ACADEMIA JOURNAL OF BIOLOGY 2023, 45(4): 83–92
DOI: 10.15625/2615-9023/18944

CHEMICAL CONSTITUENTS OF SOME ESSENTIAL OILS BEARING PLANTS GROWING WILD IN VIETNAM

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Received 18 September 2023; accepted 25 December 2023

ABSTRACT

The essential oils of three plants were isolated by hydrodistillation and their constituents were analyzed by gas chromatography-mass spectrometry/flame ionization detection (GC/MS-FID) method. 29 compounds representing 99.00% of the total oil of inflorescences of *Zingiber pellitum* were identified, consisting mainly of *α*-pinene (5.34%), *β*-caryophyllene (39.13%) and *α*-humulene (36.69%). For *Basilicum polystachyon*, 41 and 37 components were identified in the leaf and flower oils, accounting for 91.32% and 92.44% of the total oils, respectively. Germacrene D (16.33% and 16.32%), germacrene B (14.95% and 16.23%), *α*-cubebene (13.38% and 19,04%), *α*-copaene (5.81% and 5.77%), *δ*-cadinene (4.78% and 4.18%), β-cis-elemene (4.34% and 4.66%), and **γ**-elemene (4.31% and 4.72%) were the major constituents in both oils. For the leaves of *Litsea bavienes*, 40 components were identified, representing 91.73% of the total oil contents, in which hepten-3-one (70.46%) was the most abundant compound in this oil. The chemical constituents of essential oils from *Z. pellitum* (inflorescences), *B. polystachyon* (leaves and flowers) and *L. baviensis* (leaves) were being reported for the first time.

Keywords: *Zingiber pellitum*, *Basilicum polystachyon*, *Litsea baviensis*, hydrodistillation, essential oil.

Citation: Nguyen Phuong Hanh, Nguyen Thi Hien, Nguyen Quoc Binh, Dinh Thi Thu Thuy, 2023. Chemical constituents of some essential oils bearing plants growing wild in Vietnam. *Academia Journal of Biology*, 45(4): 83–92. https://doi.org/10.15625/2615-9023/18944

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INTRODUCTION

This study aimed to identify the contents and the chemical composition of the essential oils from three plants, as part of our continued interest in the phytochemical investigation of the flora of Vietnam (Binh et al., 2021; Hanh et al., 2022a, 2022b, 2022c, 2023a, 2023b; Thai et al., 2012). *Zingiber pellitum* Gagnep. (Vietnamese name: Gừng bọc da) is one of the eight *Zingiber* species known to produce terminally flowering inflorescences, belongs to the Zingiberaceae family. The species was found growing under the canopy on moist soils. The plant was distributed in Vietnam and Laos. Previous analysis of the rhizome essential oil was found to be rich in terpinen-4-ol (35.9%), p-cymene (19.8%), and sabinene (7.1%) (Giang et al., 2011), while the aerial parts consist mainly of β-pinene (5.32%), caryophyllene (23.45%) and caryophyllene oxide (4.00%) (Thien et al., 2022*)*. They were remarkable, considering that *Z. pellitum*, apart from the rhizomes and aerial parts, no other reports exist on the essential oil constituents of inflorescences (Hanh et al., 2023b).

Basilicum polystachyon (L.) Moench is an annual medicinal herb distributed mostly in the tropical areas of Asia, Africa and Australia (Ban, 2003; Phuong, 2000; Vinay et al., 2018). It is the only species of the genus *Basilicum*, which belongs to the family Lamiaceae. *Moschosma polystachyon* is one of its synonyms. As a medicinal plant, *B. polystachyon* is used in India, Indonesia, East Africa, Kenya, Nigeria, and Ghana to treat rheumatism, neuropathy, convulsion, sores in the mouth, and as a sedative (Thoppil, 1997; Phuong, 2000). According to a limited number of studies to date, *B. polystachyon* contains essential oil and phytochemicals, which showed antibacterial, antifungal, cytotoxic, antioxidant, and mosquitocidal activities (Cui et al., 2017; Chakraborty et al., 2007; Thoppil, 1997; Rajkumar et al., 2004; Madhavan et al., 2013; Tan et al., 2019). The plant is known in Vietnamese name as Thiến thảo, É sạ, Ba si lích, Phòng phong thảo, Thổ hoắc hương. The species was found growing in open places on moist soils in the provinces Lang Son, Thai Nguyen, Ha Noi, Nam Dinh, Thua Thien Hue, Khanh Hoa, Ba Ria - Vung Tau, Dong Thap, An Giang, Can Tho, and Ho Chi Minh City (Ban, 2003; Phuong, 2000). We recently also found this species growing wild in Lai Chau province, which was an addition to its distribution areas in Vietnam. In the present study, we report on the chemical composition of essential oils obtained from the leaves and the flowers of *B. polystachyon* collected in Lai Chau province. According to our knowledge, this is the first investigation on essential oils from this plant species in Vietnam.

Litsea baviensis Lecomte, which belongs to the family Lauraceae. The plant was found in China, Thailand and Vietnam (Ban, 2003; Wu et al., 2008). In Vietnam, this species has been recorded in Lao Cai, Son La, Ha Noi, Ninh Binh, Thanh Hoa, Nghe An, Khanh Hoa provinces (Vietnamese as Bời lời Ba Vì). Notably, in the present study, this plant also was added distribution area in Vietnam (Dak Nong province). On the other hand, remarkably, considering that *L. baviensis* was discovered over a century ago but no reports on the phytochemistry of this plant, apart from the taxonomy and *L. baviensis* extracts were screening for antiviral activities (Yifu et al., 2016). Therefore, this is the first study on the chemical constituents of essential oil from leaves of *L. baviensis*.

MATERIALS AND METHODS

Plant materials

The inflorescences of *Z. pellitum* were collected from Binh Chau Phuoc Buu Nature Reserve, Ba Ria - Vung Tau province in 2023. The samples of *B. polystachyon* were collected from Lai Chau province and *L. baviensis* were collected from Dak Nong province, Vietnam in 2019. Voucher specimens were BCPB2023, LC2019 and DN2019 respectively, and were deposited at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

Isolation of essential oils

The fresh inflorescences of *Z. pellitum* (550 g), leaves (960 g) and flowers (500 g) of *B. polystachyon* and leaves of *L. baviensis* (1,500 g) were used for isolation of the essential oils by hydrodistillation for 3.5 hours at normal pressure using a Clevenger type apparatus. The essential oils were dried over anhydrous sodium sulfate, stored in dark glass vials and kept under refrigeration at 4 ^{o}C before analysis. The yield of the essential oil was calculated on a dry weight basis.

Gas chromatography/Mass spectrometry, flame ionization detector (GC/MS-FID) analysis

The correct analysis of the essential oils was carried out on an Agilent Technologies HP7890A GC equipped with a mass spectrum detector (MSD) Agilent Technologies HP5975C and an HP5-MS column (60 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies). The injector and detector temperature was set at 250 ^{o}C and 280 ^{o}C respectively. The column temperature progress initiated at 60 ^{o}C, followed by an increase to 240 ^{o}C at 4 ^{o}C/min. The carrier gas was Helium at a flow rate of 1 mL/min. Samples were injected by splitting. The split ratio was 100:1. The volume injected was 1 µL of essential oils. The MSD conditions were as follows: ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range 35–450 amu under full scan. A homologous n-alkane series was used as the standard to calculate the retention time indices (RI) of each component. MassFinder 4.0 software connected to the HPCH1607, W09N08 libraries, and the NIST Chemistry WebBook were used to match mass spectra and retention indices. To confirm these results, a further comparison was made with data from authentic compounds reported in the original literature.

The quantitative analysis of the essential oils was carried out on an Agilent Technologies HP7890A GC equipped with a flame ionization detector (FID) Agilent Technologies and an HP5-MS column (60 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies) with same conditions above. The relative amounts of individual components were calculated based on the GC peak area (FID response) without correction.

RESULTS AND DISCUSSION

By hydrodistillation, essential oils from the fresh inflorescences of *Z. pellitum*, from leaves and flowers of *B. polystachyon* and leaves of *L. baviensis* were obtained in a yield of 0.20%, 0.1%, 0.08% and 0.1%, respectively (v/w, calculated on a dry weight basis). All oils were light yellow liquids having lower densities than water.

By GC-MS analysis, 29, 41, 37 and 40 components were identified and quantified in the essential oils of *Z. pellitum* (inflorescences), *B. polystachyon* (leaves and flowers) and *L. baviensis* oils (leaves). These accounted for 99.0%, 91.32%, 92.44% and 91.73% of the essential oil contents, respectively (Tables 1, 2, 3).

The data from Table 1 show that, the oils of *Z. pellitum* inflorescences contained monoterpene hydrocarbons (8.86.4%), oxygenated monoterpenes (0.66%), sesquiterpene hydrocarbons (85.14%), oxygenated sesquiterpenes (4.13%), aromatic compounds (0.55%) and others (0.13%). The main compounds in inflorescences essential oil were β-caryophyllene (39.13%), α-humulene (36.69%) and germacrene D (5.18%). Some other compounds such as safrole (0.28%), chavibetol (0.27%), pentadecanal (0.13%) were also detected in sizeable amounts. These results were compared with data available on the rhizomes and aerial parts essential oils previously reported from Vietnam (Giang et al., 2011; Thien et al., 2022) showing that the essential oils of this plant exhibited chemical diversity. The present essential oil samples contained larger amounts of sesquiterpene (85.14%) while the rhizome oil from this plant was reported to be rich of monoterpenoids (80.1%) including terpinen-4-ol (35.9%), p-cymene (19.8%), and sabinene (7.1%) as major constituents. In addition, terpinen-4-ol and p-cymene from the rhizomes oil (Giang et al., 2011) were not found in inflorescences essential oil. Cyclofenchene (11.31%), 3-carene (6.21%), sylvestrene (5.35%), and zerumbone (4.99%) presented in the aerial parts (Thien et al., 2022) were not found in this studied essential oil. Conversely, several other constituents were found in the inflorescence but absent in the aerial parts such as safrole, α-humulene and germacrene D. Thus, different parts of this species were diverse in chemical compositions.

Table 1. Chemical composition of the essential oil from the inflorescences of *Zingiber pellitum*

No.	RI	Compounds	Content (%)
1	938	α-pinene	2.60
2	954	Camphene	0.24
3	977	Sabinene	0.16
4	983	β-pinene	5.34
5	991	Myrcene	0.26
6	1033	Limonene	0.26
7	1102	Linalool	0.40
8	1175	Borneol	0.26
9	1297	Safrole	0.28
10	1365	Chavibetol	0.27
11	1388	α-copaene	0.25
12	1402	cis-β-elemene	0.56
13	1438	β-caryophyllene	39.13
14	1472	α-humulene	36.69
15	1489	γ-muurolene	0.20
16	1490	Ar-curcumene	0.26
17	1497	Germacrene D	5.18
18	1502	α-zingiberene	0.87
19	1512	Bicyclogermacrene	0.79
20	1533	β-sesquiphellandrene	0.65
21	1536	δ-cadinene	0.23
22	1569	E-nerolidol	0.33
23	1596	Spathulenol	0.16
24	1603	Caryophyllene oxide	1.44
25	1618	Humulene epoxide I	0.25
26	1630	Humulene Epoxide II	1.27
27	1656	Caryophylla-3(15),7(14)-dien-6-ol	0.35
28	1672	α-cadinol	0.22
29	1716	Pentadecanal	0.13
Total			99.00
Monoterpene (1-6) (monoterpene hydrocarbons)			8.86
Monoterpenoid (7,8) (oxygenated monoterpenes)			0.66
Sesquiterpene (11-22) (sesquiterpene hydrocarbons)			85.14
Sesquiterpenoid (23-28) (oxygenated sesquiterpenes)			4.13
Aromatic compound (9,10)			0.55
Others (29)			0.13

The data in Table 2 further showed that the main classes of compounds identified in the leaf and flower essential oil of *B. polystachyon* were sesquiterpene hydrocarbons (86.54% and 88.52%, respectively). Oxygenated monoterpenes (0.47% and 0.30%) and oxygenated sesquiterpenes (4.31% and 3.62%) were presented only in small amounts in both oils. The main components (those with percentages higher than 3.0%) of both leaves and flower oils were relatively similar, but the contents of these compounds were a little different, for example, germacrene B (14.95% and 16.23%), *α*-cubebene (13.38% and 19.04%), germacrene D (16.33% and 16.32%), *α*-copaene (5.81% and 5.77%), *δ*-cadinene (4.78% and 4.18%), *β*-*cis*-elemene (4.34% and 4.66%), and *γ*-elemene (4.31% and 4.72%). Thus, both essential oils obtained from the leaves and the flowers of the Vietnamese *B. polystachyon* were similar in chemical composition and characterized by the dominance of sesquiterpene hydrocarbons with *α*-cubebene as the major compound (13.38% and 19.04%, respectively). This report was the first phytochemical study of the leaf and flower essential oils from *B. polystachyon*.

Table 2. Chemical constituents found in the leaf and flower oils of *Basilicum polystachyon*

No.	RI	Compounds	Content (%)	
			Leave	Flower
1	979	1-octen-3-ol	0.28	0.14
2	1103	Linalool	0.19	0.16
3	1348	δ-elemene	0.37	0.33
4	1361	α-cubebene	13.38	19.04
5	1382	Cyclosativene	0.50	0.15
6	1389	α-copaene	5.81	5.77
7	1400	β-bourbonene	0.64	0.42
8	1402	β-cubebene	1.40	2.34
9	1404	β-cis-elemene	4.34	4.66
10	1425	α-gurjunene	0.75	0.32
11	1434	β-copaene	0.18	0.13
12	1437	(*E*)-caryophyllene	0.87	0.84
13	1445	γ-elemene	4.31	4.72
14	1451	α-guaiene	1.40	1.12
15	1456	Guaia-6,9-diene	0.61	0.39
16	1461	Allo-aromadendrene	0.27	-
17	1471	α-humulene	0.47	-
18	1479	(*E*)-9-epi-caryophyllene	0.59	-
19	1488	δ-patchoulene	0.36	-
20	1490	γ-muurolene	0.76	0.50
21	1494	α-amorphene	0.38	0.25
22	1499	Germacrene D	16.33	16.32
23	1505	β-selinene	1.90	1.55
24	1511	γ-amorphene	0.89	0.83
25	1514	Bicyclogermacrene	2.42	1.55
26	1521	α-bulnesene	1.57	1.43
27	1530	γ-cadinene	0.92	0.67
28	1533	7-epi-α-selinene	0.98	1.06
29	1537	δ-cadinene	4.78	4.18

No.	RI	Compounds	Content (%)	
			Leave	Flower
30	1548	trans-cadina-1,4-diene	0.97	1.06
31	1551	(*E*)-α-bisabolene	1.04	1.07
32	1561	Selina-3,7(11)-diene	2.39	1.59
33	1561	Elemol	1.52	1.54
34	1565	Germacrene B	14.95	16.23
35	1594	Scapanol	0.29	0.31
36	1598	Spathulenol	0.35	0.18
37	1627	Copaborneol	1.30	0.78
38	1647	1-epi-cubenol	0.17	0.20
39	1659	epi-α-cadinol	0.14	0.10
40	1661	epi-α-muurolol	0.17	0.16
41	1675	α-cadinol	0.38	0.35
Total			91.32	92.44
Monoterpenoid (1,2) (oxygenated monoterpenes)			0,47	0,30
Sesquiterpene (3-33) (sesquiterpene hydrocarbons)			86,54	88,52
Sesquiterpenoid (34-41) (oxygenated sesquiterpenes)			4,31	3,62

Our literature search resulted in only two reports on volatile compounds obtained from *B. polystachyon* growing in India and China (Cui et al., 2017; Thoppil, 2017). The first study was based on the essential oils obtained from the leaves and flowers of *B. polystachyon* cultivated at the Genetics and Plant Breeding Division, Department of Botany, University of Calicut, Kerala, India. The oil yield of leaves and flowers by hydrodistilation was 0.6% (dry weight). The oil contains 7.4% monoterpene hydrocarbons, 13.5% oxygenated monoterpenes, 17.7% sesquiterpene hydrocarbons, and 47.7% phenylpropanoids (39.3% methyl eugenol, 8.4% methyl isoeugenol). The main components of the monoterpene hydrocarbons was limonene (7,4%); of the oxygenated monoterpenes were 1,8-cineole (5,3%), citronellal (3,5%), isobornyl acetate (1,8%), and geranyl acetate (2,9%); of the sesquiterpene hydrocarbons were *β*-elemene (5,1%), *β*-caryophyllene (4,8%), *β*-selinene (3,8%), *α*-humulene (2,4%), and *γ*-cadinene (1,6%); and of the phenylpropanoids were methyl eugenol (39,3%) and methyl isoeugenol (8,4%) (Thoppil et al., 1997). The significant contents of methyl eugenol and methyl isoeugenol (phenylpropanoids) were characteristic of this oil. The second work investigated an essential oil of this plant collected in August 2011 from Sanya, Hainan, China. Hydrodistillation of the dry powder gave an essential oil with a 2.6% yield. By GC-MS analysis, 64 compounds have been identified, accounting for 99.75% of the total peak area. The oil was dominated by sesquiterpenes, followed by diterpenes and monoterpenes. No phenylpropanoids were detected in this analysis. The major components of the oil were ylangene (33.43%), epiglobulol (31.52%), copaene (6.14%), verticiol (5.95%), caryophyllene oxide (3.01%), limonene (2.93%), caryophyllene (2.13%), α-pinene (1.93%), and muurola-4,9-diene (1.52%). The sesquiterpene hydrocarbon ylangene (33.43%) and the oxygenated sesquiterpene epiglobulol (31.52%) were characteristic constituents of this oil (Cui et al., 2017).

Comparing the above data with the data obtained in this study, it is obvious that all the oils showed differences, not only in oil yield but also in oil compositions. The oil yields of the plant samples from Vietnam were much lower than those of the others. As for the oil compositions, the Indian oil was remarkably different from the others by containing phenylpropanoid compounds as major constituents. The Vietnamese oil was qualitatively more similar to the Chinese oil by the absence of phenylpropanoids and by the dominance of sesquiterpene compounds. It differed from the Chinese oil by containing mainly sesquiterpene hydrocarbons, while the Chinese oil contained both sesquiterpene hydrocarbons and oxygenated sesquiterpenes with nearly the same percentages. In our opinion, there was still little data to make any conclusion about the cause of these differences and similarities.

From Table 3, it is clearly seen that 40 constituents of essential oil from the leaves of *L. baviensis* belong to oxygenated monoterpenes (79.61%), sesquiterpene hydrocarbons (3.08%), oxygenated sesquiterpenes (6.98%), diterpenoid (1.68%) and aromatic esters (0.38%). The main constituents of the essential oil were 1-hepten-3-one (70.46%), linalool (3.31%), phytol (1.68%), β-caryophyllene (1.48%), and minor components (less than 1.0%). In the present study, the chemical composition of the essential oil from *L. baviensis* leaf was reported for the first time. However, the compositions of essential oils from some Litsea species have been added as reference. But, remarkably considering that *L. baviensis* content 1-hepten-3-one up to 70.46% which has not been found in other Litsea species oils in Vietnam.

Table 3. Chemical constituents found in the leaves of *Litsea baviensis*

No.	RI	Compounds	Content (%)
1	851	(2E)-hexenal	0.19
2	854	(3Z) -hexenol	0.36
3	863	(Z)-hex-2-en-1-ol	1.92
4	981	1-hepten-3-one	70.46
5	987	(3)-octanone	0.31
6	996	(3)-octanol	0.83
7	1014	(3Z)-hexenyl acetate	0.13
8	1071	(n)-octanol	0.16
9	1103	Linalool	3.31
11	1200	α-terpineol	0.86
12	1204	Methyl salicylate	0.38
13	1233	Nerol	0.29
14	1257	Geraniol	0.59
15	1265	(2E)-decenal	0.20
16	1403	(cis)-b-elemene	0.12
17	1437	β-caryophyllene	1.48
18	1453	Striatene	0.17
19	1471	α-humulene	0.30
20	1496	(E)-β-ionone	0.30
21	1512	(E,E)-α-farnesene	0.61
22	1537	δ-cadinene	0.22
23	1565	Occidentalol	0.33
24	1570	E-nerolidol	0.87
25	1598	Spathulenol	0.26
26	1605	Caryophyllene oxide	0.49
27	1614	Guaiol	0.23
28	1627	Cedrol	0.75

No.	RI	Compounds	Content (%)
29	1647	1-epi-cubenol	0.20
30	1651	γ-eudesmol	0.31
31	1658	(E,E)-germacradiene-11-ol	0.76
32	1661	epi-α-muurolol	0.26
33	1672	β-eudesmol	0.26
34	1673	α-cadinol	0.60
35	1675	α-eudesmol	0.20
36	1677	neo-intermedeol	0.55
37	1691	Caryophyllene	0.18
38	1697	epi-α-bisabolol	0.16
39	1699	α-bisabolol	0.23
40	1848	6,10,14-trimethylpentadecan-2-one	0.22
41	2117	Phytol	1.68
Total			91.73
Monoterpenoid (1- 11, 13-15)			79.61
Sesquiterpene (16-19, 21,22,37)			3.08
Sesquiterpenoid (10, 23-36, 38-40)			6.98
Diterpenoid (41)			1.68
Aromatic compounds (12)			0.38

Note: RI: Retention indices.

CONCLUSION

The present study provides the first information on the essential oil chemical compositions of three plants growing wild in Vietnam.

The chemical components of the inflorescences of Z. pellitum oil were dominated by β-caryophyllene (39.13%) and α-humulene (36.69%). The main compounds in the leaf and flower oils of B. polystachyon were germacrene D (16.32% and 16.32%), α-cubebene (13.38% and 19.04%), germacrene B (14.95% and 16.23%), α-copaene (5.77% and 5.81%). From the essential oil of L. baviensis, 1-hepten-3-one (70.46%) was found as the major compound. Comparison with the literature data shows that three plants of Vietnamese flora were diverse in chemical compositions.

Acknowledgements: This work is funded by the Vietnam Academy of Sciences and Technology via project number VAST04.05/23–24.

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