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Volatile Constituents of Essential Oils from the Leaves, Stems, Roots and Fruits of Vietnamese Species of *Alpinia malaccencis*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The essential oils obtained from different parts of *Alpinia malaccencis* (Burm f.) (Zingiberaceae) collected from Kỳ Sơn Districts, Nghệ An Province, Vietnam, has been studied. Determination of essential oil components from the leaves, stems, roots and fruits of *A. malaccencis* was performed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography mass spectrometry (GC-MS). β-Pinene (leaf: 56.%; stem: 46.0%; root: 31.7% and fruit: 18.5%) and α-pinene (leaf: 10.3%; stem: 9.8%; root: 6.3% and fruit: 5.9%) were the major constituents of the oils. In addition, β-phellandrene was present in the amount of 12.1%, 12.9% and 12.9% in the stem, root and fruit oils but absent in the leaf. Methyl cinnamate (27.8%) was identified in higher quantity only in the fruit oil but absent in the leaf while α-phellandrene (5.7%) was present in the stem and α-selina-6-en-4-ol (5.5%) was a significant compound of the root oil. The compositions of the root and fruit essential oils were reported for the first time.

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Aims: The aim of the research is to investigate for the first time the volatile constituents from *A. malaccencis* collected from Kỳ Sơn Districts, Nghệ An Province, Vietnam.
Study Design: Extraction of essential oils from the air-dried leaves, stems, roots and fruits samples of *A. malaccencis* and investigation of their chemical constituents.
Place and Duration of Study: Leaves, stems, roots and fruits of *A. malaccaencis* were collected from plants growing in Kỳ Sơn Districts, Nghệ An Province, Vietnam, in May 2014.
Methodology: Air-dried and pulverized samples were hydrodistilled in a Clevenger-type apparatus according to Vietnamese Pharmacopoeia to obtained volatile oils whose chemical constituents were analyzed by GC and GC/MS.
Results: Monoterpene hydrocarbons were the dominant class of compound in the leaf oil (74.0%) and stem oil (81.3%) of *A. malaccencis*. Sesquiterpene compounds (21.5%) were identified in appreciable quantity in the roots oil, although monoterpene hydrocarbons (59.3%) are abundant. Oxygenated monoterpenes (31.1%) and monoterpene hydrocarbons (46.7%) constituted the main classes of compounds identified in the fruit oil.
Conclusion: The literature about the oils of *A. malaccencis* indicates a high variability in the chemical composition of the essential oils.

Keywords: Alpinia malaccencis; essential oil composition; monoterpenes; sesquiterpenes.

1. INTRODUCTION

Alpinia is a rather large genus of plants, with more than 230 species. *Alpinia malaccensis* (Burm. f.) R., is a plant in the Zingiberaceae family cultivated for ornamental and medicinal purposes. It grows to over 4 m tall. It has long lush green leaves, which have fragrance. In November and December the flowers emerge above the leaves enclosed in a conical sheath, which splits to reveal a sumptuous cluster of fat pink and white buds [1]. It is a native of Indonesia and Malaysia. It has many medicinal properties. *Alpinia malaccensis* is used to cure wounds and sores [2]. It is chewed to make the voice strong and clear and used for bathing feverish people [3,4]. The leaf extracts was shown to possess antimicrobial [5], antioxidant [5,6] and potent cytotoxic [7] activities. Phytochemical investigation has led to the isolation of 5,6-dehydrokawain, coronarin E, coronarin A, (E)-8(17), 12-labdadiene-15,16-dial, hedyforrestin B, cardamonin, pinocembrin and alpinetin [8].

The volatile compositions of *A. malaccencis* grown in few countries have been reported. The rhizome oil from Bangladesh had α-phellandrene (31.80%), eucalyptol (13.76%), *o*-cymene (11.45%) and β-pinene (11.34%) as its major compounds [1]. The essential oil from the rhizome of Malaysia grown *A. malaccencis* contained methyl (*E*)-cinnamate (78.2%) as the major constituent [9]. The oil sample analysed from Kerala, India [10] was rich in α-phellandrene (36.4%) and *p*-cymene (14.9%). The major constituents of the rhizome oil collected from Phulbani, Odisha, India were α-phellandrene (43.9%), *p*-cymene (31.7%) and β-pinene (4.6%). The essential oil displayed significant antioxidant and antimicrobial activities [11]. The presence of 1,8-cineole (21.14%), camphor (18.7%) and sabinene (11.64%) have been reported in the rhizome oil from kottayam, India [12]. The leaf oil from Indonesia contained an abundant of α-pinene (30.57%), β-pinene (11.41%), 1, 8-cineole (21.39%) and methyl cinnamate (9.24%) while methyl cinnamate (30.24%), α-pinene (13.04%), β-pinene (12.38%) and 1, 8-cineole (16.58%) were present in the stem with methyl cinnamate (64.4%), α-pinene (14.90%), β-pinene (12.44%) and 1, 8-cineole (9.89%) identified in the rhizome [13]. The rhizome had stronger locomotor inhibition activity compare to the stem and leaf oils [13]. However, 1,8-cineole (11.9%), linalool (9.0%), fenchyl acetate (8.6%) and *trans*-nerolidol (5.7%) were present in the sample from Thailand [14]. The leaf oil of sample from Guandong, China was rich in methyl cinnamate (75%) while the seed oil contained 1,8-cineole, citronellol, 4-phenyl-3-buten-2-one, decanoic acid, geranyl acetate, nerolidol, lauric acid, α-farnesol, β-farnesol, myristic acid and palmitic acid [15]. The essential oil was thought to contained hydrocarbon which belongs to the pinene group [16]. (*E*)-Methyl cinnamate (64.4%-88.0%) was the main compound in the leaf, rhizome and stem oils of *Alpinia malaccencis* var. *nobilis* from Malaysia [17].

There are no previous references in literature about the essential oil of this plant from Vietnam. The objective of the present study was to examine the constituents of the leaf, stem, root

and fruit oils of *A. malaccencis* in details, and to compare the results obtained with those reported earlier.

2. MATERIALS AND METHODS

2.1 Plant Collection

Leaves, stems, roots and fruits of *A. malaccaencis* were collected from randomly selected plants growing in Kỳ Sơn Districts, Nghệ An Province, Vietnam, in May 2014. A voucher specimen, LTH 428, was deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2 Isolation of Volatile Oils

Aliquots of 0.5 kg each of air-dried plant samples were subjected to separate hydrodistillation for 4 h at normal pressure, according to the Vietnamese Pharmacopoeia [18]. The yields of essential oils were 0.25%, 0.19%, 0.32% and 0.25% (v/w, calculated on dry weight basis). Oil samples were light yellow in colouration.

2.3 Gas chromatography (GC) Analysis

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m x 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were: carrier gas H_2 (1 mL/min), injector temperature (PTV) 250°C, detector temperature 260°C, column temperature programmed from 60°C (2 min hold) to 220°C (10 min hold) at 4°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. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

2.4 Gas Chromatography- mass Spectrometry (GC/MS) Analysis

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for the gas chromatography- mass spectrometry (GC/MS) analysis, under the same conditions as those used for GC analysis. Helium (1 mL/min) was the carrier gas. 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.

2.5 Identification of the Constituents

The identification of constituents was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes, under identical experimental conditions, co-injection with standards or known essential oil constituents from home made library and by comparing with MS literature data [19-21].

3. RESULTS AND DISCUSSION

The identities and percentages of the compounds present in the studied oil samples could be seen in Table 1. Monoterpene hydrocarbons (74.0%) were the dominant class of compound in the leaf oil of *A. malaccencis*. The sesquiterpene compounds occurred in lesser amount (15.2%). β-Pinene (560%) and α-pinene (10.3%) were the main oil constituents. The abundance of β-pinene and α-pinene makes the oil similar to the leaf sample from Indonesia [12]. However, the oil differs from the Indonesian [13] and China [15] samples to its low content of methyl cinnamate. Moreover, 1,8-cineole a significant component of Indonesian sample was absent in the present study.

Also, monoterpene hydrocarbons (81.3%) predominate in the stem oil while the sesquiterpene compounds occurred in the amount of 10.4%. The compounds occurring in higher quantities were β-pinene (46.0%), β-phellandrene (12.1%), α-pinene (9.8%) and α-phellandrene (5.7%). This compositional pattern differs from data obtained from Indonesia [13] and Malaysia [17] oil samples where methyl cinnamate, 1,8-cineole, α-pinene and β-pinene predominates. The proportion of methyl cinnamate in our oil sample was insignificant (0.5%) while 1,8-cineole was not detected.

Sesquiterpene compounds (21.5%) were identified in appreciable quantity in the roots oil, although monoterpene hydrocarbons (59.3%) are abundant. The main constituents were β-pinene (31.7%), β-phellandrene (12.9%), α-pinene (6.3%) and α-selina-6-en-4-ol (5.5%). This data may represent the first analysis of the root oil.

Table 1. Constituents of *Alpinia malaccencis* oil samples from Vietnam

Compounds[a]	MI	RI[b]	RI[c]	Percent composition (%)			
				Leaf	Stem	Root	Fruit
α-Thujene	MS, RI	930	921	0.2	0.3	0.2	0.2
α-Pinene	MS, RI, Co	939	932	10.3	9.8	6.3	5.9
Camphene	MS, RI	953	946	0.9	0.7	1.0	1.1
β-Pinene	MS, RI, Co	980	974	56.0	46.0	31.7	18.5
β-Myrcene	MS, RI	990	988	0.9	1.5	1.0	1.1
α-Phellandrene	MS, RI	1006	1002	0.3	5.7	3.0	3.5
δ-3-Carene	MS, RI	1011	1008	3.3	-	-	0.1
α-Terpinene	MS, RI	1017	1014	0.2	0.6	0.4	0.3
o-Cymene	MS, RI	1024	1022	0.4	2.5	1.1	1.3
β-Phellandrene	MS, RI, Co	1028	1025	-	12.1	12.9	12.9
(*E*)-β-Ocimene	MS, RI	1052	1044	0.2	0.1	0.1	0.8
γ-Terpinene	MS, RI	1061	1054	0.4	1.0	1.0	0.5
α-Terpinolene	MS, RI	1090	1086	0.2	1.0	0.6	0.4
Linalool	MS, RI	1100	1095	-	-	-	0.7
Fenchyl alcohol	MS, RI	1122	1118	-	-	0.1	-
allo-Ocimene	MS, RI	1128	1128	0.7	-	-	0.1
trans-Pinocarveol	MS, RI	1131	1135	0.2	0.1	-	0.1
Camphor	MS, RI	1145	1141	-	-	0.1	0.3
Pinocarvone	MS, RI	1165	1160	0.2	0.4	0.2	-
Borneol	MS, RI	1167	1165	-	-	-	0.3
Terpinen-4-ol	MS, RI	1177	1174	-	0.3	0.2	0.5
α-Terpineol	MS, RI	1189	1186	-	0.2	0.2	0.4
Myrtenal	MS, RI	1209	1195	0.9	0.4	0.4	0.2
trans-Carveol	MS, RI, Co	1217	1215	-	-	0.3	-
exo-Fenchyl acetate	MS, RI, Co	1228	1229	-	-	3.7	0.3
Cumin aldehyde	MS, RI	1236	1238	-	0.1	-	-
(*Z*)-Citral	MS, RI, Co	1240	1242	0.2	0.1	-	0.2
Methyl hydrocinnamate	MS, RI	1280	1276	-	-	-	0.3
Myrtenyl acetate	MS, RI	1334	1324	0.4	-	-	-
Bicycloelemene	MS, RI, Co	1327	1338	-	0.2	-	0.3
δ-Elemene	MS, RI, Co	1340	1335	-	-	0.2	-
α-Cubebene	MS, RI	1351	1345	-	0.1	-	0.2
α-Copaene	MS, RI	1377	1374	0.3	0.2	-	0.7
(*E*)-Methyl cinnamate	MS, RI, Co	1379	1376	2.2	0.5	1.3	27.8
β-Cubebene	MS, RI	1386	1387	0.8	0.7	2.3	0.3
β-Elemene	MS, RI	1391	1389	0.4	0.5	0.3	-
Longifolene	MS, RI	1404	1407	-	-	-	0.2
α-Gurjunene	MS, RI	1412	1409	0.3	0.2	-	0.2
β-Caryophyllene	MS, RI	1419	1417	0.8	0.7	0.5	1.9
Widdrene	MS, RI	1430	1430	0.3	0.5	-	-
β-Gurjunene	MS, RI	1434	1431	-	0.4	-	0.5
Aromadendrene	MS, RI	1441	1439	0.7	-	-	-
α-Humulene	MS, RI	1454	1452	0.5	0.5	-	0.7
Selina-4(15),7(11)-diene	MS, RI, Co	1473	1470	-	-	3.1	-
γ-Gurjunene	MS, RI, Co	1477	1475	-	-	-	0.4
Germacrene D	MS, RI	1485	1484	-	-	-	1.4
α-Amorphene	MS, RI	1485	1487	-	-	-	0.2
β-Selinene	MS, RI	1489	1489	-	-	-	0.2
Zingiberene	MS, RI	1494	1493	1.0	1.9	-	-
Cadina-1,4-diene	MS, RI	1496	1496	-	-	-	0.1
Bicyclogermacrene	MS, RI	1500	1500	-	-	-	1.0
β-Bisabolene	MS, RI	1506	1505	-	0.8	-	0.4
γ-Cadinene	MS, RI, Co	1513	1513	-	0.2	-	-

Compounds[a]	MI	RI[b]	RI[c]	Percent composition (%)			
				Leaf	Stem	Root	Fruit
trans-γ-Bisabolene	MS, RI, Co	1516	1514	-	0.3	-	-
β-Agarofuran	MS, RI, Co	1520	1516	0.9	-	1.7	-
β-Sesquiphellandrene	MS, RI	1524	1521	-	-	0.7	-
δ-Cadinene	MS, RI, Co	1525	1522	0.7	1.1	1.4	0.8
Calacorene	MS, RI	1546	1544	0.3	-	-	-
Selina-3,7(11)-diene	MS, RI	1547	1545	-	-	0.6	-
α-Agarofuran	MS, RI	1548	1548	0.2	-	0.6	-
Germacrene B	MS, RI	1561	1559	-	-	-	0.4
(*E*)-Nerolidol	MS, RI	1563	1561	-	0.2	-	0.1
1,5-Epoxysalvial-4(14)-ene	MS, RI, Co	1564	1557	-	-	-	0.1
Spathulenol	MS, RI	1578	1577	0.2	-	-	2.0
Caryophyllene oxide	MS, RI	1583	1581	0.6	0.4	4.1	2.3
Globulol	MS, RI	1585	1590	2.1	-	-	-
Viridiflorol	MS, RI	1593	1592	2.3	-	-	-
Guaiol	MS, RI	1601	1600	-	0.4	0.4	-
Aromadendrene epoxide	MS, RI	1623	1639	-	-	-	0.5
τ-Muurolol	MS, RI, Co	1646	1640	0.4	0.5	-	0.6
α-Selina-6-en-4-ol	MS, RI, Co	1648	1650	0.5	-	5.5	-
α-Cadinol	MS, RI	1654	1652	0.8	0.6	-	0.9
10-nor-Calamenen-10-one	MS, RI, Co	1702	1702	-	-	-	0.2
Vulgarol A	MS, RI, Co	1708	1708	-	-	-	0.2
Valerenol	MS, RI	1711	1711	-	-	-	0.2
Farnesyl acetate	MS, RI	1726	1722	1.0	-	-	-
Benzyl benzoate	MS, RI	1760	1759	1.3	-	0.2	-
Benzyl salicylate	MS, RI	1866	1864	-	-	0.9	-
8,9-Dehydro-9-formyl-cycloisolongifolene	MS, RI, Co	2082	2082	-	-	1.3	-
Phytol	MS, RI	2125	2122	-	-	1.2	0.2
Total				**94.6**	**93.8**	**90.8**	**94.8**
Monoterpene hydrocarbons				**74.0**	**81.3**	**59.3**	**46.7**
Oxygenated monoterpenes				**4.1**	**2.1**	**6.5**	**31.1**
Sesquiterpene hydrocarbons				**7.3**	**8.3**	**11.4**	**9.7**
Oxygenated sesquiterpenes				**7.9**	**2.1**	**10.0**	**7.1**
Diterpenes				**-**	**-**	**2.5**	**0.2**
Aromatic esters				**1.3**	**-**	**1.1**	**-**

[a] Elution order on HP-5 MS column; [b] Retention indices on HP-5MS column; [c] Literature retention indices; - Not identified; MI, Mode of identification; MS, Mass spectrum; RI, Retention indices; Co, Co-injection with authentic sample

Oxygenated monoterpenes (31.1%) and monoterpene hydrocarbons (46.7%) constituted the main classes of compounds identified in the fruit oil. This oil had its quantitatively significant compounds to be methyl cinnamate (27.8%), β-pinene (18.5%) and β-phellandrene (12.9%).

This paper reports for the first time, the composition of the root and fruit oils of *A. malaccencis*. Although, β-pinene, α-pinene and methyl cinnamate were identified previously in *A. malaccencis*, some other compositional variations were observed. The present data were devoid of 1, 8-cineole, *p*-cymene, 13,14,15,16-tetranor-8(17)-labden-12-al and (*E*)-labda-8(17),12-diene-15,16-dial that were identified in the oils of *A. malaccencis* from Bangladesh [1], Malaysia [9], India [10-12], Indonesia [13], Thailand [14] and China [15]. α-Phellandrene, a major compound of Bangladesh [1] and India [10,11] oil samples occurred in much lower amounts (3.0%) in this study while fenchyl acetate was only identified in the root and fruit oil at lower amounts when compared with oil from Thailand [14]. Both β-phellandrene and α-selina-6-en-4-ol present in this result of *A. malaccencis* were not identified in the previous studies. Moreover, all the main compounds found in the seed oil of the plant from China [15] were not detected in the present investigated oils. The low content of camphor in these oil samples and the absence of sabinene were the other major

differences between the Vietnam and Indian [12] oils.

Although, it is well known that different parts of a plant could contain different chemical constituents [18], the observed compositional pattern may indicate that there is no homogeneity in the oil compositions of *A. malaccencis,* which exhibit chemical variability. The compositional variations between the same plant parts may be attributed to differences in the ecological and climatic conditions between Vietnam and other parts of the world as well as the age and nature of the plant, chemotype, handling procedure etc. [18].

4. CONCLUSION

For the first time the compositions of essential oils from the leaf and stem of *A. malaccencis* grown in Vietnam were being reported. The volatile constituents of the root and fruit oils from Vietnam and elsewhere were also being reported for the first time. It was noted that some compound such as β-pinene, α-pinene and methyl cinnamate were identified in this study as well as previous ones from other parts of the world. However, some other constituents such as sabinene, 1, 8-cineole, *p*-cymene, 13,14,15,16-tetranor-8(17)-labden-12-al, (*E*)-labda-8(17),12-diene-15,16-dial, citronelllol, 4-phenyl-3-buten-2-one, decanoic acid, geranyl acetate, nerolidol, lauric acid, α-farnesol, β-farnesol, myristic acid and palmitic acid that were identified in the oils of *A. malaccencis* from previous studies were absent in the present result. Also, the low camphor content in the oils conferred another compositional variation between the Vietnam oil samples and previous ones. A noteworthy observation was that both β-phellandrene and α-selina-6-en-4-ol present in this result of *A. malaccencis* were not identified in the previous studies.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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