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Constituents of essential oils from the leaves, stems and roots of *Zingiber gramineum* and *Zingiber rufopilosum*

[Los constituyentes de los aceites esenciales de las hojas, tallos y raíces de *Zingiber gramineum* y *Zingiber rufopilosum*]

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Abstract: The chemical constituents of essential oils obtained from leaves, stems and roots of Zingiber gramineum Noronha ex Blume and Zingiber rufopilosum Gagnep collected from Vietnam have been studied. The determination of essential oil components was performed by Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography- Mass Spectrometry (GC-MS). The main constituents of the leaves oil of Zingiber gramineum were zingiberene (19.5%), β-cubebene (12.9%), β-sesquiphellandrene (12.9%) and β-elemene (11.6%) while the stems oil was dominated by benzyl benzoate (22.6%), β-elemene (9.7%) and α-selinene (8.8%). However, γ-terpinene (17.9%), α-terpinene (17.1%), terpinen-4-ol (13.0%) and 1,8-cineole (12.8%) were the present in the root oil. In addition, β-agarofuran (13.7%), α-humulene (8.8%) and α-pinene (8.7%) were the main compounds identified in the leaves of *Zingiber rufopilosum*. The stems comprised of α-cadinol (15.1%), τ-muurolol (12.1%) and endo-1-bourbonanol (9.9%) while (E,E)-farnesol (11.6%), α-pinene (10.0%), bornyl acetate (6.6%) and β-pinene (6.2%) were the significant compounds of the root oil. This is the first report on the volatile compositions of these plant species.

Keywords: *Zingiber gramineum, Zingiber rufopilosum,* essential oil, monoterpenes, sesquiterpenes

Resumen: El presente estudio se llevó a cabo para evaluar el efecto del extracto metanólico acuoso a partir de los componentes químicos de los aceites esenciales obtenidos de las hojas, tallos y raíces de *Zingiber gramineum* Noronha ex Blume y *Zingiber rufopilosum* Gagnep recogidos de Vietnam. La determinación de componentes de aceites esenciales se realizó por cromatografía de gases-detector de ionización de llama (GC-FID) y cromatografía de gases espectrometría de masas (GC-MS). Los principales componentes del aceite de hojas de Zingiber gramineum fueron zingibereno (19,5%), β-cubebene (12,9%), β-sesquiphellandrene (12,9%) y β-elemene (11,6%), mientras que el aceite de tallos fue dominada por benzoato de bencilo (22,6%), β-elemene (9,7%) y α-selineno (8,8%). Sin embargo, γ-terpineno (17,9%), α-terpineno (17,1%), terpinen-4-ol (13,0%) y 1,8-cineol (12,8%) fueron los presentes en el aceite de la raíz. Además, β-agarofuran (13,7%), α-humuleno (8,8%) y α-pineno (8,7%) fueron los principales compuestos identificados en las hojas de *Zingiber rufopilosum*. Los tallos componen de α-cadinol (15,1%), τ-muurolol (12,1%) y endo-1-bourbonanol (9,9%), mientras que (E, E)-farnesol (11,6%), α-pineno (10,0%), acetato de bornilo (6,6%) y β-pineno (6,2%) fueron los compuestos significativos del aceite de la raíz. Este es el primer informe sobre las composiciones volátiles de estas especies de plantas.

Palabras clave: *Zingiber gramineum, Zingiber rufopilosum,* aceites esenciales, monoterpenos, sesquiterpenos.

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INTRODUCTION

Zingiberaceae, the ginger family of flowering plants is the largest family of the order Zingiberales, containing about 52 genera and more than 1,300 species. These aromatic herbs grow in moist areas of the tropics and subtropics, including some regions that are seasonably dry. Members of the family are perennials that have fleshy rhizomes (underground stems). They may grow to 6 metres (20 feet) in height. A few species are epiphytic—i.e., supported by other plants and having aerial roots exposed to the humid atmosphere. The rolled-up sheathing bases of the leaves sometimes form an apparent short aerial stem. The Zingiberaceae flower resembles an orchid because of its labellum (two or three fused stamens) joined with a pair of petal-like sterile stamens. Nectar is present in the slender flower tubes. Many species are economically valuable for their spices and perfume (Flora of China, 2000; Kress *et al*., 2002).

The genus *Zingiber* is native to Southeast Asia, China, the Indian Subcontinent, and New Guinea. The genus *Zingiber* is in the family Zingiberaceae in the major group Angiosperms (Flowering plants). It contains the true gingers, plants grown the world over for their medicinal and culinary value. The rhizomes are branched, tuberous and aromatic. The oblong or lanceolate leaves are erect held in plane parallel to rhizomes. They have persistent flowers. The often black seeds are covered by arils. There are over 200 species of plants in this genus (Ravindran & Babu, 2004; Tripathi & Singh, 2006).

In continuation of an extensive research into the volatile constituents of Vietnamese flora as they are made available (Thang *et al*., 2015), we report herein the constituents of the leaves, stems and roots oils of *Zingiber gramineum* Noronha ex Blume and *Zingiber rufopilosum* Gagnep, an engendered species (IUCN, 1997). Published literature is scanty on the biological activities as well, as the volatile and non-volatile chemical constituents already isolated from these plants. However, both plants are used in ethnomedicine for the treatment of ulcer, inflammation, bacterial infections, fever and febrigue (Nguyen, 2000).

The chemistry of volatile compounds of *Zingiber* species grown in Vietnam have been reported (Duñg *et al*., 1993; Duñg *et al*., 1995; Giang *et al*., 2011; Dai *et al*., 2013; Chau *et al*., 2014). Several terpenoid and non-terpenoid compounds have been described from the essential oils of several species in the genus. The objectives of the present study was to examine the constituents of the leaves, stems and roots essential oils of *Zingiber gramineum* and *Zingiber rufopilosum* grown in Vietnam and compare the results with data from other *Zingiber* plants.

MATERIALS AND METHODS

Plants collection

Leaves, stems and roots of both plants were collected from Pù Mát National Park, Nghệ An Province, Vietnam, in May 2014. Botanical identification was performed at the Botany Museum, Vinh University, Vietnam, where voucher specimens DND 420 and DND 430, respectively for *Z. gramineum* and *Z. rufopilosum* were deposited. Plant samples were air-dried prior to extraction.

Isolation of the essential oils

500 g of air-dried and pulverized samples of each plant were subjected to separate hydrodistillation for 3h at normal pressure, according to the standard procedure (Vietnamese Pharmacopoeia, 1997). The plant samples afforded lower yields of oils: 0.23%, 0.17% and 0.22% (v/w, *Z. gramineum*; leaves, stems and flowers respectively) and 0.25%, 0.21% and 0.32% (v/w; respectively for *Z. rufopilosum* leaves, stems and flowers), calculated on a dry weight basis. All the oil samples were light yellow coloured.

Gas chromatography (GC)

GC analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS columns (30 m x 0.25 mm i.d., film thickness 0.25 μm, Agilent Technology, Berkshire, United Kingdom). The analytical conditions were: carrier gas H_2 (1 mL/min), injector temperature 250º C, detector temperature 260º C, column temperature programmed from 40º C (2 min hold) to 220º C (10 min hold) at 4º C/min. Samples were injected by splitting and the split ratio was 10:1. The volume injected was 1.0 μL. Inlet pressure was 6.1 kPa.

Gas chromatography-Mass spectrometry (GC-MS)

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm i.d., x 0.25 μm film thickness) and interfaced with a mass spectrometer HP 5973 MSD (ion trap mass detector) was used for the GC/MS analysis, under the same conditions as those used for GC analysis. The conditions were the same as described above with He (1 mL/min) as carrier gas and transfer line temperature 260º C. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range of 35-350 amu at a sampling rate of 1.0 scan/s.

Identification of the constituents

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST 08 Libraries and Wiley 9th Version and the home-made MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature values (Joulain & König, 1998; NIST, 2011; Adams, 2007). The relative amounts of individual components were calculated based on the percentage relative area (FID response) without using correction factors.

RESULTS AND DISCUSSION

The identities and percentage composition of the compounds present in the oil samples could be seen in Table 1. Sesquiterpene (76.2%) and monoterpene hydrocarbons (13.6%) were the main class of compounds present in the leaves oil of *Z. gramineum*. The major compounds were zingiberene (19.5%), β-cubebene (12.9%), β-sesquiphellandrene (12.9%), β-elemene (11.6%), bicycloelemene (9.8%) and β-pinene (7.4%). Aromatic esters (22.6%), along with sesquiterpene (44.8%) and monoterpene hydrocarbons (11.5%) were the classes of compounds in the stems oil. The main constituents of the oil were benzyl benzoate (22.6%), β-elemene (9.7%), α-selinene (8.8%) and β-pinene (7.5%). Monoterpenes (92.5%) was the dominant class of compound in the roots oil of *Z. gramineum*. The main constituents were γ-terpinene (17.9%), α-terpinene (17.1%), terpinen-4-ol (13.0%) and 1,8-cineole (12.8%). There were significant quantities of β-pinene (7.2%) and α-terpinolene (6.6%).

Table 1
Chemical constituents of essential oils of *Zingiber gramineum* and *Zingiber rufopilosum*

Compounds[a]	RI[b]	RI[c]	*Z.gl*	*Z.gs*	*Z.gr*	*Z.rl*	*Z.rs*	*Z.rr*
Tricyclene	926	921	-	-	-	-	-	0.1
α-Thujene	930	926	-	-	2.1	-	-	-
α-Pinene	939	932	3.1	1.0	4.1	8.7	5.4	10.0
Camphene	953	946	0.1	-	1.7	0.8	-	17
Verbenene	967	967	-	-	-	0.3	-	-
Sabinene	976	974	-	2.1	-	-	-	-
β-Pinene	980	974	7.4	7.5	7.2	4.5	3.5	6.2
β-Myrcene	990	988	0.1	-	3.0	0.3	-	0.6
α-Phellandrene	1006	1002	0.1	-	0.8	-	-	0.4
α-Terpinene	1017	1014	0.1	-	17.1	-	-	0.2
o-Cymene	1022	1022	-	-	0.1	0.5	-	-
Limonene	1032	1024	0.4	-	-	-	-	4.0
1,8-Cineole	1034	1026	-	-	12.8	-	-	-
(*Z*)-β-Ocimene	1043	1032	0.1	-	-	0.1	-	1.0
(*E*)-β-Ocimene	1052	1044	0.3	-	-	0.1	-	3.2
γ-Terpinene	1061	1056	0.1	0.9	17.9	-	-	0.4
α-Terpinolene	1090	1086	0.1	-	6.6	-	-	0.3
Linalool	1100	1095	0.1	-	-	0.2	-	0.8
1,3,8-*p*-Menthatriene	1110	1105	-	-	-	0.3	-	-
Fenchyl alcohol	1122	1118	-	-	0.2	-	-	-
allo-Ocimene	1128	1128	1.7	-	-	-	-	0.4
trans-*p*-Menth-2-ene-1-ol	1142	1142	-	-	0.3	-	-	-

Camphor	1145	1141	-	-	0.8	1.0	-	0.2
Pinocarvone	1165	1164	-	-	-	0.2	-	-
Borneol	1165	1165	-	-	0.1	-	-	0.2
p-Mentha-1,5-dien-8-ol	1166	1168	-	-	-	0.3	-	
Terpinen-4-ol	1177	1174	0.1	4.2	13.0	-	-	0.1
α-Terpineol	1189	1187	-	-	1.5	-	-	0.2
Myrtenal	1200	1195	-	-	-	0.3	-	-
Piperitol	1204	1204	-	-	0.8	-	-	-
Verbenone	1205	1204	-	-	-	0.1	-	-
trans-Carveol	1217	1217	-	-	-	0.2	-	
exo-Fenchyl acetate	1228	1229	-	-	1.9	-	-	0.4
Bornyl acetate	1289	1287	-	-	0.5	0.1	0.4	6.6
2-Undecanone	1291	1293	-	0.6	-	-	-	-
Bicycloelemene	1327	1338	9.8	3.4	0.1	2.1	1.3	0.3
α-Cubebene	1351	1345	0.1	-	-	0.2	-	-
α-Copaene	1377	1374	0.6	1.5	0.1	0.3	-	0.1
β-Bourbonene	1385	1384	-	-	-	0.6	0.4	-
β-Patchoulene	1386	1388	-	-	-	-	-	0.7
Methyl cinnamate	1388	1388	-	-	-	-	-	2.4
β-Elemene	1389	1389	11.6	9.7	0.6	0.8	1.1	0.3
β-Cubebene	1390	1390	12.9	-	-	0.5	0.3	0.1
Cyperene	1399	1398	-	-	0.1	-	-	-
α-Gurjunene	1412	1409	0.1	-	0.1	0.1	6.1	-
β-Caryophyllene	1419	1417	-	3.0	0.3	3.7	2.8	1.0
trans-α-Bergamotene	1430	1431	-	-	-	-	-	0.1
β-Gurjunene	1434	1432	-	-	-	6.5	-	-
Widdrene	1437	1433	-	1.4	-	-	-	-
Aromadendrene	1441	1439	0.1	1.8	0.1	-	-	-
α-Humulene	1454	1452	-	3.3	0.2	8.8	7.0	2.5
allo-Aromadendrene	1469	1467	-	-	-	1.3	1.0	0.4
Aristolochene	1484	1487	-	-	-	-	-	1.6
Germacrene D	1485	1484	-	-	-	4.5	3.2	1.2
α-Amorphene	1485	1485	1.9	-	-	3.6	0.8	0.9
β-Selinene	1486	1489	-	4.8	0.9	-	1.5	-
Zingiberene	1494	1493	19.5	-	0.9	-	-	-
Valencene	1496	1496	-	1.1	-	-	1.1	-
α-Selinene	1498	1498	-	8.8	-	-	-	-
Bicyclogermacrene	1500	1500	-	2.1	-	-	-	1.9
(*E*,*E*)-α-Farnesene	1505	1505	-	-	-	-	-	3.7
β-Bisabolene	1506	1506	6.5	1.3	0.3	-	-	1.3
Germacrene A	1509	1508	-	-	-	-	-	0.2
γ-Cadinene	1514	1513	-	-	-	0.2	1.0	-
β-Agarofuran	1516	1516	-	-	-	13.7	4.0	1.4
endo-1-Bourbonanol	1520	1520	2.6	-	-	5.9	9.9	-
β-Sesquiphellandrene	1524	1521	12.9	-	0.3	-	-	-
δ-Cadinene	1525	1522	-	3.6	-	5.8	5.7	-
α-Cadinene	1539	1539	0.2	-	-	1.5	1.4	0.7
α-Agarofuran	1543	1548	-	-	-	2.0	1.7	0.5

Elemol	1550	1548	-	-	-	-	-	0.4
Ledol	1560	1560	0.3	-	-	-	-	-
(*E*)-Nerolidol	1563	1561	0.4	-	0.1	-	-	1.7
Spathulenol	1578	1577	-	2.4	0.1	-	-	0.7
Caryophyllene oxide	1583	1581	-	1.2	0.1	0.9	-	0.6
Viridiflorol	1585	1585	0.3	-	-	-	-	-
Salvial-4(14)-en-1-one	1587	1586	1.7	-	-	-	-	-
Globulol	1590	1590	-	-	-	-	-	0.7
Longiborneol	1599	1592	-	-	-	0.4	-	-
α-Guaiol	1600	1602	-	-	-	0.7	-	0.8
β-Oplopenone	1608	1608	-	-	-	3.9	-	1.1
10-epi-γ-Eudesmol	1613	1622	-	-	-	1.0	1.1	-
Isospathulenol	1636	1631	-	-	-	-	-	0.7
Aromadendrene epoxide	1639	1639	-	-	-	-	-	1.8
τ-Muurolol	1646	1640	0.7	1.8	-	0.9	12.1	3.9
β-Eudesmol	1651	1649	-	-	-	6.8	-	0.3
α-Cadinol	1654	1652	0.9	2.0	-	-	15.1	4.9
α-Santalol	1671	1671	-	-	0.1	-	-	-
Bulnesol	1672	1672	-	-	-	-	1.0	1.0
Vulgarol B	1688	1688	-	-	0.1	-	0.8	-
Calamenene	1702	1702	-	-	-	1.2	-	-
(*E*,*E*)-Farnesol	1718	1722	-	-	-	-	4.6	11.6
β-Maaliene	1732	1732	-	-	-	-	-	0.8
α-Sinensal	1752	1752	-	2.1	-	-	-	-
Benzyl benzoate	1760	1759	-	22.6	-	0.6	-	0.9
β-Costol	1778	1776	-	-	-	0.1	-	-
Nootkatone	1819	1806	-	-	-	-	-	0.2
8,9-Dehydro-9-formyl-cycloisolongifolene [d]	2082	2082	-	-	-	-	1.4	-
Phytol	2125	2122	0.1	-	0.1	0.2	-	0.2
Total			**97.0**	**90.8**	**97.1**	**96.8**	**95.7**	**90.6**
Monoterpene			**13.6**	**11.5**	**60.6**	**15.6**	**8.9**	**28.5**
Oxygenated monoterpenes			**0.2**	**4.2**	**31.9**	**2.4**	**0.4**	**10.9**
Sesquiterpene			**76.2**	**44.8**	**4.0**	**57.4**	**40.4**	**19.7**
Oxygenated sesquiterpenes			**6.9**	**9.5**	**0.5**	**20.6**	**44.6**	**30.2**
Diterpenes			**0.1**	**-**	**0.1**	**0.2**	**1.4**	**0.4**
Aromatic esters			**-**	**22.6**	**-**	**0.6**	**-**	**0.9**
Others			**-**	**0.6**	**-**	**-**	**-**	**-**

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5 MS column; [c] Literature retention indices; [d] Tentative assignment; - Not identified; *Z.gl, Zingiber gramineum* (leaves); *Z.gs, Zingiber gramineum* (stems); *Z.gr, Zingiber gramineum* (roots); *Z.rl, Zingiber rufopilosum* (leaves); *Z.rs, Zingiber rufopilosum* (stems); *Z.rr, Zingiber rufopilosum* (roots)

The leaves oil of *Z. rufopilosum* was characterized by large amounts of sesquiterpene compounds (78.0%) and monoterpene hydrocarbons (15.6%). The main constituents include β-agarofuran (13.7%), α-humulene (8.8%) and α-pinene (8.7%). There were significant amounts of β-eudesmol (6.8%), β-gurjunene (6.5%), δ-cadinene (5.8%) and *endo*-1-bourbonanol (5.9%). Sesquiterpene (84.6%) were the main compounds in the stems oil. The representative compounds were α-cadinol (15.1%), τ-muurolol (12.1%), *endo*-1-bourbonanol (9.9%), α-humulene (7.0%), α-gurjunene (6.1%), δ-cadinene

(5.7%) and α-pinene (5.4%). The roots oil contained monoterpene and sesquiterpene compounds represented by (*E*,*E*)-farnesol (11.6%), α-pinene (10.0%), bornyl acetate (6.6%) and β-pinene (6.2%).

Regardless of *Zingiber* being large family, with mostly aromatic plants, the essential oils of *Z. gramineum* and *Z. rufopilosum* have not been previously investigated. Therefore, the present results may represent the first of its kind aimed at the comprehensive characterization of the volatile constituents of both plant. The chemical composition of the leaves oil of *Z. gramineum* has similarity with the rhizomes of *Z. officinale* in the high content of zingiberene and β-sesquiphellandrene, (Pellerin, 1991; Pino *et al*., 2004; Sacchetti *et al*., 2005), although some other quantitative and qualitative variations could be observed between the oil compositions.

In previous studies on *Zingiber* oils Vietnam and other countries, high accumulation of α-pinene (50.2%) and β-pinene (23.6%) were found in the rhizomes oil of *Z. collinsii* (Chau *et al*., 2014), while (*Z*)-citral (30.1%) and camphene (9.7%) were the main compounds of *Z. rubens*. A large amount of (*Z*)-citral (26.1%), camphene (16.3%) and sabinene (14.6%) were present in *Z. zerumbet* (Dai *et al.*, 2013). The main constituents of *Z. pellitum* (Giang *et al.*, 2011) were identified as terpinen-4-ol (35.9 %) and *p*-cymene (19.8 %) while zerumbone (72.3%) was reported previously as the main compound of *Z. zerumbet* (Duñg *et al.*, 1993). Also, the chemical composition of the leaves oil of *Z. zerumbet* from another report (Duñg *et al.*, 1995) was dominated by zerumbone (21.3%), *trans*-phytol (12.6%) and β-caryophyllene (11.2%) while (*Z*)-nerolidol (22.3%) was found in the stems, with (*Z*)-nerolidol (36.3%) and β-caryophyllene (13.2%) making up the composition of the flowers. However, β-phellandrene (45.3 %) was the main constituent in the inflorescences oil of *Z. spectabile* (Zoghbi *et al*., 2005). The main compound of the rhizomes oil of *Z. roseum* (Prakash *et al*., 2006) was linalool (53.3 %) while β-caryophyllene (42.2 %) was the significant constituent in the rhizomes of *Z. nimmonii* (Sabulal *et al*., 2006). It could be seen that the essential oils of *Zingiber* plants exhibited high chemical variability. Each species has its own compositional pattern different from one another. The high content of compounds identified in the essential oils of *Z. graminuem* and *Z. rufopilosum* may be an important chemical and economic characteristic of these oil samples.

In the comparison of chemical constituents of essential oils from the studied *Zingiber* plants with essential constituents from other genus and family, it was discovered the stems oil of *Z. gramineum* had composition pattern similar to the leaves oil of Croton *kongensis* (Chau *et al*., 2014) while the root oil was also similar to the leaves of *Melaleuca alternifolia* (Pereira *et al*., 2014).

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