge expansion on *Clerodendrum* species EOs from China.

Keywords: *Clerodendrum cyrtophyllum* Turcz.; *Clerodendrum fortunatum* L.; essential oil; GC-MS; chemical compositions; biological activities. © 2025 ACG Publications. All rights reserved.

1. Introduction

Natural products have been assumed an increasingly pivotal role in human health and daily life [1]. Among them, essential oil (EO) predominates as a prominent category. EO typically encapsulates the quintessence of plants with delightful fragrances. Preceding papers have revealed the EOs derived from numerous medicinal plants manifesting remarkable antioxidant, anti-acetylcholinesterase, antibacterial, and other therapeutic attributes [2-4].

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The family Lamiaceae encompasses 288 genera and over 7000 species, predominantly distributed across Mediterranean regions, South America, Europe, Australia, and Southwest Asia [5]. In recent years, Lamiaceae plants' EO has garnered increasing attention in natural medicine and aromatherapy [6]. *Clerodendrum*, a representative genus within the Lamiaceae family, consists of approximately 570 species. It has been demonstrated to be effective in treating pain, inflammation, rheumatism, and fever [7]. Moreover, using ethanol and methanol, natural products extracted from these species have exhibited a broad spectrum of biological properties, containing antioxidant, anticholinesterase, and antibacterial activities [8]. Considering this genus' significance in natural products research along with the resemblance in biological activities between its extracts and other medicinal plants' EOs, we selected the two typical *Clerodendrum* species EOs to investigate their chemical compositions, as well as their antioxidant, anti-acetylcholinesterase, and anti- β -lactamase activities in details.

Free radicals are defined as atoms or groups with unpaired electrons. The accumulation of some free radicals can disrupt the redox balance in both extracellular and intracellular environments, thereby triggering oxidative stress [9]. Furthermore, some free radicals can exist in a free-state and engage in interactions with various components present within tissues. These interactions are endowed with the capacity to undermine the organism's intricate homeostatic equilibrium. This disruption consequence could develop the chronic dysfunctions and the facilitation of bacterial invasion, manifested as Alzheimer's disease (AD) and bacterial infections. [10]. Antioxidants are substances inhibiting free radicals' activities [11]. Therefore, antioxidants play an indispensable role in preventing diseases triggered by free radicals.. Some synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are extensively applied in pharmaceutical and food industries [12]. However, they were proven to have toxic effects by research [12]. Consequently, there has been increasingly more attention to obtaining compounds boasting antioxidant potential from natural products.

AD primarily affects older people and is characterized by increased free radicals around nerve cells, leading to oxidative stress and subsequent neuronal damage [13]. Furthermore, the over-accumulated free radicals may result in the release and ensuing aggregation of intracellular amyloid β -peptide (A β). Some of these peptides are converted into fibriform deposition, while others are inserted into the cell membrane, further promoting the peroxidation of proteins [14]. Eventually, these processes cause neuron death and, therefore, trigger AD [14]. One of the critical strategies in treating AD involves reducing the reactive oxygen species production and alleviating oxidative stress. Additionally, according to the cholinergic hypothesis [15], the low level of acetylcholine (ACh) in AD patients' brains hinders the signal transmission between synapses of nerve cells [16]. Acetylcholinesterase (AChE), crucial enzyme presenting among nerve cells, serves as a pivotal role in terminating signal transmission at cholinergic synapses by hydrolyzing ACh to inhibit its activity, resulting in an increased acetylcholine (ACh) level between synapses, and leading to heightened neural activity [17]. Accordingly, natural products with antioxidant and anti-AChE properties could be therapeutic candidates for AD.

Bacterial infections present a substantial threat to physical health. *Streptococcus pneumoniae*, a gram-positive bacterium, can activate Mixed lineage kinase domain-like pseudokinase (MLKL) and Receptor-interacting protein kinase 3 (RIPK3). This activation can be triggered by releasing bacterial pore-forming toxins (PFTs), which initiates the cell necroptosis program and leads to pneumonia [18]. In this complex process, reactive oxygen species (ROS) could act as initiators, which enhance the PFTs' combination to their targets, thereby facilitating the activation of MLKL and increasing cellular susceptibility to PFT-induced necroptosis [18]. Moreover, most gram-positive bacteria can secrete β -lactamase, an enzyme breaks amide bonds in antibiotic drugs, conferring drug resistance to these bacteria [19]. Therefore, researching natural products with antioxidant and anti- β -lactamase properties without side effects holds significant value for the bacterial infections treatment.

In order to discover effective natural products to solve the above problems, the present research selected *C. cyrtophyllum* and *C. fortunatum* EOs to study. *C. cyrtophyllum*, characterized as shrubs or macrophanerophytes, is endemic to China, South Korea, Vietnam, and Malaysia [20]. This species earned a reputation for addressing many human ailments, including colds, high fever, pharyngitis, epidemic encephalitis, furuncles, rheumatic arthritis, carbuncles, and snakebites [20,21]. *C. fortunatum*, a shrub widely distributed in China, Vietnam, Malaysia, and South Korea, is known for its potent medicinal properties in traditional medicine, including heat dissipation, fire reduction, inflammation alleviation, and

pain relief [22]. Although the two *Clerodendrum* genus plants contribute to treating diseases and human health, the natural products, especially their EOs have been investigated rarely. Therefore, the present study aimed to evaluate the chemical composition and biological activities of *C. cyrtophyllum* and *C. fortunatum* EOs. The insights gained from this study could make significant contributions to study the biological and pharmacological natural products originating from *Clerodendrum* genus plants.

2. Materials and Methods

2.1. Plant Materials

Aerial parts of *C. cyrtophyllum* was collected from Pingnan County, Guigang City, Guangxi Province, China (23.555 N, 110.356 E), in October 2022, while aerial parts of *C. fortunatum* was collected from Dianbai County, Maoming City, Guangxi Province, China (23.555 N, 110.356 E) in November 2022. The plants were confirmed as *Clerodendrum cyrtophyllum* Turcz. and *Clerodendrum fortunatum* L., by Hong Zhao, plant taxonomist of Marine College (Shandong University), where the herbarium specimens of *Clerodendrum cyrtophyllum* Turcz. (PE 02243290) and *Clerodendrum fortunatum* L. (PE 02244446) are stored in the Herbarium of the Institute of Botany, Chinese Academy of Sciences (PE). Plant samples were stored at -18 degrees Celsius until EO extraction.

2.2. Extraction of EOs

Aerial parts of the plants (1.5 kg) were washed using pure water and then pulverized into tiny fragments. The pieces were transferred to 5.0 L round-bottom flask and mixed with approximately 3.0 L ultrapure water. The EOs were extracted through hydrodistillation using a Clevenger-type apparatus for about four hours. Then they were partitioned from the aqueous layer through the utilization of diethyl ether. Subsequently, the extracted essential oils (EOs) underwent a desiccation procedure using a Termovap Sample Concentrator and anhydrous sodium sulfate. The EOs thus obtained were kept at 4 °C condition for further analysis.

2.3. GC-MS and GC-FID analysis

The EO components and content were measured using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID) methods according to previous papers [23-25].

2.4 Antioxidant Activity Evaluation

2.4.1. DPPH method

The experimental protocol for 2,2-diphenyl-1-picrylhydrazyl (DPPH) was adapted from previous studies [26,27,28]. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Butylatedhydroxytoluene (BHT) were through the utilization as the positive control. The DPPH ethanolic solution was prepared at 0.17 mM concentration. In the ethanolic system, Trolox, BHT and mother solution of EO concentration was 50, 25, 10, 5, 2.5, 1, and 0.5 mg mL⁻¹. 200 µL DPPH solution and aliquots of 50 µL EO solutions or positive control were transferred into the 96-well microplate. After reacting for 30 minutes under the dark condition at a temperature of 25°C, the absorbance was determined at 516 nm wavelength by Epoch microplate absorbance spectrophotometer (Biotek, USA). Radical scavenging capacity (RSC%) was calculated as follows:

$$RSC\% = \left(1 - \frac{A_{Sample} - A_{Sample\ Blank}}{A_{Control}}\right) \times 100\%$$

Where $RSC\%$ represents the “radical scavenging activity” of the DPPH radical, A_{Sample} is the solution absorbance in the microplate with the sample at different concentrations, $A_{Control}$ is the DPPH solution absorbance without the EO samples, and $A_{Sample\ Blank}$ is the ethanol sample absorbance without DPPH.

2.4.2. ABTS Method

The 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) scavenging capacity was measured by previous methods[27, 28]. Equal volumes of ABTS stock solution (7.4 mM) and $K_2S_2O_8$ stock solution (2.6 mM) were mixed and reacted at room temperature for at least 12 hours under dark conditions. The ethanolic solution of essential oil (EO) was formulated in accordance with the following gradients: 0.5, 1, 2.5, 5, 10, and 25 mg/mL. Then we mixed 200 μ L aliquot of $ABTS^{++}$ reagent with 50 μ L ethanol solution in 96-well microplates, shake for 10s to make it mix well and stand for 6 minutes. The absorbance was determined with Epoch microplate absorbance spectrophotometer at 734 nm wavelength as follows:

$$RSC\% = \frac{A_0 - A}{A_0} \times 100\%$$

Where $RSC\%$ assesses the “radical scavenging activity” of the $ABTS^{++}$ radical, A_0 is diluted $ABTS^{++}$ solution (200 μ L) absorbance mixed with 50 μ L ethanol at 734 nm, and A is diluted $ABTS^{++}$ solution (200 μ L) absorbance mixed with 50 μ L sample solution at 734 nm. Then IC_{50} was calculated using GraphPad Prism 9.5.

2.4.3. FRAP Method

The ferric reducing antioxidant power (FRAP) assay was determined using the method described in the previous publication [29]. In these assays, the absorbance was measured at 593 nm. Trolox was chosen to construct the standard curve, and the ferric-reducing antioxidant power (FRAP) values were determined with Trolox serving as the standard. By bringing the absorbance A of the sample at the determined concentration back to the standard curve equation to acquire the equivalent value of 1.0 μ mol mL^{-1} of the Trolox as the standard value (also the antioxidant value X μ M). When the concentration is Y μ g mL^{-1} , the compound's antioxidant capacity is determined to be $20 X/Y$ μ mol/g.

2.5. Acetylcholinesterase Inhibition Activity Test

The inhibitory effect on acetylcholinesterase was measured by employing a method that was previously reported with minor modifications [27,30]. The EO sample was prepared with ethanol at a series of concentrations of 5, 10, 25, 50, 100, 500, 1000 μ g/mL. Subsequently, in a microplate, 125 μ L of 0.1 mM phosphate-buffered saline (PBS) at pH 8.0, 20 μ L of the sample, and 35 μ L of an acetylcholinesterase solution with a concentration of 0.28 U/mL were thoroughly mixed. The mixture was then incubated at 4 °C for 20 minutes. Then, 10 μ L 2 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) and 10 μ L 15 mM s-acetyl choline iodide (ATCI) (15 mM) were added to the cells. The absorbance at 412 nm wavelength was read in 60-second intervals standing for 6 minutes. Taking the absorbance as the ordinate and the time as transverse, the initial reaction rate K of enzyme inhibition was obtained after linear regression. Replacing 20 μ L of the sample solution with 20 μ L of the solvent of the sample to be tested and repeating the above steps, then we obtained the initial reaction rate K_0 of the enzyme without inhibitory conditions. The acetylcholinesterase inhibitory rate was calculated shown below:

$$\text{Inhibition\%} = \frac{K_0 - K}{K_0} \times 100\%$$

Where K_0 is the enzyme initial reaction rate without inhibition, K is the initial reaction rate of the inhibited enzyme. The IC_{50} value was computed via nonlinear regression analysis performed with GraphPad Prism 9.5.

2.6. Anti- β -Lactamase Inhibition Activity Test

The β -lactamase inhibitory test was adapted from a previous method [31]. The ethanolic essential oil (EO) solutions were serially diluted to concentrations of 50, 100, 250, 500, 1000, 2500, and 5000 $\mu\text{g/mL}$ with phosphate-buffered saline (PBS) at pH 7.0. 20 μL samples, 30 μL 0.1 M pH 7.0 PBS, and 100 μL β -lactamase solution (1000 U/mL) were mixed and then incubated at 30 $^{\circ}\text{C}$ standing for 10 minutes. Then, 50 μL 0.1 mg/mL substrate nitrocefin was added and maintained for another 10 minutes at 30 $^{\circ}\text{C}$. The absorbance was measured at 489 nm wavelength. Clavulanate Potassium was used as the positive control. The β -lactamase inhibitory rate was calculated using the formula:

$$\text{Inhibition\%} = \left(1 - \frac{A_s - A_{sb}}{A_e - A_b}\right) \times 100\%$$

Where A_s is the absorbance of samples, A_{sb} is the blank reaction absorbance of the samples with 130 μL PBS replacing 100 μL β -lactamase solution, A_e is the absorbance of enzymatic determination, and A_b is the absorbance of blank reaction with 100 μL PBS replacing 100 μL β -lactamase solution. The IC_{50} value was calculated using nonlinear regression by using GraphPad Prism 9.5.

3. Results and Discussion

3.1. Chemistry

The *C. cyrtophyllum* and *C. fortunatum* EOs obtained by hydrodistillation were light yellow oily liquids. Total yields of *C. fortunatum* and *C. cyrtophyllum* are 0.10 and 0.30 mL/kg, respectively. The total ion chromatograms (TICs) of *C. fortunatum* and *C. cyrtophyllum* are shown in supporting information.

A total of 89 and 63 components were identified into the *C. cyrtophyllum* and *C. fortunatum* EOs, accounting for 94.9% and 96.2% of their compositions, respectively. The EOs from *C. cyrtophyllum* was primarily composed of linalool (10.0%), 1-octen-3-ol (6.9%), phytol (6.6%), (Z)-2-hexenol (4.6%), edulan III (3.8%), caryophyllene (3.5%), hexahydrofarnesyl acetone (3.5%), β -isocomene (3.3%) and pentadecanal (3.0%). In contrast, the EO from *C. fortunatum* was dominated by 1-octen-3-ol (18.2%), linalool (16.8%), benzaldehyde (6.6%), palmitic acid (5.1%), hexahydrofarnesyl acetone (3.8%) and β -ionone (3.5%). As presented in Table 1, the EOs derived from *Clerodendrum cyrtophyllum* and *Clerodendrum fortunatum* exhibited distinct chemical compositions. Linalool, with relative abundances of 10.0% in *C. cyrtophyllum* EO and 16.8% in *C. fortunatum* EO, is well-known for its remarkable antioxidant activity. It can counteract H_2O_2 - induced oxidative stress in PC12 cells, as proven in previous studies [32,33]. Moreover, further analysis has demonstrated that linalool could significantly alleviate neuropathic pain by dephosphorylating phosphatidylinositol-3,4,5-trisphosphate (PIP3) to inhibit the activity of serine/threonine kinase (AKT), thereby providing neuroprotective effects [34]. Comparative studies have also revealed a substantial amount of linalool in the EOs of *Clerodendrum* species, highlighting its indispensable status within the *Clerodendrum* genus [35-37]. Another prevalent compound, 1-octen-3-ol, accounted for 6.9% in *C. cyrtophyllum* EO and 18.2% in *C. fortunatum* EO. It has been proven to possess various biological activities, such as cytotoxicity against human embryonic stem cells [38]. Additionally, it can readily react with the sensors of mosquito olfactory receptor neurons, suggesting its potential application on pest trapping [39]. 1-octen-3-ol shows a dominant role in some *Clerodendrum* species' volatile organic compounds, when compared with the volatile composition of *Clerodendrum infortunatum* L. (where 1-octen-3-ol ranges from 48.7% to 68.7%)[35]. Phytol, characterized as diterpene, is widely distributed in *Clerodendrum* EOs, presented at 6.6% in *C. cyrtophyllum* EO and 2.4% in *C. fortunatum* EO [35-37]. It constitutes a relatively high proportion in *C. cyrtophyllum* and *C. fortunatum* EOs and can be typically synthesized via the 2-C-methylerythritol-4-phosphate (MEP) or mevalonic acid (MVA) pathways from chlorophyll molecules [40,41]. Moreover, phytol manifests a diverse array of biological activities, including anti-inflammatory and antimicrobial properties, making it a promising candidate for further investigations into the biological activities of EOs [42,43]. Terpenoids were present at 42.3% in *C. cyrtophyllum* EO and 26.1% in *C. fortunatum* EO, showing a relatively notable proportion when compared with *Clerodendrum bungei* (smelly peony) and *Clerodendrum infortunatum* L. EOs (30.5% and 8.6%),

respectively [35,36]. Furthermore, the EOs from *C. cyrtophyllum* and *C. fortunatum* are relatively rich in unsaturated reducing compounds, such as enols, with relative abundances of 36.9% and 44.7%, respectively. A previous study indicated that the antioxidant activity of compounds containing enol groups was significantly higher than other compounds [44], suggesting the potential antioxidant activity of the EOs from these two *Clerodendrum* species. Considering the EOs' chemical diversity and potential biological activities from *C. cyrtophyllum* and *C. fortunatum*, we evaluated *in vitro* antioxidant capacity and enzyme inhibitory activities.

Table 1. Chemical composition of EOs distilled from *C. cyrtophyllum* and *C. fortunatum*.

No.	RI ^{calc.}	RI ^{Lib} (range)	Compounds*	<i>C. Cyrtophyllum</i> (%)	<i>C. Fortunatum</i> (%)
1	856	855-857	Leaf alcohol	1.2	1.3
2	871	864-872	(Z)-2-Hexenol	4.6	-
3	876	864-872	1-Hexanol	-	1.7
4	937	937	α -Pinene	1.1	-
5	964	959-965	Benzaldehyde	1.2	6.6
6	983	978-983	1-Octen-3-ol	6.9	18.2
7	992	991-995	2- <i>n</i> -Pentylfuran	2.6	-
8	999	999-1003	(<i>E</i>)-2-(2-Pentenyl)furan	1.1	-
9	1000	991-997	3-Octanol	-	1.6
10	1014	1008-1016	(<i>E,E</i>)-2,4-Heptadienal	0.5	-
11	1033	1031	(-)-Limonene	1.3	-
12	1035	1027-1033	2-Ethyl-1-hexanol	-	1.9
13	1049	1041-1049	Benzeneacetaldehyde	0.5	0.9
14	1075	1070-1078	(<i>Z</i>)-Linalool oxide	1.1	0.7
15	1091	1081-1091	(<i>E</i>)-Furan linalool oxide	-	0.4
16	1098	1081-1101	3,5-Octadien-2-one	-	0.3
17	1104	1062-1125	Linalool	10.0	16.8
18	1106	1105	2-Nonen-1-ol	1.6	-
19	1109	1099-1109	Nonanal	-	0.6
20	1132	1123-1127	α -Campholenal	0.2	-
21	1139	1127-1139	2-Propyl-1,1,3-trimethylcyclohexane	-	0.2
22	1144	1138-1146	3-Nonen-2-one	-	0.2
23	1146	1145	Lilac aldehyde A	-	0.5
24	1155	1154	Lilac aldehyde B	0.4	0.4
25	1157	1155	Cucumber aldehyde	0.3	0.4
26	1163	1159-1165	(<i>E</i>)-2-Nonenal	0.3	0.4
27	1169	1163-1171	Lilac aldehyde C	0.2	0.2
28	1180	1164-1196	4-Ethylbenzaldehyde	-	0.8
29	1185	1179-1185	L-4-terpinenol	0.5	-
			α -Terpineol	-	1.2
31	1197	1190-1194	Methyl salicylate	1.3	1.3
32	1198	1190-1204	γ -Terpineol	1.8	-
33	1202	1197-1205	Safranal	-	0.4
34	1208	1204-1208	Decanal	0.3	0.2
35	1223	1219-1223	β -Cyclocitral	0.6	0.7
36	1233	1226-1230	Neryl alcohol	-	0.6
37	1253	1252-1258	Geraniol	0.4	1.1
38	1260	1254	β -Homocyclocitral	0.3	-
39	1266	1260-1266	(<i>E</i>)-2-Decenal	0.2	-

Essential oils from *Clerodendrum cyrtophyllum* Turcz. and *Clerodendrum fortunatum*

40	1280	1285	Edulan III	-	0.9
41	1285	1285-1305	2,6-Diisopropylanisole	3.8	-
42	1293	1293	Dihydroedulan II	0.3	0.4
43	1321	1314-1320	(<i>E,E</i>)-2,4-Decadienal	0.3	-
44	1356	1350-1356	α -Longipinene	0.2	-
45	1357	1346-1362	Dehydro-ar-ionene	0.5	0.4
46	1359	1346	1,1,4,7-Tetramethylindan	-	0.3
47	1382	1375-1389	Aromandendrene	0.7	-
48	1388	1381-1391	Damascenone	-	0.3
49	1392	1389-1393	β -Elemene	0.4	-
50	1400	1400	Tetradecane	0.2	-
51	1402	1402	Petasitene	0.2	-
52	1407	1403-1409	Longifolene	-	0.2
53	1411	1408-1414	α -Cedrene	0.2	-
54	1416	1408-1416	β -Isocomene	3.3	-
55	1423	1412-1418	(<i>Z</i>)- α -Bergamotene	0.5	-
56	1425	1414-1424	Caryophyllene	3.5	1.0
			α -Ionone	-	0.9
58	1431	1429-1435	(<i>Z</i>)- β -Copaene	0.3	-
59	1436	1430-1438	γ -Elemene	0.3	-
60	1447	1446	Spiranol	1.3	-
61	1450	1451-1455	Geranylacetone	1.8	-
62	1455	1455-1459	(<i>E</i>)- β -Farnesene	1.3	-
63	1456	1451-1461	Dihydropseudoionone	-	1.2
64	1462	1459-1463	Humulene	0.7	-
65	1465	1467	Patchoulene	0.3	-
66	1482	1482-1490	(<i>E</i>)- β -Ionone	2.8	-
67	1484	1480-1486	α -Curcumene	1.3	-
68	1491	1489-1493	β -Ionone	-	3.5
69	1495	1489-1497	Viridiflorene	0.4	-
70	1499	1500	Pentadecane	-	0.2
71	1501	1501	Aciphyllene	0.3	-
72	1504	1506-1510	α -Farnesene	0.2	-
73	1507	1503-1513	Cashmeran	0.1	-
74	1512	1511-1517	β -Curcumene	1.0	-
75	1518	1515	Cubebol	0.2	-
76	1519	1518	Jasmololon	0.2	-
77	1527	1522-1526	δ -Cadinene	0.2	0.2
78	1530	1520-1530	3-(2-Pentenyl)-1,2,4-cyclopentanetrione	-	0.3
79	1563	1560-1568	Nerolidol	0.7	-
80	1583	1579-1583	Caryophyllene oxide	0.5	0.9
81	1588	1584-1588	Presilphiperfolan-8-ol	0.9	-
82	1597	1596	Fokienol	0.6	-
83	1599	1600	Hexadecane	-	0.3
84	1599	1600-1612	Geranyl isovalerate	0.2	-
85	1614	1611-1615	Tetradecanal	0.5	-
86	1651	1651-1655	α -Cadinol	0.3	-
87	1661	1658-1662	Neointermedeol	0.3	0.2
88	1666	1665-1669	Intermedeol	1.5	-

89	1678	1675-1681	Aromadendrene oxide-(2)	0.2	-
90	1697	1694-1702	2-Pentadecanone	0.2	-
91	1699	1700	Heptadecane	-	0.2
92	1716	1712-1718	Pentadecanal	3.0	0.5
93	1727	1723-1727	Methyl tetradecanoate	-	0.2
94	1730	1727-1731	(+)-Nuciferol	0.2	-
95	1818	1811-1823	Hexadecanal	0.2	-
96	1832	1812-1832	Farnesoic acid	0.2	-
97	1848	1840-1848	Hexahydrofarnesyl acetone	3.5	3.8
98	1860	1850-1860	Di-sec-butyl phthalate	0.2	-
99	1888	1884-1888	(8Z,11Z)-Heptadecadienal	0.3	-
100	1893	1892	Methyl 7,10-hexadecadienoate	0.2	-
101	1916	1914-1924	Farnesyl acetone	2.0	1.0
102	1920	1921-1921	Heptadecanal	0.7	-
103	1925	1925-1927	Methyl palmitate	0.4	-
104	1928	1924-1928	Methyl pentadecanoate	-	2.0
105	1946	1946-1950	Isophytol	-	0.2
106	1954	1955-1975	Dibutyl phthalate	0.2	-
107	1965	1961-1975	Palmitic acid	2.0	5.1
108	1996	1990-1996	Ethyl palmitate	-	0.5
109	2023	2032-2036	Geranyl linalol	0.3	-
110	2057	2054-2058	Manool	0.2	-
111	2079	2066-2080	(Z)-10-Heptadecenoic acid	0.3	-
112	2096	2088-2096	Methyl linoleate	0.3	1.1
113	2102	2104-2106	γ -Palmitolactone	0.2	-
114	2103	2096-2100	Methyl linolenate	-	2.2
115	2110	2105	Methyl petroselinat	-	0.3
116	2110	2109-2119	Phytol	6.6	2.4
117	2135	2121-2145	Linoleic acid	0.2	-
118	2138	2116-2162	(Z)-Vaccenic acid	0.3	-
119	2144	2130-2152	Oleic acid	0.2	2.9
120	2153	2111-2151	9-Octadecenoic acid	-	0.5
121	2171	2159-2179	Ethyl linolenate	-	0.6
122	2498	2500	Pentacosane	-	0.2
123	2699	2700	Heptacosane	-	0.7
124	2899	2900	Nonacosane	-	0.8
125	3107	3100	Hentriacontane	-	0.2
Terpenoids(%)				42.3	26.1
Aliphatic compounds(%)				40.7	56.5
Aromatic compounds(%)				8.9	10.9
Other compounds(%)				3.0	2.7
Total identified(%)				94.9	96.2

Concentration calculated from total ion chromatogram; RI^{Calc}: Calculated average retention index; RI^{Lib}(range): Retention index range obtained from NIST/EPA/NIH 2023 Mass Spectral Database on the HP-5MS column. Compounds*: Compounds identification method based on the relative retention indices (RRI) of authentic compounds on the HP-5MS column and computer matching of the mass spectra with NIST/EPA/NIH 2023 Mass Spectral Database and comparison with literature data.

3.2. Biological Activity

3.2.1. Antioxidant Activity

As depicted in Figure 1a, at a concentration of 20 mg/mL, the DPPH scavenging activities of essential oils (EOs) extracted from *Clerodendrum cyrtophyllum* and *Clerodendrum fortunatum* were $43.49\% \pm 0.95\%$ and $27.23\% \pm 0.40\%$, respectively. Meanwhile, at a concentration of 0.1 mg/mL, Trolox exhibited a DPPH scavenging activity of $95.38\% \pm 1.63\%$. Although the EOs from *C. cyrtophyllum* and *C. fortunatum* showed relatively lower DPPH scavenging activities compared to the *Hygrophila salicifolia* EO ($IC_{50} = 2.13 \pm 0.17$ mg/mL) [45], they demonstrated comparatively noticeable ABTS free-radical scavenging activities. The IC_{50} values were determined to be 2.31 ± 0.05 mg/mL, 7.43 ± 1.80 mg/mL for the EOs of *C. cyrtophyllum* and *C. fortunatum*, respectively, and 0.79 ± 0.12 mg/mL for Trolox (Figure 1b, 1c, and 1d). The scavenging rate exhibited an upward trend in a dose-dependent manner as the EOs concentration increased. Consistent with previous research on the antioxidant activities of fruits, vegetables, and beverages, EO samples assayed using the ABTS method exhibited higher antioxidant capacities than those assayed using the DPPH method [46]. Previous studies reported that the IC_{50} values of the ABTS scavenging rate for the Lamiaceae EO from *Origanum vulgare* subsp. *vulgare* [47] and *Nepeta transcaucasica* EO [48] were 176.41 ± 0.22 mg/mL and 57.48 ± 1.21 mg/mL, respectively. These results suggest that the EOs from the two *Clerodendrum* plants possess noticeable ABTS free-radical scavenging activities. In the FRAP assay, the Trolox equivalent antioxidant concentration (TEAC) values of the EOs from *C. fortunatum* and *C. cyrtophyllum* were 23.39 ± 1.58 μ mol/g and 55.61 ± 2.56 μ mol/g, respectively. Compared with the secondary metabolites from Redartfulplum tea (TEAC: 19.57 ± 0.02 μ mol/g) [49] and *H. salicifolia* EO (TEAC: 170.97 ± 3.68 μ mol/g) [45], the *Clerodendrum* EOs displayed moderate antioxidant activities.

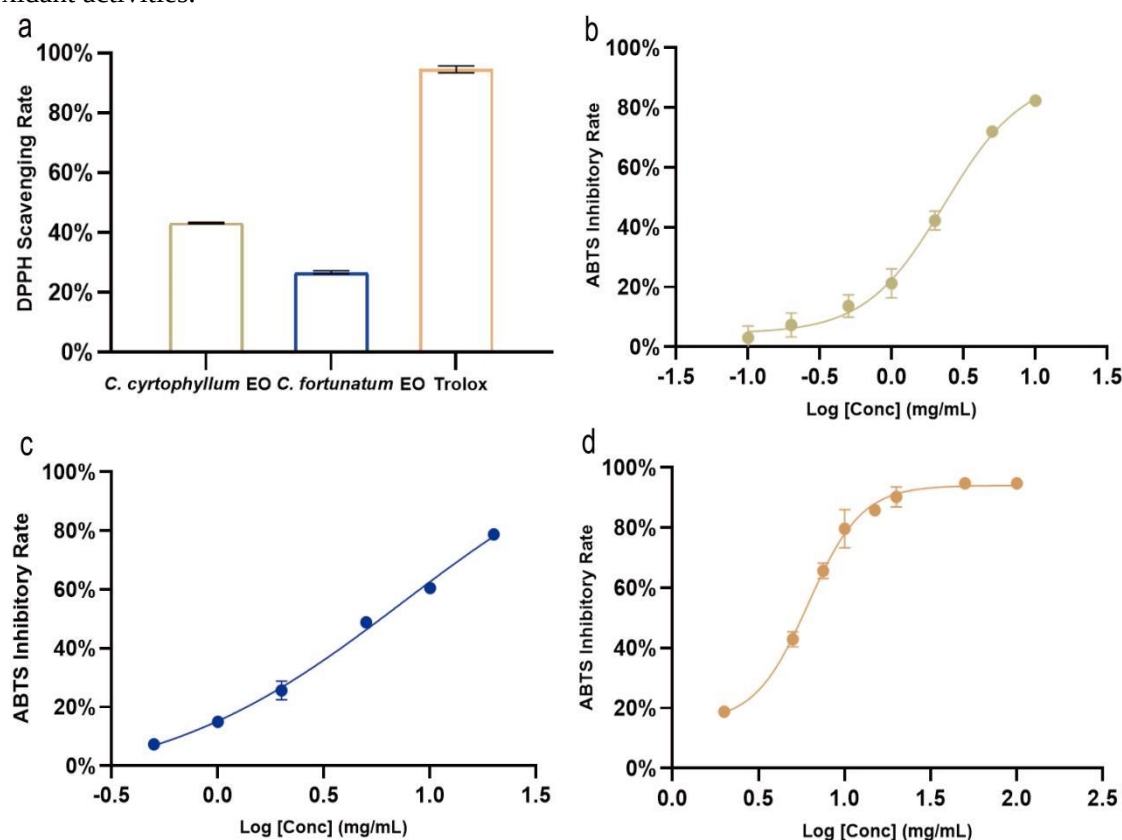


Figure 1. (a) Bar plot of DPPH scavenging rate of *C. cyrtophyllum* EO, *C. fortunatum* EO at 20 mg/mL, and Trolox at 0.1 mg/mL; the regression curve of ABTS scavenging activity of (b) *C. cyrtophyllum* EO, (c) *C. fortunatum* EO, and (d) Trolox.

EOs (essential oils) present a diverse range of chemical compositions, and different compounds within them exhibit distinct mechanisms that influence antioxidant activity. Unsaturated aldehyde compounds, specifically benzaldehyde (1.2% in *Clerodendrum cyrtophyllum* and 6.6% in *Clerodendrum fortunatum*), showed a relatively high inhibition rate in the DPPH assay in a previous study (52.9% inhibition at 8.0 mmol) [50]. *Clerodendrum buchholzii* (Gürke), a species belonging to the *Clerodendrum* genus, also demonstrated positive free-radical scavenging activity, with benzaldehyde accounting for 96% of its composition [51]. Unsaturated terpenoids, like α -pinene (1.1% in *C. cyrtophyllum* and not available in *C. fortunatum*), function as antioxidants because of their high reactivity with ROO $\cdot$ radicals. During these reactions, the resultant α -pinene oxide further reacts with oxygen to form hydroperoxyl radicals (HOO $\cdot$). These HOO $\cdot$ radicals can accept electrons transferred from ROO $\cdot$, thereby enhancing the efficiency of the free-radical scavenging reaction [52]. Phenolic compounds, such as methyl salicylate (1.3% in both *C. cyrtophyllum* and *C. fortunatum*), could eliminate ROO $\cdot$ by transferring hydrogen atoms [52]. Moreover, the generated phenoxyl radical can rapidly neutralize ROO $\cdot$ in fast radical-radical reactions without propagating the radical chain [52,53]. Therefore, we infer that unsaturated terpenoids and phenolic compounds served as significant contributors to the relatively strong antioxidant capacities of the EOs from the two *Clerodendrum* plants. Ketene compounds, including (*E*)- β -ionone (2.8% in *C. cyrtophyllum* and NA in *C. fortunatum*) and β -ionone (NA in *C. cyrtophyllum* and 3.5% in *C. fortunatum*), are also recognized as antioxidants. This is due to the β -unsaturated carbonyl substructure's highly reactive nature, which can accept electrons from free radicals and exhibit positive antioxidant activity [54]. Although the content of (*E*)- β -ionone is relatively low, it occupies a small proportion in *Clerodendrum* genus plant [36]. Previous studies have shown that α,β -unsaturated carbon-based compounds mediate the well-known NRF2/KEAP1 antioxidant signaling pathway [54]. In conclusion, compounds containing the above-mentioned groups may be responsible for the remarkable antioxidant capacity of the EOs from the two *Clerodendrum* plants. These findings may contribute to a more in-depth understanding of the biological activities of EOs derived from *Clerodendrum* genus plants.

Peroxyl radicals (ROO $\cdot$), secondary ROS derived from adding superoxide to a carbon-centered radical, such as the hydroperoxyl or perhydroxyl radical, are common products of fatty acid peroxidation in living organisms [55]. Although DPPH and ABTS served as synthetic free radicals, their reaction mechanisms with antioxidants are analogous to the ROO $\cdot$. In this process, antioxidants could donate hydrogen to reduce ROO $\cdot$ to the non-radicals [55,56]. Consequently, DPPH and ABTS scavenging activities may indicate similar free radical scavenging capacities of EO in organisms, suggesting potential therapeutic applications and *in vivo* activity. In contrast to the previous methods focusing on free radical scavenging, FRAP is not a specific reaction, which implied any half-reaction could be less positive than the Fe²⁺/Fe³⁺-TPTZ half-reaction with a redox potential will facilitate the Fe³⁺ reduction, including reactions between Fe³⁺ and compounds from plant EOs [57,58]. Therefore, this analysis approach puts more emphasis on analyzing the overall antioxidant activity of the EO system. Another advantage could be that since the reaction is conducted under acidic conditions, some of the interfering factors of endogenous nature can be inhibited, drastically minimizing the impact of errors on the experiment [58].

3.2.2. Enzyme Inhibitory Activities

The investigation revealed that the AChE inhibitory effects of EOs from *C. cyrtophyllum* and *C. fortunatum* were 289.10 ± 0.43 $\mu\text{g/mL}$ and 1060.00 ± 0.82 $\mu\text{g/mL}$, as exhibited in Figure 2. In the previous studies, EO from *Pseuduvaria macrophylla* [59] and *Syzygium variolosum* [60] showed comparatively lower inhibitory activity against AChE (32.50% and 35.20% at 1000 $\mu\text{g/mL}$) than our tested two *Clerodendrum* plant EOs. Therefore, the results indicated that the EOs from two *Clerodendrum* plants showed relatively notable anti-AChE activity.

The results showed that the anti-AChE activity of *C. cyrtophyllum* EO is relatively higher than *C. fortunatum* EO. One of the reasons showed the bicyclic monoterpenoids like α -pinene (1.1% and NA) from *C. cyrtophyllum* and *C. fortunatum* EOs can potentially be essential sources of anti-AChE activity, as it displayed a low IC₅₀ value of 0.40 mmol by a preceding study [61]. Additionally, benzylbenzofuran and its derivatives could be another possible factor with comparatively low inhibitory activity against AChE (IC₅₀ = 12.55-85.43 μmol). They exhibited an affinity with the amino acid residues situated at the enzyme's active site entrance, then combine with it [62]. Therefore, we speculate that 2-*n*-pentylfuran (2.6% and NA) and

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(*E*)-2-(2-pentenyl)furan (1.1% and NA) from *C. cyrtophyllum* and *C. fortunatum* EOs might have played a similar and indispensable role in our anti-AChE experiment. Listed in Table 1, these compounds' contents from *C. cyrtophyllum* EO demonstrated relatively higher percentage than *C. fortunatum* EO, consistent with our experiments' results on the anti-AChE activity of two *Clerodendrum* plant EOs.

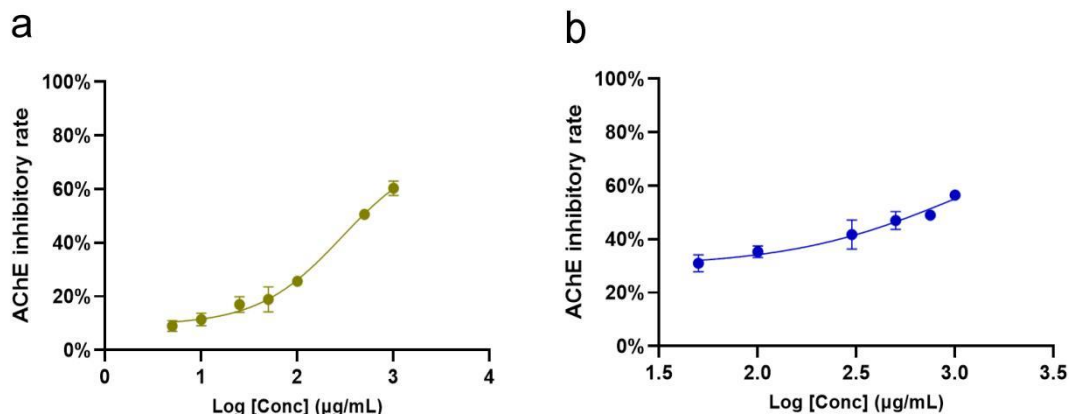


Figure 2. The regression curve of AChE inhibitory activity of *C. cyrtophyllum* EO (a) and *C. fortunatum* (b) EO.

Furthermore, the present study found the EOs exhibited inhibitory effects on β -lactamase with IC_{50} values $41.34 \pm 0.84 \mu\text{g/mL}$ and $673.50 \pm 1.27 \mu\text{g/mL}$ for *C. cyrtophyllum* and *C. fortunatum* (Figure 3). EOs from two *Clerodendrum* plants showed relatively noticeable anti- β -lactamase activity compared with *Dorstenia convexa* De Wild. (The inhibitory rate is less than 50% at a concentration of 4 mg/mL) [63]. The previous article stated some terpenoids such as linalool, α -pinene and limonene displayed superior antibacterial activity of bacterium that can secrete β -lactamase [64]. These compounds possess comparatively considerable content in EOs from two *Clerodendrum* plants, which implied the reason for the noticeable β -lactamase inhibitory effects of our tested EOs.

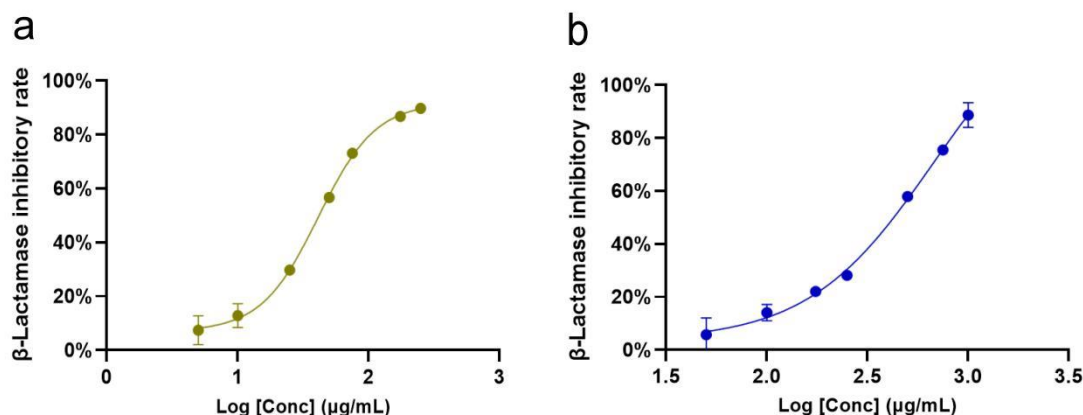


Figure 3. The regression curve of β -lactamase inhibitory activity of *C. cyrtophyllum* EO (a) and *C. fortunatum* (b) EO.

4. Conclusion

The present study investigated the chemical composition of EOs from *C. cyrtophyllum* and *C. fortunatum*, including 89 and 63 chemical compounds, respectively. The major components of *C. fortunatum* EO include 1-octen-3-ol, benzaldehyde, linalool, β -ionone, palmitic acid, and hexahydrofarnesyl acetone. In contrast, the primary compounds in *C. cyrtophyllum* EO are linalool, 1-octen-3-ol, phytol, (*Z*)-2-hexenol, edulan III, caryophyllene, hexahydrofarnesyl acetone, β -isocomene and pentadecanal. The EO from *C. cyrtophyllum* and *C. fortunatum* displayed moderate DPPH radical-

scavenging activity ($43.49\% \pm 0.95\%$ and $27.23\% \pm 0.40\%$ inhibition at 20 mg/mL, respectively), ABTS radical-scavenging activity (IC_{50} values: 2.31 ± 0.05 mg/mL and 7.43 ± 1.80 mg/mL, respectively) and FRAP total antioxidant capacity (TEAC: 55.61 ± 2.56 μ mol/g and 23.39 ± 1.58 μ mol/g, respectively). Therefore, the *Clerodendrum* plant EOs can be used as antioxidants for food and cosmetics. The study also showed the potential AChE inhibitory activity of *C. cyrtophyllum* and *C. fortunatum* EOs with IC_{50} values 289.10 ± 0.43 μ g/mL and 1060.00 ± 0.82 μ g/mL, respectively, indicating that EOs derived from the *Clerodendrum* plants can function as viable sources for the development of drugs aimed at treating AD. Besides, the present research exhibited comparatively noticeable anti- β -lactamase activity (IC_{50} values: 41.34 ± 0.84 μ g/mL and 673.50 ± 1.27 μ g/mL, respectively) from *C. cyrtophyllum* and *C. fortunatum* EOs, which hints at the EO's antibacterial properties. The results demonstrated the EOs from two *Clerodendrum* plants displayed potential pharmacological properties. However, we still need to understand further potential mechanisms for treating diseases regarding the *Clerodendrum* plant EOs, which can be achieved through *in vivo* experiments, molecular docking, and molecular dynamics simulations.

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