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Chemical Composition, and Antioxidant and Cholinesterase Inhibitory Activities of *Lindera glauca* Fruit Essential Oil and Molecular Docking Studies of Six Selected Compounds

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Abstract: *Lindera glauca* is a shrub or small tree mostly distributed in China, Japan and Korea. However, reports on the biological activities of *Lindera glauca* fruit essential oil (LGFEO) are limited. The study on its chemical composition, and antioxidant and cholinesterase inhibitory activities were performed, along with molecular docking of six selected compounds. The LGFEO was extracted by hydro distillation and analyzed by GC-MS and GC-FID. Antioxidant activities of LGFEO were evaluated by three methods with different mechanisms. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of LGFEO were tested. A total of 48 components were identified representing 95.74% of the total composition of LGFEO in which the major compounds were (E)-β-ocimene (41.53%), α-copaene (13.17%), δ-cadinene (6.20%), 3-carene (5.89%) and eucalyptol (3.57%). Weak antioxidant activities of LGFEO in three assays (9.52, 11.36 and 38.98 μmol TE/g, respectively) were observed. LGFEO showed obvious cholinesterase inhibitory activities at the final concentrations of 50 and 20 μg/mL. IC_{50} values for acetylcholinesterase and butyrylcholinesterase were 46.48 and 34.85 μg/mL, respectively. Molecular docking revealed that geranyl acetate, β-caryophyllene and limonene had lower binding affinities in the range of −7.1 to −6.1 kcal/mol through hydrophobic interactions and hydrogen bond. Six compounds including 3-carene, limonene, eucalyptol, (E)-β-ocimene, geranyl acetate and β-caryophyllene could contribute together to cholinesterase inhibitory activities of LGFEO. This essential oil indicated low potential as natural antioxidant, but it could be potentially used as cholinesterase inhibitor with possible application in food, aromatherapy and pharmaceutical industries.

Keywords: antioxidant; β-caryophyllene; cholinesterase inhibitor; essential oil; geranyl acetate; limonene; *Lindera glauca*; molecular docking

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1. Introduction

The genus *Lindera* belongs to Lauraceae family and contains more than 100 species that are widely spread in tropical and subtropical areas throughout the world. *Lindera* plants have found their various uses as ornamental plants or herbal medicines [1]. *Lindera glauca* (Siebold et Zucc.) Blume (*L. glauca*) is a shrub or small tree which can grow up to 8 m. It is mostly distributed in China, Japan, Korea and Vietnam. This plant is known in Chinese as Shan hu jiao and could be easily confused with *Litsea cubeba* (*L. cubeba*) which has quite large annual yield in fruit and essential oil, as one of the Chinese names of *L. cubeba* is the same as *L. glauca*. The leaves, roots and fruits of *L. glauca* have been traditionally used as herbal medicines. The leaves could be used for detoxification and hemostasis. The roots have been used for the treatment of contusion, extravasation and rheumatic arthritis. The fruits also found their uses in relieving several symptoms of abdominal and cardiac pain [2].

In the past decade, *L. glauca* has been received considerable attention due to its abundant valuable molecules. Phytochemical investigations have revealed different chemical compounds in this plant, such as essential oils, flavonoids [2], sesquiterpenoids [3–5], sesterterpenoids [6], lignans [7,8], sterols [9], fatty acids [10,11], diarylpropanoids [12] and alkaloids [13]. In previous studies, the compounds from *L. glauca* have been reported with various pharmacological effects, including anti-tumor activity [14–17], antiviral activity [18], antioxidant activity [2,19], anti-inflammatory effects [4,20] and neuroprotection effect [21].

Essential oils with unique flavors and various biological activities have aroused great interest in application in food, pharmaceutical, tobacco and cosmetical industries. Essential oils existed in different parts of *L. glauca*. The chemical constituents of essential oils from the fruits and leaves of *L. glauca* have been previously reported. The main compounds of *L. glauca* fruit essential oil (LGFEO) from Hubei province were n-carpric acid (25.39%), germacrene A (10.71%) and n-dodecanoic acid (10.08%) [22]. Volatile compounds analysis from leaves and fruits of *L. glauca* from Guizhou province revealed that D-germacrene (45.56%), (+)-ledene (5.76%) and caryophyllene (5.75%) were the most abundant volatile compounds in leaves, while β-ocimene (31.90%), copaene (12.75%) and α-caryophyllene (8.06%) were the major volatiles in fruits [23]. Volatile constituents from the fruits of *L. glauca* from Henan province with different maturities were studied and β-ocimene was found to be the major compound, with the content varying from 12.99% to 37.4% [24]. In another study on LGFEO from Henan province, the major compound was (E)-β-Ocimene (30.54%), followed by (E)-β-caryophyllene (4.87%), δ-guaiene (4.76%) and limonene (4.20%) [25]. For the leaf essential oil of *L. glauca* from Vietnam, β-caryophyllene (29.2%), α-humulene (18.0%) and caryophyllene oxide (14.6%) were the significant compounds [26]. Similarity and differences were observed in chemical components of *L. glauca* essential oils from different plant parts, collection locations and maturities.

Despite of some studies on chemical components, reports on the biological activities of essential oils from *L. glauca* were quite limited. The antimicrobial activities of essential oils from the leaves and fruits of *L. glauca* were reported [22,25,26]. The fruit essential oil showed promising antimicrobial activity, especially against *Shigella flexneri* and antimicrobial mechanism was investigated [25]. To the best of our knowledge, no reports on the antioxidant and cholinesterase inhibitory activities of LGFEO was available. The evaluation of these activities could provide some insights into the potential application of LGFEO in food, pharmaceutical and other industries.

The objective of the current research was to investigate the chemical composition of LGFEO, and to evaluate its antioxidant and cholinesterase inhibitory activities. We also aimed to study the interactions of chemical compounds of LGFEO with cholinesterases by molecular docking.

2. Materials and Methods

2.1. Plant Materials

The fruits of *L. glauca* were collected in September 2021 in Wudang District of Guiyang (Latitude 26°39′58″ N, longitude 106°46′7″ E, altitude 1158 m). The fruits were naturally dried in the shade for about three weeks before isolation of essential oil. Water content was detected as 6.2 ± 0.3%. Associate Prof. Yazhou Zhang from Guizhou University of Traditional Chinese Medicine identified this plant according to the Flora of China and voucher specimens were deposited at the laboratory of Pharmaceutical engineering, Guizhou Institute of Technology.

2.2. Extraction of Essential Oil

The dry fruits (200 g) of *L. glauca* were ground and then subjected to hydro distillation in Clevenger-type apparatus for 5 h using 3 L of deionized water. Extraction of essential oil was performed in triplicates. The essential oil yield was calculated (*w*/*w*). The essential oil was stored in amber-colored glass bottles at −20 °C for further analysis.

2.3. GC-MS and GC-FID Analysis

The LGFEO was diluted 1:50 v/v in n-hexane and analyzed according to the method previously reported in our study [27] with some changes. TG-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used for GC-MS and GC-FID analysis. Column temperature was initially set at 50 °C and hold for 3 min. It was increased to 140 °C with a rate of 3 °C/min and hold for 2 min. Then it was increased to 190 °C with a rate of 2 °C/min and hold for 2 min. Finally, it was increased to 220 °C with a rate of 10 °C/min. The other parameters were the same as the previous study. The identification of essential oil components was made based on the comparison of retention index and mass spectrum. The relative percentage (%) of each essential oil individual component was given by peak area normalization of GC chromatogram. The analysis was conducted in three replications.

2.4. Antioxidant Activity Assays

Three methods with different mechanisms, including DPPH radical scavenging assay, ABTS cation radical scavenging assay and ferric reducing antioxidant power assay, were employed to evaluate the antioxidant activities of LGFEO. The experiments were carried out according to the previously reported method [27]. The antioxidant activity of essential oil was expressed in µmol Trolox equivalents (TE)/g of essential oil. BHT was used as the positive control.

2.5. Cholinesterase Inhibitory Activities

Inhibitory activity evaluation of LGFEO against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were conducted using the methods previously described in our study [27]. LGFEO was tested for a preliminary screening at three final concentrations of 50, 20 and 2 µg/mL. The IC_{50} values (µg/mL, concentration of essential oils that inhibits the hydrolysis of substrates by 50%) were also determined. Tacrine and tetraisopropyl pyrophosphoramide (iso-OMPA) were employed as the reference for AChE and BuChE inhibitor, respectively.

2.6. Molecular Docking Study

Six chemical compounds in the LGFEO, which had relative percentages of more than 1% and have been reported to have cholinesterase inhibitory activities in previous studies, were selected as ligands. The interactions between ligands and cholinesterases were simulated by molecular docking using AutoDock Vina 1.1.2 [28]. Two-dimensional structures of ligands in sdf format were obtained from PubChem website and converted to three-dimensional structures in mol2 format using Chem 3D 19.0 after energy minimization. The ligands were then prepared by AutoDockTools 1.5.7 and saved in pdbqt format. The protein structures of acetylcholinesterase [29] and butyrylcholinesterase [30] were downloaded from the Protein Data Bank database, with PDB ID codes as 4M0E and 6QAA, respectively. The protein structures were processed using PyMOL 2.5.4 and AutoDockTools 1.5.7. Water molecules, undesired protein chains and the co-crystalized ligands were removed from the protein structure, after which hydrogen atoms and Gesteiger charges were added. The target file was finally saved in pdbqt format. The docking grid box was assigned to cover the protein structure and defined in terms of coordinates and size (Table 1). Possible conformations were docked at the active sites of the cholinesterases using AutoDock Vina software. Docking results were presented as binding affinity values (kcal/mol), where the more negative value indicated stronger binding possibilities. Three replications were performed for each ligand. Docking results were visualized, and the interactions between the ligands and the action site of cholinesterases were analyzed using PyMOL and online tool named Protein-Ligand Interaction Profiler (PLIP) [31].

Table 1. Molecular docking parameters for cholinesterases.

Protein	PDB ID	Centre Coordinates	Size
acetylcholinesterase	4M0E	x = −0.733 y = −37.62 z = 33.673	x = 62 y = 64 z = 74
butyrylcholinesterase	6QAA	x = 18.163 y = 31.938 z = 39.042	x = 64 y = 60 z = 76

2.7. Statistical Analysis

All experiments including extraction, antioxidant activity assay, cholinesterase inhibitory activity assay, GC analysis and molecular docking, were conducted in triplicates. The results were given as the mean ± SD. For comparing average values, one-way ANOVA analysis was carried out using SPSS 25.0 software and a value of $p < 0.05$ indicated statistical significance.

3. Results

3.1. Chemical Composition of the LGFEO

The LGFEO was light yellow and transparent liquid. The yield (w/w) was (0.32 ± 0.03)%. GC and GC-MS were used to determine the chemical components of the essential oil. The results were presented in Table 2. A total of 48 components were identified, representing 95.74% of the total composition of the essential oil. (E)-β-ocimene (41.53%) was the most abundant compound, followed by α-copaene (13.17%), δ-cadinene (6.20%), 3-carene (5.89%), eucalyptol (3.57%), limonene (2.14%), myrcene (1.90%), γ-muurolene (1.83%), β-caryophyllene (1.63%), α-zingiberene (1.61%), geranyl acetate (1.17%) and (E)-nerolidol (1.00%). Except for these 12 compounds, all other compounds had the relative percentages of less than 1%. The essential oil contained predominantly monoterpene and sesquiterpene hydrocarbon compounds, which accounted for 56.97% and 28.40%, respectively. The minor contents were oxygenated monoterpenes (5.45%), oxygenated sesquiterpenes (2.36%) and other compounds (2.56%).

Table 2. Chemical composition of the LGFEO.

No.	Compound	RI Calc.	RI Lit.	Identification	Relative Percentage (%)
1	α-pinene	931	932	MS, RI	0.73 ± 0.01
2	camphene	946	946	MS, RI	0.39 ± 0.02
3	β-pinene	977	974	MS, RI	0.22 ± 0.01
4	6-methyl-hept-5-en-2-one	988	986	MS, RI	0.33 ± 0.01
5	myrcene	991	988	MS, RI	1.90 ± 0.02
6	3-carene	1009	1008	MS, RI	5.89 ± 0.02
7	α-terpinene	1015	1014	MS, RI	0.14 ± 0.01
8	p-cymene	1021	1017	MS, RI	0.66 ± 0.02
9	o-cymene	1023	1022	MS, RI	0.82 ± 0.01
10	limonene	1027	1024	MS, RI	2.14 ± 0.03
11	eucalyptol	1030	1026	MS, RI	3.57 ± 0.02
12	(Z)-β-ocimene	1037	1032	MS, RI	0.74 ± 0.01
13	(E)-β-ocimene	1048	1044	MS, RI	41.53 ± 0.11
14	γ-terpinene	1058	1054	MS, RI	0.58 ± 0.02
15	isoterpinolene	1085	1081	MS, RI	0.32 ± 0.01
16	terpinolene	1087	1085	MS, RI	0.91 ± 0.01
17	linalool	1100	1095	MS, RI	0.33 ± 0.01

Table 2. *Cont.*

No.	Compound	RI Calc.	RI Lit.	Identification	Relative Percentage (%)
18	n-nonanal	1104	1099	MS, RI	0.27 ± 0.02
19	fenchol	1112	1110	MS, RI	0.03 ± 0.00
20	isopinocarveol	1162	1160	MS, RI	0.03 ± 0.01
21	terpinen-4-ol	1176	1174	MS, RI	0.55 ± 0.02
22	α-terpineol	1190	1186	MS, RI	0.11 ± 0.01
23	geraniol	1254	1249	MS, RI	0.84 ± 0.03
24	trans-2-decenal	1261	1260	MS, RI	0.37 ± 0.01
25	bornyl acetate	1285	1280	MS, RI	0.19 ± 0.03
26	2-undecanone	1294	1293	MS, RI	0.11 ± 0.01
27	methyl geranate	1324	1322	MS, RI	0.12 ± 0.01
28	ylangene	1372	1372	MS, RI	0.10 ± 0.01
29	α-copaene	1376	1374	MS, RI	13.17 ± 0.06
30	geranyl acetate	1384	1379	MS, RI	1.17 ± 0.03
31	β-caryophyllene	1419	1416	MS, RI	1.63 ± 0.02
32	α-guaiene	1439	1438	MS, RI	0.47 ± 0.01
33	α-humulene	1453	1448	MS, RI	0.25 ± 0.04
34	γ-muurolene	1476	1474	MS, RI	1.83 ± 0.02
35	α-amorphene	1479	1483	MS, RI	0.16 ± 0.01
36	β-selinene	1486	1486	MS, RI	0.51 ± 0.01
37	α-zingiberene	1495	1493	MS, RI	1.61 ± 0.03
38	α-bulnesene	1506	1505	MS, RI	0.51 ± 0.02
39	α-farnesene	1508	1508	MS, RI	0.52 ± 0.03
40	γ-cadinene	1514	1513	MS, RI	0.64 ± 0.01
41	δ-cadinene	1524	1522	MS, RI	6.20 ± 0.08
42	trans-cadina-1,4-diene	1532	1533	MS, RI	0.27 ± 0.02
43	α-cadinene	1537	1537	MS, RI	0.27 ± 0.01
44	α-calacorene	1542	1544	MS, RI	0.27 ± 0.01
45	(E)-nerolidol	1563	1561	MS, RI	1.00 ± 0.03
46	di-epi-1,10-cubenol	1627	1623	MS, RI	0.13 ± 0.01
47	τ-cadinol	1640	1638	MS, RI	0.68 ± 0.01
48	α-cadinol	1653	1652	MS, RI	0.56 ± 0.01
	Compounds identified				48
	Total identified (%)				95.74
	Monoterpene hydrocarbons				56.97
	Oxygenated monoterpenes				5.45
	Sesquiterpene hydrocarbons				28.40
	Oxygenated sesquiterpenes				2.36
	Others				2.56

RI Calc.: retention indices calculated against n-alkane series on TG-5MS column; RI Lit.: retention indices from literature on similar columns with the same polarity; MS: mass spectrum.

3.2. Antioxidant Activity of the LGFEO

The application of different antioxidant assays is necessary as there are several mechanisms for antioxidant activities [32]. In order to give a comprehensive prediction of antioxidant efficacy of the LGFEO, three different methods including DPPH radical scavenging assay, ABTS cation radical scavenging assay and ferric reducing antioxidant power assay were employed. The results were expressed as Trolox equivalents (TE) and presented in Table 3. It seems that FRAP assay gave higher Trolox equivalent values than DPPH and ABTS assays. In comparison with positive control, the LGFEO showed significantly lower radical scavenging capacities against DPPH and ABTS radicals and reducing ability, indicating very weak antioxidant activity.

Table 3. Antioxidant activity of the LGFEO.

Sample	DPPH	ABTS	FRAP
	μmol TE/g	μmol TE/g	μmol TE/g
LGFEO	9.52 ± 0.35	11.36 ± 0.63	38.98 ± 1.57
BHT	2123.68 ± 33.65	4673.64 ± 32.14	2566.40 ± 53.33

3.3. Cholinesterase Inhibitory Activity of the LGFEO

Acetylcholinesterase and butyrylcholinesterase inhibitory activity assays were performed to evaluate the potential effect of the LGFEO on neurodegenerative disease. The essential oil at three final concentrations (50, 20 and 2 μg/mL) were tested for a preliminary screening. The cholinesterase inhibitory activities expressed by inhibition percentage (%) were given in Table 4.

Table 4. Cholinesterase inhibitory activity of the LGFEO by inhibition percentage.

Sample	Concentration (μg/mL)	Acetylcholinesterase Inhibition (%)	Butyrylcholinesterase Inhibition (%)
LGFEO	50	63.82 ± 1.15	69.72 ± 1.11
	20	35.57 ± 2.44	24.15 ± 2.70
	2	10.13 ± 1.66	NA

NA: not active.

The LGFEO showed inhibitory effects on acetylcholinesterase at all three tested concentrations. At the final concentration of 50 μg/mL, the inhibitory percentages of the LGFEO against acetylcholinesterase and butyrylcholinesterase could reach 63.82% and 69.72%, respectively. At 50 and 20 μg/mL, the LGFEO displayed obvious cholinesterase inhibition activities. However, no inhibition effect on butyrylcholinesterase was observed at 2 μg/mL.

The IC_{50} values of the LGFEO were also determined. Tacrine and tetraisopropyl pyrophosphoramide (iso-OMPA) were employed as the reference for acetylcholinesterase and butyrylcholinesterase inhibitor, respectively. The results could be seen in Table 5. IC_{50} values of the LGFEO for acetylcholinesterase and butyrylcholinesterase were 46.48 and 34.85 μg/mL, respectively. In comparison with the positive controls, the cholinesterase inhibitory activities of the LGFEO were weaker.

Table 5. Cholinesterase inhibitory activity of the LGFEO by IC_{50}.

Sample	Acetylcholinesterase (μg/mL)	Butyrylcholinesterase (μg/mL)
LGFEO	46.48	34.85
Tacrine	0.14	/
iso-OMPA	/	0.60

3.4. Molecular Docking

AutoDock Vina is a powerful tool for studying the interactions between ligands and protein targets. Six chemical compounds, which had relative percentages of more than 1% and have been reported to have cholinesterase inhibitory activities in previous studies, were selected for molecular docking to study the interactions between ligands and cholinesterases. The binding affinity values (kcal/mol) for each compound was displayed in Table 6. For acetylcholinesterase, the binding affinities of six compounds ranged from −7.1 to −5.9 kcal/mol. Among these compounds, geranyl acetate exhibited the lowest binding affinity (−7.1 kcal/mol), followed by β-caryophyllene (−6.8 kcal/mol) and limonene (−6.7 kcal/mol). For butyrylcholinesterase, there was no significant difference ($p > 0.05$) between binding affinities of geranyl acetate (−6.3 kcal/mol) and β-caryophyllene (−6.2 kcal/mol).

The bind affinity of limonene was close to these two values. The more negative values indicated stronger binding possibilities, which means that geranyl acetate, β-caryophyllene and limonene could have stronger binding abilities with cholinesterases.

Table 6. Binding affinities of six major compounds in the LGFEO against acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE).

No.	Compound	PubChem ID	Binding Affinities (kcal/mol)	
			AChE	BuChE
	tacrine (positive control)	1935	−9.1 ± 0.0	−8.3 ± 0.0
1	3-carene	26049	−6.2 ± 0.0	−6.0 ± 0.0
2	limonene	22311	−6.7 ± 0.0	−6.1 ± 0.0
3	eucalyptol	2758	−5.9 ± 0.2	−5.9 ± 0.1
4	(E)-β-ocimene	5281553	−6.3 ± 0.1	−5.6 ± 0.1
5	geranyl acetate	1549026	−7.1 ± 0.1	−6.3 ± 0.1
6	β-caryophyllene	5281515	−6.8 ± 0.0	−6.2 ± 0.1

The best ranked poses of limonene, geranyl acetate and β-caryophyllene can be seen in Figures 1 and 2, with the interactions with amino acid residues in the binding pockets of acetylcholinesterase and butyrylcholinesterase. The main interactions were hydrophobic interaction and hydrogen bond. In comparison with tacrine whose binding affinities were −9.1 and −8.3 kcal/mol, these compounds showed weaker binding abilities.

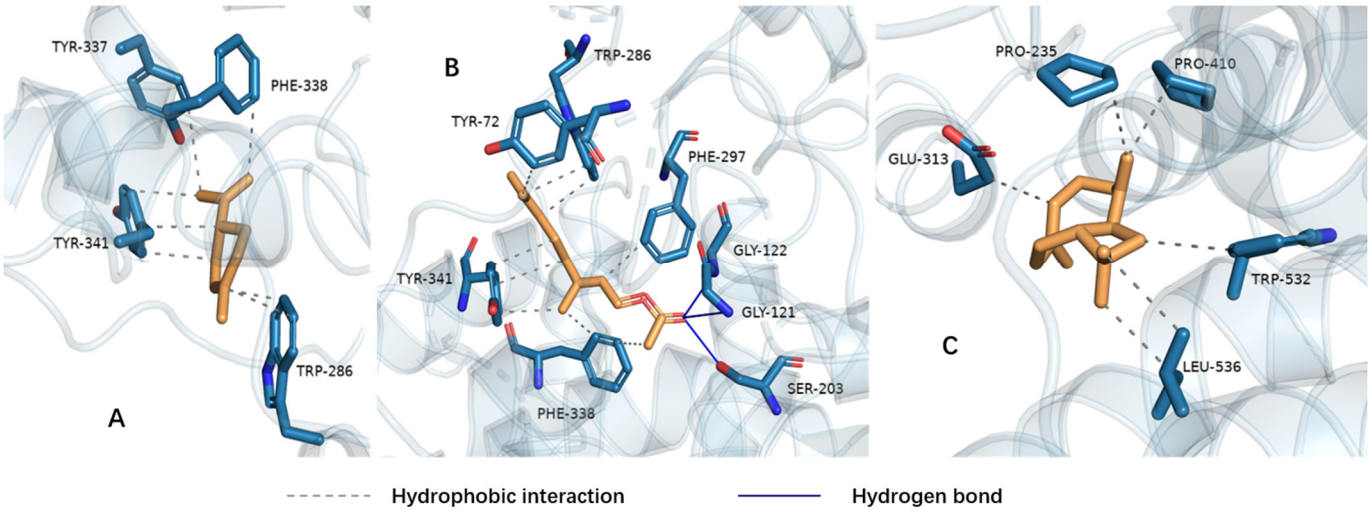

Figure 1. The interactions of acetylcholinesterase with limonene (**A**), geranyl acetate (**B**) and β-caryophyllene (**C**).

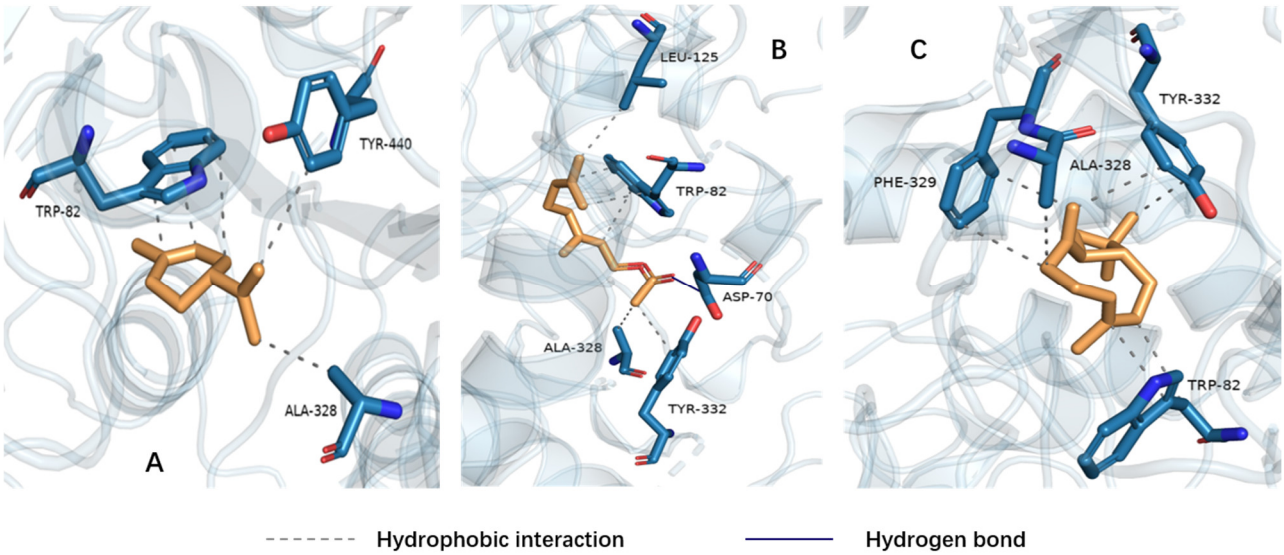

Figure 2. The interactions of butyrylcholinesterase with limonene (**A**), geranyl acetate (**B**) and β-caryophyllene (**C**).

4. Discussion

The yield (0.32%) of the LGFEO was considerably lower than that (1.91%) reported in the previous study [25]. This may be due to the difference in geographic location in which this plant grew. For the chemical composition, the most abundant compound in this study was (E)-β-ocimene (41.53%), which was in agreement with the result (30.54%) of *L. glauca* from Henan province. Except for (E)-β-ocimene, differences were observed in other major compounds. For example, α-copaene (13.17%), δ-cadinene (6.20%) and 3-carene (5.89%) were other three major compounds in this study, while the corresponding compounds in the literature [25] were (E)-β-caryophyllene (4.87%), δ-guaiene (4.76%) and limonene (4.20%). Compared with another study on fruit volatiles [23], the two most abundant compounds were the same, and copaene had similar relative percentages (13.17% versus 12.75%). If comparing the chemical components of essential oils from the fruits and leaves, significant differences could be found. The leaf essential oil from Vietnam was reported to have β-caryophyllene (29.2%), α-humulene (18.0%) and caryophyllene oxide (14.6%) as major compounds [26], which was totally different from our study. The chemical components of *L. glauca* essential oils were significantly influenced by geographic location, plant parts and other factors.

In comparison with different species in the genus *Lindera*, some similarities and differences were noticed. In the fruit essential oil of *Lindera neesiana*, *Z*-citral (15.08%), *E*-citral (11.89%), eucalyptol (8.75%), citronellal (6.72%), α-pinene (6.63%) and β-pinene (5.61%) were the major components [33], which was different from our results. However, α-pinene (0.73%), β-pinene (0.22%) and eucalyptol (3.57%) were identified in our study. In one study on essential oil from *Lindera umbellata* [34], 20 chemical compounds were identified, of which 14 compounds could be found in our study. The essential oil from the leaves of *Lindera fragrans* [35] was reported with spathulenol (27.63%), ledol (6.81%), β-caryophyllene (4.01%), (+)-cis-limonene oxide (3.69%) and α-cadinol (3.24%) as major compounds. Spathulenol and ledol were not identified in our study. In another publication on essential oil of *Lindera aggregate* [36], the major compounds, α-longifolene (15.13%), bornyl acetate (11.49%), α-eudesmol (9.14%) and α-pinene (7.88%), were also different from our results.

The most abundant compound in the LGFEO was (E)-β-ocimene which accounted for more than 40%. (E)-β-ocimene exists in many plants such as basil and lavender, and could be used in many fields. This compound is in the list of food additives as flavoring agent. Recent publication has shown that it played an important role in the interactions

between plants and other organisms [37]. LGFEO seems to be a good natural source of (E)-β-ocimene due to its high percentage in this essential oil.

With increasing concern on adverse effects of synthetic antioxidants on human health, researchers have turned their attention to plants which are good natural sources of antioxidants. On the other hand, these plants can bring color or flavor to food. It is interesting to evaluate the antioxidant activity of extracts from plants. One recent publication reported the application of encapsulated *Indigofera tinctoria* extract as a natural antioxidant and colorant in ice cream [38]. Many essential oils have been reported to have strong antioxidant activity and have been used as antioxidant additive in food products, such as clove essential oil [39] and oregano essential oil [40]. These essential oils provided not only flavors but also antioxidant protection. In our research, however, the weak antioxidant activity of LGFEO indicated a low potential of this essential oil as natural antioxidant.

The antioxidant activities of essential oils are closed connected with their components, especially phenolic compounds which showed good antioxidant activities due to their high reactivity with radicals [41]. In this study, no phenolic compounds were detected, which could be the reason for the weak antioxidant activity of the LGFEO. However, the LGFEO showed a certain degree of antioxidant activity especially in the FRAP assay. The antioxidant activity could be partially explained by the presence of some main components in the essential oil. It has been reported that monoterpenes such as β-ocimene, 3-carene, limonene and myrcene showed antioxidant activities [42,43]. Eucalyptol (or 1,8-cineole) also exhibited antioxidant activity in the oxygen radical absorbance capacity assay [44]. (E)-β-ocimene (41.53%), 3-carene (5.89%), eucalyptol (3.57%), limonene (2.14%) and other compounds could contribute together to the antioxidant activity of the LGFEO.

Cholinesterase inhibitors are often used for the treatment of neurodegenerative disease. As important sources of cholinesterase inhibitor, many herbs have been investigated to evaluate their effects. The investigations of different spices have been reported [45,46], such as saffron, rosemary, cinnamon, amomum tsao-ko, pepper and ginger. Pepper essential oils were found to have potent acetylcholinesterase inhibitory activity with IC_{50} values of 8.54 μg/mL (black pepper essential oil) and 5.02 μg/mL (white pepper essential oil). In our study, LGFEO had an IC_{50} value of 46.48 μg/mL, which means that acetylcholinesterase inhibitory activity of LGFEO is weaker than pepper essential oil.

The LGFEO exhibited obvious cholinesterase inhibitory effects at the final concentration of 50 and 20 μg/mL, which could be attributed to some main components in the essential oil. Cholinesterase inhibitory activity of the essential oils and their individual constituents can be found in many reports in the literature [47]. Some monoterpenes and monoterpenoids from essential oils have been reported as inhibitors of cholinesterase, such as 3-carene [48], limonene [49], cis-ocimene [50], 1,8-cineole (or eucalyptol) [51] and geranyl ester [52]. Some sesquiterpenes also showed cholinesterase inhibitory activities, such as β-caryophyllene [48] and α-humulene [53]. These compounds could contribute together to the activities displayed by the LGFEO. In order to provide insight into different contributions of these compounds to cholinesterase inhibitory activities, molecular docking was used to study the interactions of these compounds with acetylcholinesterase and butyrylcholinesterase.

Six compounds including 3-carene, limonene, eucalyptol, (E)-β-ocimene, geranyl acetate and β-caryophyllene were selected for molecular docking. The interactions of limonene, geranyl acetate and β-caryophyllene with cholinesterases were analyzed. It can be seen in Figures 1 and 2 that limonene and β-caryophyllene mainly interacted with cholinesterases by hydrophobic interactions. Limonene was stabilized in the active site of acetylcholinesterase by hydrophobic interactions with residues Trp 286, Tyr 337, Phe 338 and Tyr 341. β-caryophyllene had interactions with residues Pro 235, Glu 313, Pro 410, Trp 532 and Leu 536 of acetylcholinesterase. For the binding models of butyrylcholinesterase, limonene interacted with three resides including Trp 82, Ala 328 and Tyr 440 while β-caryophyllene had seven interactions with four residues (Trp 82, Ala 328, Phe 329 and Tyr 332). The docking analysis of geranyl acetate revealed strong interactions by forming three

hydrogen bonds with residues Gly 121, Gly 122 and Ser 203 of acetylcholinesterase, and by forming one hydrogen bond with residue Asp 70 of butyrylcholinesterase. On the other hand, hydrophobic interactions were also observed for geranyl acetate, which had interactions with residues Tyr 72, Trp 286, Phe 297, Phe 338 and Tyr 341 of acetylcholinesterase. The hydrogen bond and hydrophobic interactions could explain lower binding affinity of geranyl acetate.

Compared with geranyl acetate, β-caryophyllene and limonene, the other three compounds including 3-carene, eucalyptol and (E)-β-ocimene exhibited higher binding affinities, which indicated weaker binding ability with cholinesterases. Based on the interaction analysis, 3-carene, eucalyptol and (E)-β-ocimene were enfolded in the active site of cholinesterases mainly by hydrophobic interactions. However, relative percentages of these three compounds in the LGFEO were quite high, with 5.89%, 3.57% and 41.53%, respectively. On the other hand, the relative percentages of geranyl acetate, β-caryophyllene and limonene were 1.17%, 1.63% and 2.14%, respectively. Considering this, it is difficult to predict the real contributions of these six compounds to the cholinesterase inhibitory activities. It could be concluded that geranyl acetate, β-caryophyllene, limonene, 3-carene, eucalyptol and (E)-β-ocimene were the main contributors to the cholinesterase inhibitory activities of the LGFEO through hydrophobic interactions and hydrogen bond forming.

LGFEO showed obvious cholinesterase inhibitory activities at the final concentrations tested. However, this is only the result of in vitro experiments, and its effect has yet to be confirmed in animal experiments. In comparison with the positive control, the activity of LGFEO is much lower, indicating that it is difficult to develop it directly into medicine. However, it could find its use in adjuvant therapy for the related diseases. It could be developed into functional foods. The synergistic effects between LGFEO and tacrine or other commercial cholinesterase inhibitor can also be evaluated. If synergistic effects can be observed, it is possible to develop compound preparations. Another interesting field is aromatherapy. In fact, some essential oils have been proved to be efficacious in non-pharmacological aromatherapy for dementia [54]. By inhaling the LGFEO, some improvement could be perhaps observed for the neurodegenerative diseases.

5. Conclusions

In the present study, chemical composition, antioxidant and cholinesterase inhibitory activities of the LGFEO were investigated. A total of 48 components were identified, representing 95.74% of the total composition of the essential oil, in which the major compounds were (E)-β-ocimene (41.53%), α-copaene (13.17%), δ-cadinene (6.20%), 3-carene (5.89%), eucalyptol (3.57%), etc. LGFEO seems to be a good natural source of (E)-β-ocimene. The LGFEO displayed weak antioxidant activities in different assays, indicating its low potential as natural antioxidant. However, the LGFEO showed obvious cholinesterase inhibitory activities at the concentrations of 50 and 20 μg/mL. IC_{50} values of the LGFEO for acetylcholinesterase and butyrylcholinesterase were 46.48 and 34.85 μg/mL, respectively. Six compounds including 3-carene, limonene, eucalyptol, (E)-β-ocimene, geranyl acetate and β-caryophyllene were selected for molecular docking. Geranyl acetate, β-caryophyllene and limonene showed lower binding affinities. Considering the relative percentages in the LGFEO, these six compounds could be main contributors to the cholinesterase inhibitory activities of the essential oil through hydrophobic interactions and hydrogen bond forming. Confirmation of cholinesterase inhibitory activity in animal experiments, synergistic effect evaluation between LGFEO and commercial cholinesterase inhibitor, as well as study on effect of aromatherapy on patients with Alzheimer's disease, could be conducted in the future. The essential oil could find its potential use as a cholinesterase inhibitor with possible application in food, aromatherapy and pharmaceutical industries.

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