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Article

Chemical Constituents of the Leaf Essential Oil of *Vitex axillariflora* (Merr.) Bramley from Vietnam

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Abstract: Essential oil from the leaves of *Vitex axillariflora* (Merr.) Bramley distributed in Sao La Nature Reserve - Quang Nam was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). The main chemical constituents of the essential oil were (*E*)-β-caryophyllene (54.3 %), α-humulene (15.6 %), bicyclogermacrene (7.9 %) and α-copaene (3.3 %). For the first time, the chemical composition of *Vitex axillariflora* (Merr.) Bramley was studied.

Keywords: Lamiaceae, *Vitex axillariflora,* essential oil, (*E*)-β-Caryophyllene.

Introduction

Since 2009, the genus *Tsoongia* was merged into the genus *Vitex* of the family Lamiaceae [1], hence the species *Tsoongia axillariflora* Merr. became the synonym of *Vitex axillariflora* (Merr.) Bramley. *V. axillariflora* is distributed in Myanmar, China and Vietnam [2]. The leaves are borne from one to three leaflets, which are oval or oblong, upper surface smooth, lower surface sparsely hairy and glandular; lateral tendons 5-7 pairs. Inflorescences grow in leaf axils, small bracts, bell-shaped calyx. The corolla is pale yellow. Fruit is black-brown when mature, nearly ovoid, shiny, sparsely glandular [3]. In folk medicine, the roots are used to treat tuberculosis, the whole plant is used to treat hepatitis, and the leaves are

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used to treat scabies [4]. This report aims to provide data on the essential oil of *Vitex axillariflora* distributed in Sao La Nature Reserve - Quang Nam.

Materials and methods

Plant material

Leaves (2.0 kg) of the species *Vitex axillariflora* (Merr.) Bramley were collected in Sao La Nature Reserve, Quang Nam Province (15°572 483 N 107°312 063 E, 613 m elevation) in September 2019. The specimen (DND787) of this species was identified by Dr. Do Ngoc Dai and Associate Professor Le Thi Huong stored in the plant sample room, laboratory practice center, Vinh University.

Isolation of essential oil

The fresh leaves (0.5 kg), immediately after collection, were shredded and hydrodistilled for 4 h using a Clevenger type apparatus (Witeg Labortechnik, Wertheim, Germany), and were performed in triplicate. The mass of essential oil obtained for each replicate was 0.765, 0.946 and 0.985 g, respectively. The essential oil was dried over anhydrous Na_2SO_4 and stored in a sealed glass vial at 4°C until analyzed.

Gas chromatography-Mass spectrometry

The essential oil of *Vitex axillariflora* was analyzed by gas chromatographic (GC)-mass spectrometry (MS) using a Shimadzu GCMS-QP2010 Ultra (Shimadzu Scientific Instruments, Columbia, Maryland, USA) operated in the electron impact mode (electron energy = 70 eV, scan range = 40-400 atomic mass units, scan rate = 3.0 scans/s), and using GC-MS solution software. The GC column was a ZB-5ms fused silica capillary column (Phenomenex®, Torrance, CA, USA, 60 m length, 0.25 mm diameter) with a (5 % phenyl)-polymethylsiloxane stationary phase and a film thickness of 0.25 μm. The carrier gas was helium with a column head pressure of 208.3 kPa and a flow rate of 2.0 mL/min. The injector temperature was 260°C and the ion source temperature was 260°C. The GC oven temperature program was programmed for 50°C initial temperature and the temperature was increased at a rate of 2°C/min to 260°C. A 5 % w/v solution of the sample in CH_2Cl_2 was prepared, and 0.1 μL of the sample was injected using a splitting mode (25:1).

Identification of the oil components was based on their retention indices determined by reference to a homologous series of *n*-alkanes and by comparison of their mass spectral fragmentation patterns with those reported in the databases [5-8]. Quantification done was done by external standard method. Calibration curves of representative compounds from each class were drawn and used for quantification.

Results and discussion

The yield of essential oil distillation reached 0.18 % (w/w) of fresh leaf mass. The essential oil was colorless and lighter than water. The results of the essential oil analysis are presented in Table 1. The essential oil of the leaves of *Vitex axillariflora* was dominated by sesquiterpene hydrocarbons (90.5 %), the main chemical constituents of which were (*E*)-β-caryophyllene (54.3 %), α-humulene (15.6 %) and bicyclogermacrene (7.9 %). To the best of our knowledge, this is the first time that the results of the chemical composition analysis of *Vitex axillariflora* leaf essential oil have been presented.

(*E*)-β-Caryophyllene, has shown insecticidal activities against *Aedes aegypti* [9], *Rhyzopertha dominica*, *Sitophilus oryzae, Tribolium castaneum* [10], *Lycoriella ingénue* [11], *Aphis gossypii* [12] and *Tyrophagus putrescentiae* [13]. (*E*)-β-Caryophyllene has also shown acaricidal activities against *Dermatophagoides farinae* and *D. pteronyssinus* [14], *Dermanyssus gallinae* and *Hyalomma dromedarii* [15]. The essential oil of *Lantana montevidensis*, whose main component was (*E*)-β-caryophyllene, has shown acaricidal activity against *Rhipicephalus microplus* [16]. Furthermore, (*E*)-β-caryophyllene has shown toxicity against the spider mite *Tetranychus urticae* [17]. The essential oil of *Psidium guajava* containing 39.0 % (*E*)-β-caryophyllene demonstrated acaricidal activity against *Rhipicephalus microplus* [18]. Thus, the traditional use of *V. axillariflora* leaves to treat scabies may be due to the main component (*E*)-β-caryophyllene. In addition, a synergistic effect between the ingredients may also play an important role.

Table 1. Chemical composition of essential oil from leaves of *Vitex axillariflora*

No.	Compound	RT (min)	RI_{calc}	RI_{db}	Concentration %
1	α-Pinene	12.5	935	932	0.5
2	β-Pinene	15.0	979	978	0.2
3	Bicycloelemene	38.6	1332	1334	0.4
4	**α-Copaene**	41.4	1376	1375	**3.6**
5	β-Elemene	42.3	1390	1390	2.8
6	**(*E*)-β-Caryophyllene**	44.2	1419	1417	**54.3**
7	**α-Humulene**	46.5	1455	1453	**15.6**
8	Germacrene D	48.1	1481	1480	2.5
9	β-Selinene	48.6	1489	1489	1.5
10	**Bicyclogermacrene**	49.0	1495	1497	**7.9**
11	δ-Cadinene	50.3	1518	1518	1.9
12	Spathulenol	53.8	1576	1576	0.5
13	Caryophyllene oxide	54.1	1581	1577	1.5
14	Humulene epoxide I	55.1	1598	1594	0.2
15	Humulene epoxide II	55.8	1609	1613	2.0
16	Selin-6-en-4β-ol	56.2	1617	1624	0.4
17	Caryophylla-4(12),8(13)-dien-5α-ol	57.1	1632	1630	1.4
18	Caryophylla-4(12),8(13)-dien-5β-ol	57.3	1637	1636	0.5
19	α-Eudesmol	58.3	1655	1655	0.9
20	Selin-11-en-4α-ol	58.5	1658	1658	1.4
	Monoterpene hydrocarbons (Sr. Nos. 1, 2)				0.7
	Sesquiterpene hydrocarbons (Sr. Nos. 3-11)				90.5
	Oxygenated sesquiterpenoids (Sr. Nos. 12-20)				8.8
	Total identified				100.0

RT = Retention Time (min)

RI_{calc}= Retention Indices determined with respect to a homologous series of *n*-alkanes on a ZB-5ms column

RI_{db}= Retention Indices from the database

Conclusion

The chemical composition of the essential oil from the leaves of *Vitex axillariflora* was dominated by (*E*)-β-caryophyllene (54.3 %). In addition to insecticidal and acaricidal effects, (*E*)-β-caryophyllene has also shown local anesthetic, anticarcinogenic, antioxidant, antibiotic, antiinflammatory, anxiolytic and anti-alcoholic, antidepressant, neuroprotective, and antinociceptive activities [19]. Therefore, essential oil from the leaves of *V. axillariflora* may be an important source of (*E*)-β-caryophyllene.

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Conflict of interest

The authors declare no conflict of interest.

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