ISSN 2462-2052
DOI: https://doi.org/10.37134/jsml.vol11.1.9.2023
Journal of Science and Mathematics Letters
Volume 11, Issue 1, 64-67, 2023

SHORT COMMUNICATION

Chemical Composition of the Essential Oil of *Litsea resinosa* Blume and Acetylcholinesterase Activity

Abubakar Siddiq Salihu[1,2], Nurunajah Ab Ghani[3*,4], Nurulfazlina Edayah Rasol[3*,4], Shamsul Khamis[5]

[1]Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjong Malim, Perak, Malaysia
[2]Department of Pure and Industrial Chemistry, Faculty of Natural and Applied Sciences, Umaru Musa Yar'adua University, Katsina, Nigeria
[3]Atta-ur-Rahman Institute for Natural Product Discovery, Universiti Teknologi MARA, 42300 Bandar Puncak Alam, Selangor, Malaysia
[4]Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia
[5]Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
*Corresponding author: nurunajah@uitm.edu.my

Received: 2 January 2023; **Accepted:** 5 March 2023; **Published:** 21 March 2023

ABSTRACT

The essential oil of *Litsea resinosa* Blume (Lauraceae) was obtained by hydrodistillation and was fully characterized by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). A total of twenty-eight components were identified in the essential oil, which made up 90.8% of the total oil. The essential oil consists mainly of β-caryophyllene (22.5%), α-humulene (15.4%), caryophyllene oxide (9.2%), germacrene D (5.6%), and β-selinene (5.0%). The essential oil showed significant activity against acetylcholinesterase with a percentage inhibition of 72.4%.

Keywords: *Litsea resinosa*, Lauraceae, essential oil, caryophyllene, acetylcholinesterase

1. INTRODUCTION

Litsea is a genus of evergreen and deciduous trees or shrubs belonging to the laurel family, Lauraceae. It is mainly distributed in the tropical and subtropical regions and has been used in traditional and indigenous Chinese medicines for the treatment of diarrhea, stomachache, diabetes, edema, cold, arthritis, asthma, pain, and traumatic injury (Azhar et al., 2022). The presence of essential oil is one of the characteristic chemosystematic features of *Litsea* species. Most species of the genus *Litsea* produce an essential oil that can be extracted from different parts of the plant including fruits, leaves, stems, roots, and flowers, mainly composed of oxygenated monoterpenes and sesquiterpenes. These data are essential in promoting the bioactivity and industrial applications of *Litsea* essential oils (Kong et al., 2015). The present study reports the chemical composition and acetylcholinesterase inhibitory activity of the essential oil from the leaves of *L. resinosa.*

2. MATERIALS AND METHODS

2.1. Plant material and isolation of the essential oil

A sample of *L. resinosa* was collected from Behrang, Perak in September 2019, and identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (SA03-12) was deposited at UKMB Herbarium, UKM. The fresh leaf (300 g) was subjected to hydrodistillation in Clevenger-type apparatus for 4 hours. The essential oil obtained was dried over anhydrous magnesium sulfate and stored at 4-6°C.

2.2. Analysis of the essential oil

Gas chromatography (GC-FID) analysis was performed on an Agilent Technologies 7890B equipped with HP-5MS capillary column (30 m long, 0.25 μm thickness and 0.25 mm inner diameter). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was kept at 50°C, then gradually raised to 280°C at 5°C/min and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 μL were injected manually (split ratio 50:1). The injection was repeated three times and the peak area percent were reported as means ± SD of triplicate. The calculation of peak area percentage was carried out by using the GC HP Chemstation software (Agilent Technologies). Gas chromatography-mass spectrometry (GC-MS) analysis was recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with an HP-5 column. Helium was used as the carrier gas at a flow rate of 1 mL/min. The injector temperature was 250°C. The oven temperature was programmed from 50°C (5 min hold) to 280°C at 10°C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50-400 amu (Salleh et al., 2016). For identification of essential oil components, co-injections with the standards (major components) were used, together with the correspondence of retention indices and mass spectra with respect to those reported in Adams (2007). Semi-quantification of essential oil components was made by peak area normalization considering the same response factor for all volatile components. Percentage values were the mean of three chromatographic analyses.

2.3. Acetylcholinesterase activity

Acetylcholinesterase (AChE) inhibitory activity of the essential oil was measured by slightly modifying the reported spectrophotometric method (Salleh et al., 2021; Abdullah et al., 2022). Electric eel AChE (0.22 U/mL) was used, while acetylthiocholine iodide was employed as substrate of the reaction. DTNB acid was used for the measurement of the activity. Percentage of inhibition (I%) of AChE was determined by comparison of the rates of reaction of samples relative to a blank sample (EtOH in phosphate buffer, pH 8) using the formula: I% = [E - S / E] × 100; where E is the activity of enzyme without test sample and S is the activity of the enzyme with the test sample. Galantamine (conc. of 100 μg/mL) was used as a reference.

3. RESULTS AND DISCUSSION

A list of chemical components identified in the essential oil is shown in Table 1. The essential oil yield is 0.12% as calculated from the fresh weight of the leaves. The GC-FID and GC-MS analysis of the essential oil showed the occurrence of twenty-eight components

representing 90.8% of the essential oil components. Sesquiterpene hydrocarbons were the most dominant components in the essential oil, accounting for 62.7%, followed by oxygenated sesquiterpenes (24.9%). The major components of the essential oil were β-caryophyllene (22.5%), α-humulene (15.4%), caryophyllene oxide (9.2%), germacrene D (5.6%), and β-selinene (5.0%). The other minor components detected in the essential oil in more than 2% were α-bisabolol (2.8%), δ-cadinene (2.8%), (*E*)-nerolidol (2.6%), viridiflorol (2.5%), aromadendrene (2.3%), (*E*)-β-farnesene (2.2%), globulol (2.2%), ledol (2.2%), t-muurolol (2.2%), and α-zingiberene (2.1%). As a comparison with a previous study, the leaf oil of *L. resinosa* showed high contents of bulnesol (14.9%), β-caryophyllene (10.2%), and β-elemene (10.2%) (Ahmad et al., 2005). The differences in the chemical components of this study were probably due to the different environmental and genetic factors, chemotypes, and nutritional status of the plants, which can influence the oil composition. In fact, these factors influence plant biosynthetic pathways and consequently, the relative proportion of the main characteristic compounds (Salleh et al. 2021).

Table 1. Chemical components identified in the essential oil of *L.resinosa*

No.	Components	KI[a]	KI[b]	Percentage (%)	Identification[c]
1	α-Pinene	0935	0932	0.2	RI, MS
2	β-Pinene	0975	0974	0.5	RI, MS
3	α-Terpinene	1015	1014	1.0	RI, MS
4	Linalool	1095	1095	1.2	RI, MS
5	Borneol	1165	1162	0.3	RI, MS
6	Isoledene	1374	1375	0.2	RI, MS
7	α-Copaene	1376	1375	1.2	RI, MS
8	β-Patchoulene	1380	1381	0.4	RI, MS
9	α-Gurjunene	1410	1409	1.5	RI, MS
10	**β-Caryophyllene**	1415	1417	**22.5**	RI, MS, Std
11	γ-Elemene	1432	1434	0.5	RI, MS
12	Aromadendrene	1440	1442	2.3	RI, MS
13	**α-Humulene**	1450	1452	**15.4**	RI, MS, Std
14	(*E*)-β-Farnesene	1454	1455	2.2	RI, MS
15	**Germacrene D**	1485	1484	**5.6**	RI, MS, Std
16	**β-Selinene**	1490	1492	**5.0**	RI, MS, Std
17	α-Zingiberene	1495	1495	2.1	RI, MS
18	δ-Cadinene	1520	1522	2.8	RI, MS
19	Selinα-3,7(11)-diene	1545	1546	1.0	RI, MS
20	(*E*)-Nerolidol	1562	1562	2.6	RI, MS
21	Palustrol	1567	1565	0.4	RI, MS
22	**Caryophyllene oxide**	1580	1582	**9.2**	RI, MS, Std
23	Globulol	1590	1590	2.2	RI, MS
24	Viridiflorol	1595	1596	2.5	RI, MS
25	Ledol	1602	1602	2.2	RI, MS
26	β-Eudesmol	1650	1649	0.8	RI, MS
27	t-Muurolol	1635	1635	2.2	RI, MS
28	α-Bisabolol	1685	1686	2.8	RI, MS
	Monoterpene hydrocarbons			**1.7**	
	Oxygenated monoterpenes			**1.5**	
	Sesquiterpene hydrocarbons			**62.7**	
	Oxygenated sesquiterpenes			**24.9**	
	Total identified (%)			**90.8**	

[a]Linear retention index, experimentally determined using a homologous series of C_6-C_{30} alkanes; [b]Linear retention index taken from Adams; [c]Std, based on comparison with authentic compounds; MS, based on comparison with Wiley databases; RI, based on comparison of calculated RI with those reported in Adams

In this study, acetylcholinesterase inhibitory activity was tested against AChE enzyme. It was compared with that of galantamine, as a standard drug against Alzheimer's disease. The essential oil indicated significant AChE (I%: 72.4%) inhibitory activity at 1,000 µg/mL concentration, compared to galantamine which gave 95.9% inhibition. In previous reports, AChE inhibition can be explained by the presence of β-caryophyllene and caryophyllene oxide which have shown anticholinesterase activity. This study shows that the high content of these components obtained in the essential oil may contribute, at least in part, to the activity ascribed to the plant (Salleh et al. 2021). In another study, the methanol extracts from the root and stem of *L. resinosa* have shown strong antioxidant and antimicrobial properties (Wong et al., 2014).

4. CONCLUSION

The chemical composition of the essential oil from fresh leaves of *L. resinosa* growing in Malaysia was studied by gas chromatography combined with mass spectrometry. The essential oil consists mainly of β-caryophyllene (22.5%), α-humulene (15.4%), caryophyllene oxide (9.2%), germacrene D (5.6%), and β-selinene (5.0%). These results shed light on the phytochemistry of this unexplored flora species found in Malaysia. Further investigations are needed to study the safe use of the essential oil as therapeutic agents for Alzheimer's diseases.

Declaration of Interest
The authors declare that there is no conflict of interest.

Acknowledgment
The authors would like to thank the Department of Chemistry, Faculty of Science and Mathematics, UPSI for research facilities.

REFERENCES

Abdullah SA, Lathiff SMA, Jamil S. (2022). Acetylcholinesterase activity of crude extracts and flavonoids from *Artocarpus anisophyllus* Miq. and *Artocarpus lowii* King. *Journal of Science and Mathematics Letters*, 10(2), 62-66.

Adams RP. (2007). Identification of essential oil components by gas chromatography-mass spectrometry. 4th Ed. Carol Stream (IL): Allured Publishing Corporation.

Ahmad FB, Jantan IB, Bakar BA, Ahmad AS. (2005). A comparative study of the composition of the leaf oils of three *Litsea* species from Borneo. *Journal of Essential Oil Research*, 17(3), 323-326.

Azhar MAM, Salleh WMNHW, Khamis S, Ghani NA. (2022). Variation in essential oil composition of three *Litsea* species from Malaysia. *Rivista Italiana Delle Sostanze Grasse*, 99(1), 57-61.

Kong DG, Zhao Y, Li GH, Chen BJ, Wang XN, Zhou HL, Lou HX, Ren DM, Shen T. (2015). The genus *Litsea* in traditional Chinese medicine: an ethnomedical, phytochemical and pharmacological review. *Journal of Ethnopharmacology*, 164, 256-264.

Salleh WMNHW, Ahmad F, Khong HY, Zulkifli RM. (2016). Chemical composition and biological activities of essential oil of *Beilschmiedia pulverulenta*. *Pharmaceutical Biology*, 54(2), 322-330.

Salleh WMNHW, Khamis S, Abed SA. (2021). Characterization of volatile components of *Chassalia chartacea* and its acetylcholinesterase inhibitory activity. *Chemistry of Natural Compounds*, 57(2), 376-377.

Salleh WMNHW, Khamis S. (2021). Essential oil of *Thottea grandiflora* and its lipoxygenase inhibitory activity. *Chemistry of Natural Compounds*, 57(5), 959-960.

Wong MH, Lim LF, Ahmad FB, Assim ZB. (2014). Antioxidant and antimicrobial properties of *Litsea elliptica* Blume and *Litsea resinosa* Blume (Lauraceae). *Asian Pacific Journal of Tropical Biomedicine*, 4(5), 386-392.