Short note

Variation in essential oil composition of three *Litsea* species from Malaysia

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The genus *Litsea*, mainly distributed in the tropical and subtropical regions, has a long history of use in traditional and indigenous Chinese medicines. This study presents the essential oil composition of three *Litsea* species namely, *Litsea costalis*, *Litsea machilifolia*, and *Litsea globularia* collected from Malaysia. The GC-FID and GC-MS analysis of the essential oils led to the identification of 32, 14 and 28 components, representing 82.3%, 85.5% and 83.4% of the total compositions of *L. costalis*, *L. machilifolia*, and *L. globularia*, respectively. The main components of the essential oil from *L. costalis* were β-caryophyllene (12.6%) and δ-cadinene (12.1%), whereas components of the essential oil from *L. machilifolia* were β-sesquiphellandrene (29.5%), β-bisabolene (19.7%), and α-zingiberene (15.7%). The *L. globularia* oil was dominated by β-caryophyllene (25.2%) and δ-cadinene (15.8%).

Keywords: Essential oil, Lauraceae, *Litsea costalis*, *Litsea machilifolia*, *Litsea globularia*, GC-MS

1. INTRODUCTION

The genus *Litsea* (Lauracea) comprises nearly about 400 species, which are widely distributed in the tropical and subtropical Asia, North and South America [1]. Traditionally, the plant possesses medicinal properties and has been used to cure various gastro-intestinal ailments (e.g., diarrhoea, stomach-ache, indigestion, and gastroenteritis) along with diabetes, oedema, cold, arthritis, asthma, and traumatic injury [2]. *Litsea* is known for its essential oils, which have a protective action against several bacteria, possesses antioxidant and antiparasitic properties, exerts acute and genetic toxicity as well as cytotoxicity, and can even prevent several cancers [3, 4]. Previous phytochemical studies on the genus afforded flavonoids, terpenoids, alkaloids, butanolides, butenolactones, lignans, amides, steroids, and fatty acids [5]. The genus *Litsea* is represented by about 54 species in the flora of Malaysia. Of these, twenty species can be found in Peninsular Malaysia which are *L. costalis*, *L. trunciflora*, *L. magnifica*, *L. tomentosa*, *L.grandis*, *L. artocarpifolia*, *L. firma*, *L. gracilis*, *L. amara*, *L. polyantha*, *L. wrayi*, *L. cordata*, *L. glabrifolia*, *L. angulata*, *L. quercina*, *L. perakensis*, *L. machilifolia*, *L. panamonja*, *L. robusta*, and *L. johorensis* [6]. Tracing the current literature, nothing was found concerning the chemical composition on the essential oils of *L. costalis*, *L. machilifolia*, and *L. globularia* growing in Malaysia. *L. costalis* is locally known as *medang keladi,* grows in undisturbed mixed dipterocarp up to 100 m altitude [7]. *L. machilifolia* is an evergreen tree that can grow from 20-36 meters tall, known as *medang balong* [8]. However, no data was reported on *L. globularia*. As a continuation of our systematic studies on volatile oils from Malaysian plants [9-12], we aimed to investigate qualitatively and quantitatively the chemical composition of the essential oils by GC-FID and GC-MS analysis.

2. MATERIALS AND METHODS

2.1. PLANT MATERIALS

The fresh samples of *L. costalis* (SK27/19), *L. machilifolia* (SK36/19), *L. glubularia* (SK37/19) were collected from Behrang, Perak in January 2019. All samples were identified by Dr. Shamsul Khamis, a botanist from Universiti Kebangsaan Malaysia (UKM). The voucher specimens were deposited at UKMB Herbarium, Faculty of Science and Technology at UKM.

2.2. EXTRACTION OF ESSENTIAL OILS

The fresh leaves of each sample (300 g) were chopped into small pieces and thereafter subjected to hydrodistillation process in Clevenger-type apparatus for 4 hours. The essential oils obtained were then dried over anhydrous magnesium sulphate and stored at 4-6°C. The oil yield (%) is calculated based on the fresh weight (w/w). The oil yield for *L. costalis*, *L. machilifolia*, and *L. globularia* essential oils were 0.20%, 0.22% and 0.24% (w/w), respectively.

2.3. ANALYSIS OF ESSENTIAL OILS

Gas chromatography (GC-FID) analysis was performed on Shimadzu GC-2010 Plus gas chromatograph. Two types of capillary columns with different polarities were used HP-5MS or DB-Wax capillary column (30 m × 0.25 mm × 0.25 µm film thickness). 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).

GC-MS chromatograms were recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with HP-5 column. Helium was used as 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 250°C at 10°C/min and finally held isothermally for 15 min. For GC-MS detection, an electron ionisation system, with ionisation 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.

2.4. IDENTIFICATION OF CHEMICAL COMPONENTS

For the identification of the essential oil components, co-injection with the standards (major components) were used, together with correspondence of retention indices (relative to the retention times of *n*-alkanes from C_6 to C_{30}) and mass spectra with respect to those reported in Adams [13], NIST08 [14], and FFNSC2 [15] libraries. Semi-quantification of the essential oil components was undertaken by peak area normalisation considering the same response factor for all volatile components. Quantification was done by the external standard method using calibration curves generated by running the GC analysis of representative authentic compounds.

3. RESULTS AND DISCUSSION

A total of 44 volatile components were successfully identified through the hydrodistillation of fresh leaves of *L. costalis*, *L. machilifolia* and *L. globularia*. The percentages of each component are listed in Table I. The essential oil of *L. costalis* had 32 components with a percentage of 82.3%. The essential oil was characterised by a high concentration of sesquiterpene hydrocarbons (70.2%). The oil was demonstrated to be rich in β-caryophyllene (12.6%), δ-cadinene (12.1%), α-selinene (5.2%), α-amorphene (5.1%), α-cadinol (5.1%), and germacrene B (5.0%). In *L. machilifolia* essential oil, a total of 14 components were identified with the constitution of 85.5%. The oil was predominantly comprised of sesquiterpene hydrocarbons, constituting about 80.2% of the oil. The most abundant components were β-sesquiphellandrene (29.5%), β-bisabolene (19.7%), α-zingiberene (15.7%), α-bisabolol (4.5%), and (*Z*)-α-bisabolene (4.2%). As for *L. globularia*, the essential oil consisted of 28 components, representing 83.4% of the total oil. β-Caryophyllene (25.2%), δ-cadinene (15.8%), β-sesquiphellandrene (5.2%), and α-cadinol (5.2%) were identified as the main components in this oil. Based on the component analysis, ten components (isoledene, β-patchoulene, β-bourbonene, guaia-3,7-diene, γ-gurjunene, *cis*-β-guaiene, epizonarene, selinα-3,7(11)-diene, 1-*epi*-cubenol, and eudesm-7(11)-en-4-ol) were only found in *L. costalis* oil, whereas five components (italicene, α-*cis*-bergamotene, *epi*-β-santalene, (*Z*)-α-bisabolene, and (*E*)-γ-bisabolene) were only identified in *L. machilifolia* oil. Following a detailed examination of the chemical composition of the essential oils from the three *Litsea* species, all were identified to be rich in sesquiterpene hydrocarbons. On the other hand, oxygenated sesquiterpenes were in substantial amounts in *L. costalis*, *L. machilifolia* and *L. globularia* oils, which constituted 12.1%, 5.3% and 13.6%, respectively.

β-caryophyllene which is the major component in *L. costalis* and *L. globularia* oils is found in numerous edible plants that are ingested daily, and it is approved as a food additive by the Food and Drug Administration. This compound can change the inflammatory processes in humans through the endocannabinoid system [16]. Furthermore, this compound could increase the intracellular accumulation of anticancer agents, thereby potentiating their cytotoxicity due to the absorption of 5-fluorouracil across human skin. β-Caryophyllene facilitates the passage of paclitaxel through membranes and thus potentiates its anticancer activity [17]. Similar to our results,

Table I - Chemical components identified in *Litsea* essential oils

N.	Components	KI[a]	KI[b]	Percentage (%) [c]			Methods[d]
				LCLO	LMLO	LGLO	
1	α-Cubebene	1342	1463	1.3±0.1	0.4±0.2	0.2±0.1	RI, MS
2	Isoledene	1374	1459	3.2±0.2	-	-	RI, MS
3	α-Copane	1375	1495	0.8±0.1	-	0.1±0.1	RI, MS
4	β-Patchoulene	1380	1488	1.8±0.1	-	-	RI, MS
5	β-Bourbonene	1385	1528	0.4±0.1	-	-	RI, MS
6	β-Cubebene	1388	1558	0.4±0.1	-	2.5±0.2	RI, MS
7	β-Elemene	1390	1595	2.7±0.2	-	2.2±0.1	RI, MS
8	Italicene	1400	1557	-	0.7±0.1	-	RI, MS
9	Longifolene	1402	1575	0.4±0.1	-	1.0±0.1	RI, MS
10	α-Cedrene	1408	1585	0.2±0.1	1.2±0.1	-	RI, MS
11	α-*cis*-Bergamotene	1410	1601	-	0.9±0.1	-	RI, MS
12	β-Caryophyllene	1415	1605	12.6±0.2	-	25.2±0.2	RI, MS, Std
13	γ-Elemene	1434	1635	0.8±0.1	-	0.1±0.1	RI, MS
14	Aromadendrene	1440	1650	0.7±0.1	-	3.2±0.1	RI, MS
15	Guaia-3,7-diene	1442	1652	1.0±0.1	-	-	RI, MS
16	*epi*-β-Santalene	1445	1653	-	0.6±0.1	-	RI, MS
17	α-Humulene	1450	1660	2.4±0.2	-	2.4±0.2	RI, MS
18	(*E*)-β-Farnesene	1454	1665	-	3.0±0.1	0.2±0.1	RI, MS
19	γ-Gurjunene	1470	1668	1.2±0.1	-	-	RI, MS
20	α-Elemene	1475	1680	1.0±0.1	-	1.0±0.1	RI, MS
21	γ-Curcumene	1480	1690	-	0.5±0.1	0.1±0.1	RI, MS
22	α-Amorphene	1482	1692	5.1±0.2	-	1.8±0.2	RI, MS
23	β-Selinene	1490	1698	2.9±0.1	-	1.6±0.1	RI, MS
24	*cis*-β-Guaiene	1492	1702	0.7±0.1	-	-	RI, MS
25	α-Zingiberene	1495	1715	-	15.7±0.3	0.5±0.1	RI, MS, Std
26	α-Selinene	1490	1724	5.2±0.2	-	0.2±0.1	RI, MS
27	Epizonarene	1500	1702	0.8±0.1	-	-	RI, MS
28	β-Bisabolene	1505	1692	-	19.7±0.2	0.2±0.1	RI, MS, Std
29	(*Z*)-α-Bisabolene	1508	1730	-	4.2±0.1	-	RI, MS
30	β-Sesquiphellandrene	1520	1735	-	29.5±0.2	5.2±0.2	RI, MS, Std
31	δ-Cadinene	1522	1750	12.1±0.1	-	15.8±0.3	RI, MS, Std
32	(*E*)-γ-Bisabolene	1530	1773	-	3.8±0.1	-	RI, MS
33	α-Cadinene	1535	1725	2.2±0.2	-	0.1±0.1	RI, MS
34	Selinα-3,7(11)-diene	1545	1720	2.3±0.2	-	-	RI, MS
35	Germacrene B	1555	1816	5.0±0.1	-	2.4±0.1	RI, MS
36	(*E*)-Nerolidol	1560	1825	-	0.8±0.1	2.0±0.1	RI, MS
37	Germacrene D	1575	1875	3.0±0.2	-	3.8±0.2	RI, MS
38	Caryophyllene oxide	1580	1999	1.8±0.1	-	3.2±0.1	RI, MS
39	Globulol	1590	2060	1.1±0.1	-	2.0±0.1	RI, MS
40	1-*epi*-Cubenol	1625	2046	1.4±0.1	-	-	RI, MS
41	β-Eudesmol	1650	2245	1.7±0.1	-	1.0±0.1	RI, MS
42	α-Cadinol	1652	2211	5.1±0.2	-	5.2±0.2	RI, MS, Std
43	α-Bisabolol	1685	2153	-	4.5±0.2	0.2±0.1	RI, MS
44	Eudesm-7(11)-en-4-ol	1702	2241	1.0±0.1	-	-	RI, MS
	Group components						
	Sesquiterpene hydrocarbons			**70.2**	**80.2**	**69.8**	
	Oxygenated sesquiterpenes			**12.1**	**5.3**	**13.6**	
	Identified components (%)			**82.3**	**85.5**	**83.4**	

LCLO - *L. costalis* oil; LMLO - *L. machilifolia* oil; LGLO - *L. globularia* oil; RT – retention time

[a]Linear retention index on HP-5MS column, based on comparison with those reported in Adams [13]

[b]Linear retention index on DB-Wax column, based on comparison with those reported in NIST08 [14] and FFNSC2 [15]

[c]Relative percentage values are means of three determinations ±SD

[d]Identification methods: Std, based on comparison with authentic compounds; MS, based on comparison with Wiley, Adams, FFNSC2, and NIST08 MS databases; RI, based on comparison of calculated RI with those reported in Adams, FFNSC2 and NIST08

β-caryophyllene has been reported to be the major component in other *Litsea* essential oils, which are *L. deccanensis* (India: leaf oil 51.8%) [18], *L. glaucescens* (Mexico: leaf oil 21.4%) [19], *L. coreana* (China: leaf oil 15.7%) [20], *L. helferi* (Vietnam: leaf oil 14.2%) [21], *L. japonica* (Japan: seed oil 17.6%, mesocarp oil 22.9%) [22], and *L. quinqueflora* (India: leaf oil 13.3%) [18]. On the other hand, δ-cadinene that was also noticed to be dominant in the studied *Litsea* oils, has been indicated as to inhibit the growth of ovarian cancer cells via caspase-dependent apoptosis and cell cycle arrest, alongside its larvicidal activities against vectors transmitting malaria, dengue, and filariasis [23]. Meanwhile, several studies have reported monoterpenes as the foremost components from the essential oils of *L. verticillata* (linalool 23.4%) [21], *L. schaffneri* (1,8-cineole 23.7%) [24], *L. kostermanin* (α-pinene 40.0%) [25], *L. glutinosa* ((*E*)-β-ocimene 70.8%) [26], *L. akoensis* (β-phellandrene 43.7%) [27] and L. acutivena (α-phellandrene 30.4%) [28]. However, in this study, no monoterpenes were found in all *Litsea* essential oils. The chemical differences among *Litsea* species may depend on the extraction procedure, season, stage of development, and the distinct habitat from which the plant was collected [9].

4. CONCLUSIONS

The chemical composition of the essential oils of *L. costalis*, *L. machilifolia*, and *L. globularia* plants growing in Malaysia was investigated for the first time using the GC-FID and GC-MS method. To unravel the full therapeutic potential of *Litsea* species, the pharmacological investigations should be performed. This study also provides valuable and useful information and indications for further exploring of the potential nutraceutical and pharmaceutical applications of the genus *Litsea*.

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