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Zingiber vuquangensis and *Z. castaneum*: Two Newly Discovered Species from Vietnam and Their Essential Oil Constituents

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The chemical constituents of essential oils obtained by hydrodistillation from *Zingiber vuquangensis* Lý N.S., Lê T.H., Trịnh T. H., Nguyễn V.H., Đỗ N.Đ. and *Zingiber castaneum* Škorničk. & Q.B. Nguyễn collected from Vu Quang National Park, Hà Tĩnh Province, Vietnam were analysed by GC and GC-MS. β-Pinene (24.7% and 26.1%) and β-caryophyllene (12.3% and 13.9%) were the main constituents in the leaf oil and stem oil of *Zingiber vuquangensis*. On the other hand the root oil contained bornyl acetate (20.9%), zerumbone (14.1%) and α-humulene (9.6%) while β-pinene (19.6%), 1,8-cineole (15.6%), α-pinene (10.3%) and β-caryophyllene (10.4%) were the significant compounds of the fruit oil. The leaf oil *Zingiber castaneum* was dominated by β-pinene (30.6%), α-pinene (9.5%), β-caryophyllene (9.4%) and bicycloelemene (9.1%). The compounds occurring in higher quantity in the stem oil were β-caryophyllene (14.7%), δ-cadinene (9.8%), bicycloelemene (8.4%) and α-cubebene (7.8%). However, camphene (15.1%), 1,8-cineole (13.6%), linalool (11.3%) and δ-3-carene (8.5%) were the main compounds of the root oil while (*E*)-nerolidol (23.2%), (*Z*)-9-octadecenamide (17.3%) and β-caryophyllene (10.8%) were the main constituents of the fruit oil. The essential oil did not exhibit noticeable antimicrobial effects. This is the first report on the volatile compositions of *Z. vuquangensis* and *Z. castaneum*.

Keywords: *Zingiber vuquangensis*, *Zingiber castaneum*, monoterpenes, sesquiterpenes.

Zingiber Miller (Zingiberaceae) is distributed in tropical to warm-temperate Asia with the highest diversity in the monsoonal parts of Asia. It is considered the largest genus in subfamily Zingiberoideae with more than 200 names corresponding to approximately 100–150 species [1a]. The genus is traditionally divided into four sections based on the position of the inflorescence. *Zingiber vuquangensis* was so far recorded from two places in northern Vietnam, Vũ Quang NP, Hà Tĩnh Province, and Phong Nha-Kẻ Bàng NP, Quảng Bình Province, with a distance of about 120 km between two localities. The plant is a perennial rhizomatous herb that grows up to 0.6-1.8 m tall. The rhizome is fleshy and compact, dull brown externally while tinted pinkish-white internally. The leaf sheaths are reddish to tinted green-red. The flowers are translucent white externally. The seeds are irregular and pink-red which bears 16–20 seeds per fruit. The plant was observed to flower in April to May and fruit in May to August [1a]. *Zingiber castaneum* is easily recognized among other terminally flowering species by its upright inflorescence with reflexed bracts. The plant is also a rhizomatous herb forming small clumps. The creeping aromatic rhizome which grows up to 1.5 cm in diameter is externally light brown and internally cream white [1c]. The translucent light green leaves are glabrous. Flowering starts in July and extends to September. It was found growing in Ninh Bình Province.

Both *Z. vuquangensis* and *Z. castaneum* are newly described species [1b,c], nothing is known about their medicinal uses as well as the chemical constituents and biological effects. In the present paper, the compounds identified in the essential oils from various parts of both *Z. vuquangensis* and *Z. castaneum* grown in Vietnam were reported. However, previously, the chemical compounds identified in the essential oils of some other Zingiber plant grown in Vietnam were reported [2a-c]. Although, terpene compounds were prominent in these essential oils, the identities of the compounds differ from one species to another.

The yield of essential oils for the leaf, stem, root and fruit of *Z. vuquangensis* were 0.20%, 0.31%, 0.35% and 0.51 (v/w, respectively) while those from *Z. castaneum* were obtained respectively in yields of 0.15%, 0.12%, 0.24% and 0.21% (v/w), calculated on a dry weight basis. All the eight oil samples were light yellow in colouration. The chemical compounds present in the essential oils were shown in Table 1. Forty-four representing 91.5% of the volatile constituents were identified in the leaf oil of *Z. vuquangensis*. This was represented mainly by monoterpene hydrocarbons (39.5%) and sesquiterpene hydrocarbons (34.3%). The main constituents of the oil were β-pinene (24.7%) and β-caryophyllene (12.3%). The thirty-one compounds identified in the stem oil falls into the main classes of compounds represented by monoterpene hydrocarbons (22.0%), sesquiterpene hydrocarbons (30.0%) and oxygenated sesquiterpenes (11.7%). The compounds occurring in higher amounts were β-pinene (26.1%) and β-caryophyllene (13.9%), with significant quantity of α-humulene

(8.2%), α-pinene (5.2%) and elemol (4.5%). Thirty-eight accounting for 95.0% of the total oil contents were identified in the root of *Z. vuquangensis*. The representative classes of compounds include monoterpene hydrocarbons (22.0%), oxygenated monoterpenes (24.7%), sesquiterpene hydrocarbons (19.1%) and oxygenated sesquiterpenes (22.6%). The major components of the oil were bornyl acetate (20.9%), zerumbone (14.1%), α-humulene (9.6%) and β-pinene (8.0%). However, the classes of compounds identified in the fruit oil were monoterpene hydrocarbons (39.8%), oxygenated monoterpenes (27.0%), and sesquiterpene hydrocarbons (22.4%). Moreover, 42 compounds were also identified in the oil. The significant amount them being β-pinene (19.6%), 1,8-cineole (15.6%), α-pinene (10.3%) and β-caryophyllene (10.4%).

Forty-one compounds representing 95.4% of oil contents were identified in the leaves of *Z. castaneum*. These comprised of mainly monoterpene hydrocarbons (49.2%) and sesquiterpene hydrocarbons (41.1%). Oxygen containing terpene compounds are less common. The main constituents of the oil were β-pinene (30.6%), α-pinene (9.5%), β-caryophyllene (9.4%) and bicycloelemene (9.1%). Bicyclogermacrene (7.7%) and germacrene D (6.5%) were also present in sizeable amount. On the other hand, sesquiterpene hydrocarbons (73.0%) and oxygenated sesquiterpenes (16.8%) dominated the stem oil of *Z. castaneum*. Monoterpenes (10.6%) occurred in much lower quantity common among the 40 compounds identified in the oil. The major terpenes present in the oil were β-caryophyllene (14.7%), δ-cadinene (9.8%), bicycloelemene (8.4%), and α-cubebene (7.8%). Other notable compounds include γ-elemene (6.1%), α-gurjunene (6.1%), β-selinene (6.0%) and zerumbone (5.2%). However, the 38 compounds of the root oil of *Z. castaneum* comprised mainly monoterpene hydrocarbons (54.0%) and oxygenated monoterpenes (34.0%). The main oil constituents were camphene (15.1%), 1,8-cineole (13.6%), linalool (11.3%), δ-3-carene (8.5%) and α-pinene (7.8%). Moreover, sesquiterpene hydrocarbons (35.7%), oxygenated sesquiterpenes (30.8%) and amides (17.3%) were main the prominent classes of compounds present in the fruit oil. Thirty-five compounds making up 98.3% of the total volatile composition were detected in the oil. The oil was dominated by (*E*)-nerolidol (23.2%), (*Z*)-9-octadecenamide (17.3%) and β-caryophyllene (10.8%) were the main constituents of the fruit oil.

There is no readily available information on the volatile oils of both *Z. vuquangensis* and *Z. castaneum* grown in Vietnam or elsewhere. The present result therefore represents the first of its kind. The chemical constituents of essential oils from some *Zingiber* plants from Vietnam and other parts of the world have been reported. For example, the most abundant components of *Z. rubens* were (*Z*)-citral (30.1%) and camphene (9.7%) while (*Z*)-citral (26.1%), camphene (16.3%) and sabinene (14.6%) were found in *Z. zerumbet* [2a]. The leaf oil of *Z. nitens* were composed of δ-elemene (17.0 %) and β-pinene (12.8 %). The stem oil comprised mainly of δ-elemene (20.1 %), germacrene D (8.6 %) and bicyclogermacrene (8.1 %), while β-pinene (21.0 %), β-elemene (12.8 %) and bornyl acetate (11.8 %) were identified in the root oil [2b]. Although, terpene compounds were prominent in these essential oils, the identities of the compounds differ from one species to another. The essential oils of *Zingiber* plants exhibited high chemical variability. Each species has its own compositional pattern different from one another. Sesquiterpene hydrocarbons predominate in the leaf oils of *Z. gramineum* [2c] and *Z. rufopilosum* [2c] as well as the rhizome of *Z. nimmonii* [2d]. Phenylpropanoids were common in the root and rhizome of *Z. niveum* [3]. On the other hand, the stems of *Z. gramineum* and *Z. rufopilosum* [2c] as well as the rhizome of *Z. amaricans* [4] produced oils with large quantity of oxygenated sesquiterpenes. Monoterpene hydrocarbon compounds occurred in higher quantity in the rhizome of *Z. collinsii* [5a] and inflorescence of *Z. spectabile* [5b]. The rhizomes of *Z. rubens* [2a] and *Z. roseum* [5c] consisted of oxygen containing monoterpene compounds. Quantitative amounts of monoterpene hydrocarbons and oxygenated monoterpene could be seen in the root of *Z. gramineum* [2c] as well as the rhizomes of *Z. zerumbet* [2a], *Z. pellitum* [6a] and *Z. purpureum* [6b]. Phenylbutanoids were the quantitatively and qualitatively significant compounds of the rhizome oil of *Z. neesanum* [6c].

Intra-specific variations could also be observed in the essential oils of *Zingiber* plant. For example, *Z. officinale* [7a] from Malaysia contained sesquiterpene hydrocarbon compounds while the samples of *Z. officinale* [7b] from India contained quantitative amounts oxygenated monoterpenes and sesquiterpene hydrocarbon compounds. Interestingly, the oil of *Z. officinale* [7c] of north western Himalayas origin, India, was dominated by oxygenated monoterpene compounds. Likewise, while the leaf oil of *Z. cassumunar* [7d] from Bangladesh contained oxygenated sesquiterpene, the sample analysed from Indonesia [7e] was dominated by monoterpene hydrocarbon and oxygenated monoterpene compounds. On the other hand, the rhizomes oil of Bangladesh sample contained aromatic and monoterpene compounds compared to the phenylbutanoids that were observed in the Indonesia sample. The enantiomeric compositions of *Z. roseum* fruit and flower essential oils were similar, but, in contrast, the rhizome oil contained an entirely different composition [8a]. It has been found that the variety could be used as an additional new source of some natural compounds in Zingiber oils. For example zerumbone has been identified in larger amount in *Z. zerumbet* var. *darcyi* besides *Z. zerumbet* [8b].

A comparative analysis of the chemical compositions of the essential oils of both *Z. vuquangensis* and *Z. castaneum* grown in Vietnam with the constituents of previously studied *Zingiber* plants revealed some marked quantitative and qualitative variations. Although terpene compounds predominate, the large quantity of (*Z*)-9-octadecenamide was being reported for the first time in the essential oils of *Zingiber*. The leaf and stem oils of *Z. vuquangensis* as well as the leaf oil of *Z. castaneum* belongs to the class dominated by monoterpene and sesquiterpene hydrocarbons while the root oil was classed into group consisting of oxygenated monoterpene and sesquiterpene. The stem oil of *Z. castaneum* falls into group consisting mainly of sesquiterpene hydrocarbons. The root oil of *Z. castaneum* can be categorized to the group of hydrocarbons and oxygenated monoterpenes. It has been postulated that factors such as different plant parts, age of the plant, chemotype, handling procedure, environmental and climatic conditions and other edaphic factors may have contributed to the observed compositional variations in *Zingiber* oils. Although results not shown, the studied essential does not possess noticeable antimicrobial activity unlike some other *Zingiber* oils such as *Z. zerumbet* and its main compound, zerumbone, which exhibited antimalarial, antifungal and antileishmanial activities [9a]. *Zingiber officinale* essential oil displayed insecticidal [9b], larvicidal [9c], immunotoxicity [9d], antioxidant and antibacterial [9e,d], anti-inflammatory and antinociceptive [9f] activities. The antimicrobial activity of *Z. nimmonii* [2d], inecticidal potential of *Z. purpureum* [6b], anti-inflammatory action of *Z. neesanum* [6c] and antioxidant characteristic of *Z. montanum* [9g] essential oils have been reported. It should be noted that apart from essential oils, extracts of some Zingiber plants displayed biological activities. The crude extracts of *Z. zerumbet*, showed anti-acetylcholinesterase activity [10].

Experimental

Collection of plant species: The leaves, stems, roots and fruits of *Z. vuquangensis* and *Z. castaneum* were collected from Vu Quang National Park, Hà Tĩnh Province, Vietnam, in August 2014. Botanical identification was performed by Dr. Dai, D.N. Voucher specimens LTH 472 and TTH 473 respectively have been deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

Hydrodistillation of essential oils from plants: Five hundred gram each of the pulverized different parts of *Z. vuquangensis* and *Z. castaneum* was used. Essential oils were obtained by separate hydrodistillation which was carried out in an all glass Clevenger-type distillation unit designed according to the established specification [11]. The distillation time was 3 h and conducted at normal pressure. The volatile oils distilled over water and were collected in the receiver arm of the apparatus into clean and previously weighed sample bottles. The oils were kept under refrigeration (4^oC) until the moment of analyses as described previously [2a-c].

GC and GC/MS analyses: The GC and GC-MS analyses as well as the means of identification of the constituents of the essential oils were reported in our earlier publications [2a-c].

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Table 1. Chemical composition of essential oils of *Z. vuquangensis* and *Z. castaneum*

S/N	Compounds [a]	RI [b]	RI [b]	Percentage composition [d]							
				Z. v Leaf	*Z. v* tem Stem	*Z. v* Root	*Z. v* Fruit	*Z. cast* Leaf	*Z. cast* Stem	*Z. cast* Root	*Z. cast* Fruit
1	Santolina triene	910	908	-	-	2.1	-	-	-	-	-
2	Tricyclene	926	914	-	-	0.3	-	-	-	-	-
3	α-Thujene	930	921	0.2	-	-	0.3	0.1	-	1.8	-
4	α-Pinene	939	932	6.9	5.2	2.2	10.3	9.5	0.8	7.8	0.4
5	Camphene	953	946	1.4	4.8	4.3	5.4	0.5	-	15.1	1.4
6	Verbenene	955	948	0.1	0.5	-	-	-	-	-	-
7	Sabinene	976	965	-	3.3	-	-	0.2	-	4.9	0.8
8	β-Pinene	980	978	24.7	26.1	8.0	19.6	30.6	4.9	4.2	0.9
9	β-Myrcene	990	988	0.9	0.8	0.6	1.1	2.0	0.6	3.4	0.6
10	α-Phellandrene	1006	1004	0.1	-	0.1	1.3	0.7	-	2.2	0.3
11	δ-3-Carene	1011	1008	1.3	-	-	0.2	0.2	-	8.5	0.3
12	α-Terpinene	1017	1014	0.2	0.4	0.2	-	0.1	-	0.9	-
13	*p*-Cymene	1026	1020	-	0.6	0.3	0.8	-	-	3.0	-
14	Limonene	1032	1026	2.3	2.5	1.8	-	3.9	0.9	-	-
15	1,8-Cineole	1034	1032	-	-	-	15.6	-	-	13.6	3.9
16	(*Z*)-β-Ocimene	1043	1034	0.1	-	-	-	0.1	-	-	-
17	(*E*)-β-Ocimene	1052	1044	0.7	1.3	1.1	0.3	0.2	0.4	0.2	0.4
18	γ-Terpinene	1061	1056	0.4	1.4	0.5	0.5	0.2	0.9	2.0	-
19	α-Terpinolene	1090	1089	0.2	0.5	0.3	-	0.2	0.5	-	-
20	Linalool	1100	1100	1.4	1.7	0.3	2.1	-	1.6	11.3	3.2
21	Nonanal	1106	1102	-	-	-	-	0.1	-	-	-
22	α-Thujone	1106	1105	-	-	-	3.1	-	-	-	-
23	Fenchyl alcohol	1110	1112	-	-	-	0.2	-	-	-	-
24	(E)-4,8-Dimethyl-1,3,7-nonatriene	1115	1113	0.3	-	-	-	0.2	-	0.3	0.3
25	Camphor	1145	1143	-	-	-	1.1	-	-	0.2	-
26	*neoallo*-Ocimene	1465	1147	-	-	0.2	-	0.7	-	-	0.6
27	Borneol	1167	1167	-	3.2	-	2.1	-	-	0.6	-
28	Terpinen-4-ol	1177	1177	-	-	-	0.6	0.1	-	1.5	0.7
29	α-Terpineol	1189	1189	-	-	-	0.5	-	-	0.4	-
30	Myrtenal	1200	1198	0.2	-	-	-	-	-	-	-
31	Fenchyl acetate	1206	1222	-	-	0.2	-	-	-	0.4	-
32	*trans*-Carveol	1217	1217	-	-	0.5	-	-	-	-	-
33	Dihydro-Edulan I	1280	1280	-	-	-	1.0	-	-	-	-
34	Bornyl acetate	1289	1289	2.5	-	20.9	0.4	-	-	3.2	0.7
35	*trans*-Pinocarvyl acetate	1295	1297	-	-	2.8	-	-	-	-	-
36	Pulespenone	1310	1309	-	0.5	-	-	-	-	-	-
37	Myrtenyl acetate	1326	1328	-	-	-	-	-	-	2.6	-
38	Bicycloelemene	1327	1337	4.0	1.2	0.3	0.6	9.1	8.4		1.6
39	α-Cubebene	1351	1345	-	-	-	-	-	7.8	-	2.9
40	Neryl acetate	1362	1367	-	-	-	-	-	-	0.2	-
41	α-Ylangene	1375	1372	-	-	-	-	0.1	-	-	-
42	α-Copaene	1377	1374	0.4	-	0.2	0.3	0.4	0.3	-	0.3
43	Methyl cinnamate	1384	1388	-	-	-	0.3	-	-	-	-
44	β-Bourbonene	1385	1384	-	-	-	-	0.3	0.5	-	-
45	β-Cubebene	1388	1389	1.3	-	-	-	0.5	-	-	-
46	β-Elemene	1391	1390	0.8	0.5	0.6	0.3	1.8	1.7	0.2	1.2
47	α-Gurjunene	1412	1412	-	-	-	-	0.2	0.7	-	-
48	*trans*-α-Bergamotene	1413	1415	0.3	0.8	1.1	0.3	-	6.1	1.3	-
49	β-Caryophyllene	1419	1417	12.3	13.9	4.5	10.4	9.4	14.7	2.0	10.8
50	γ-Elemene	1437	1434	-	0.6	-	0.3	-	6.1	0.8	3.8
51	α-Guaiene	1440	1439	0.2	-	-	-	-	-	-	-
52	Aromadendrene	1441	1440	1.4	-	1.0	1.0	0.1	0.3	-	-
53	(*E*)-β-Farnesene	1443	1450	1.0	-	-	-	-	-	-	-
54	α-Humulene	1454	1452	7.7	8.2	9.6	2.5	1.3	3.2	0.5	2.5
55	α-Patchoulene	1457	1457	0.5	-	-	0.5	-	-	-	-
56	γ-Gurjunene	1479	1479	-	-	-	-	-	0.6	-	-
57	ar-Curcumene	1480	1480	-	1.5	-	-	-	-	-	-
58	γ-Muurolene	1480	1482	-	-	-	-	-	-	-	0.7
59	Germacrene D	1485	1484	-	-	-	0.3	6.5	0.7	0.4	2.4
60	α-Amorphene	1485	1485	0.4	-	0.2	0.3	0.4	0.4	-	1.0
61	β-Selinene	1486	1486	0.4	-	-	-	0.3	6.0	-	-
62	Zingiberene	1494	1493	1.6	-	-	2.0	-	-	-	-
63	Bicyclogermacrene	1500	1500	3.6	-	-	0.8	7.7	4.3	0.4	2.8
64	β-Bisabolene	1506	1506	0.9	1.4	0.4	-	-	0.7	0.2	-
65	(*E,E*)-α-Farnesene	1508	1505	-	-	-	0.6	-	-	-	1.8
66	γ-Cadinene	1514	1512	-	-	0.4	1.3	-	-	-	-
67	(*E*)-γ-Bisabolene	1515	1519	-	-	0.2	-	-	-	-	-
68	δ-Cadinene	1525	1522	1.0	-	0.6	1.2	1.0	9.8	-	2.2
69	β-Sesquiphelandrene	1527	1524	-	0.9	-	-	-	-	-	-
70	Elemol	1550	1548	-	4.5	3.3	-	-	-	0.6	2.1
71	Ledol	1560	1559	-	-	-	-	0.3	-	-	-
72	Germacrene B	1561	1560	0.5	0.9	-	-	-	0.7	-	-

73	(*E*)-Nerolidol	1563	1561	3.2	0.8	0.9	0.7	1.1	1.2	0.5	23.2
74	Spathulenol	1578	1577	0.8	0.5			1.5	1.1	-	0.6
75	Caryophyllene oxide	1583	1581	1.0	1.8	2.3	1.0	1.0	0.9	0.7	0.9
76	Isoledene	1594	1590	-	-	-	-	0.1	0.2	-	-
77	α-Cedrol	1601	1601	-	0.5	-	0.4	-	3.5	-	-
78	β-Oplopenone	1608	1608	-	-	-	-	-	-	0.2	-
79	Aromadendren epoxide	1623	1526	-	-	-	-	-	0.8	-	-
80	τ-Muurolol	1646	1646	0.4	-	1.1	-	0.3	0.6	0.3	-
81	β-Eudesmol	1651	1648	-	-	-	-	-	0.5	-	-
82	α-Cadinol	1654	1652	0.6	-	0.9	1.3	0.4	0.8	-	2.6
83	Bulnesol	1672	1572	-	-	-	-	-	0.6	-	-
84	Farnesol	1718	1722	0.2	-	-	-	-	1.4	-	-
85	Zerumbone	1732	1732	3.0	3.6	14.1	-	-	5.2	0.5	1.4
86	Mint sulfide	1741	1745	-	-	-	-	1.9	-	-	1.7
87	Phytol	2125	2119	0.5	-	-	-	0.1	0.4	-	-
88	(*Z*) -9-Octadecenamide	2398	2375	3.6	-	6.6	1.3	-	-	1.5	17.3
Total				**91.5**	**94.5**	**95.0**	**93.9**	**95.4**	**99.8**	**96.9**	**98.3**
Monoterpene hydrocarbons				**39.5**	**47.4**	**22.0**	**39.8**	**49.2**	**9.0**	**54.0**	**5.7**
Oxygenated monoterpenes				**4.1**	**5.4**	**24.7**	**27.0**	**0.1**	**1.6**	**34.0**	**8.5**
Sesquiterpene hydrocarbons				**34.3**	**30.0**	**19.1**	**22.4**	**41.1**	**73.0**	**5.8**	**35.7**
Oxygenatedsesquiterpenes				**9.2**	**11.7**	**22.6**	**3.4**	**4.6**	**16.8**	**2.8**	**30.8**
Diterpenes				**0.5**	**-**	**-**	**-**	**0.1**	**0.4**	**-**	**-**
Amides				**3.6**	**-**	**6.6**	**1.3**	**-**	**-**	**1.5**	**17.3**
Others				**0.3**	**-**	**-**	**-**	**0.3**	**-**	**0.3**	**0.3**

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5MS column; [c] RI (Lit.) Literature retention indices; [c] Identification by mass spectra, GC retention indices and comparison with literature data; [d] Standard deviation (SD ±) were insignificant and were excluded from the Table; - =Not identified; ***Z. v,*** *Z. vuquangensis;* ***Z. cast*** *Z. castaneum*

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