

Artículo Original | Original Article

Constituents of essential oils from the leaf, stem, root, fruit and flower of *Alpinia macroura* K. Schum

[Constituyentes de los aceites esenciales de las hojas, tallo, raíz y flores de *Alpinia macroura* K. Schum]Le T Huong¹, Do N Dai², Mai V Chung¹, Doan M Dung³ & Isiaka A Ogunwande⁴¹Faculty of Biology, Vinh University, 182-Le Duan, Vinh City, Nghe An Province, Vietnam²Faculty of Agriculture, Forestry & Fishery, Nghe An College of Economics, 51-Ly Tu Trong, Vinh City, Nghe An Province, Vietnam³Faculty of Chemistry, Vinh University, 182-Le Duan, Vinh City, Nghe An Province, Vietnam⁴Natural Products Research Unit, Department of Chemistry, Faculty of Science, Lagos State University, Badagry Expressway Ojo, P. M. B. 0001, Lasu Post Office, Ojo, Lagos, NigeriaContactos | Contacts: Do N DAI - E-mail address: daidn23@gmail.comContactos | Contacts: Isiaka A OGUNWANDE - E-mail address: isiaka.ogunwande@lasu.edu.ng

Abstract: This paper reports the chemical constituents of essential oils from the various parts of *Alpinia macroura* K. Schum (Zingiberaceae) from Vietnam. The essential oils were obtained by hydrodistillation and analysed by means of gas chromatography coupled to Flame ionization detector (GC-FID) and gas chromatography coupled to mass spectrometry (GC/MS). The main constituents of the oils were β -pinene (8.8%-16.4%), 1,8-cineole (5.5%-17.7%), γ -terpinene (5.9%-16.9%), α -pinene (4.5%-8.4%) and β -caryophyllene (1.4%-18.6%). Sabinene (9.0%) was identified only in the fruit. Overall, nineteen of the identified compounds are coming to all the essential oils. The chemical constituents of essential oils from the leaf, stem, root, fruit and flower of *A. macroura* are being reported for the first time and were found to be different from those of other *Alpinia* oils.

Keywords: *Alpinia macroura*, hydrodistillation, essential oil, monoterpenes, sesquiterpenes

Resumen: En este trabajo se presentan los componentes químicos de los aceites esenciales de las distintas partes de *Alpinia macroura* K. Schum (Zingiberaceae) de Vietnam. Los aceites esenciales se obtuvieron por hidrodestilación y se analizaron por medio de cromatografía de gases acoplada a detector de ionización de llama (GC-FID) y cromatografía de gases acoplada a espectrometría de masas (GC/MS). Los principales constituyentes de los aceites fueron β -pineno (8,8% -16,4%), 1,8-cineol (5,5% -17,7%), γ -terpineno (5,9% -16,9%), α -pineno (4,5% -8,4 %) y β -cariofileno (1,4% -18,6%). Sabineno (9,0%) fue identificado solamente en la fruta. En general, diecinueve de los compuestos identificados están llegando a todos los aceites esenciales. Los componentes químicos de los aceites esenciales de la hoja, tallo, raíz, frutas y flores de *A. macroura* están siendo reportados por primera vez y se encontró que eran diferentes de las de otros aceites de *Alpinia*.

Palabras clave: *Alpinia macroura*, hidrodestilación, aceite esencial, monoterpenos, sesquiterpenos.

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Abbreviation List: v/w- volume by weight; GC-FID Gas chromatography coupled to Flame Ionization Detector; GC-MS- Gas chromatography coupled to mass spectrometry.

INTRODUCTION

The genus *Alpinia* from the *Zingiberaceae* family of plants consists of more than 230 species (Krees *et al.*, 2005). *Alpinia macroura* is pseudostem plant of about 2-2.5 m high with oblong leaf blades. The labellium are red with yellow margin. The globular fruits are white and are about 2-2.5 cm long. Flowering takes place in August to December while fruiting occurs in November to March of later year. It is native to Vietnam, Thailand, Mianma, Laos and Cambodia (Binh, 2011). The plant is used ethnomedically in the treatment of sores, fever and intestinal infections (Huong, 2016). The authors are not aware of any information on the biological potentials and non-volatile phytochemical constituents on this plant.

There are no previous references in literature about the chemical composition of essential oil of this plant from Vietnam or elsewhere and this prompted the present investigation of the volatile constituents of *A. macroura*. In continuation of an extensive study into the chemical constituents of underutilized flora of Vietnam (Chau *et al.*, 2015; Dai *et al.*, 2016), we report the compounds identified in the essential oils obtained by hydrodistillation of the leaf, stem, root, fruit and flower of *A. macroura*.

MATERIALS AND METHOD

Plants collection

Leaves, stems, roots, fruits and flowers of *A. macroura* were collected from Pù Mát National Park, Nghệ An Province, Vietnam, in May 2014. Voucher specimen DND 389 was deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to hydrodistillation.

Hydrodistillation of the essential oils

Briefly, 500 g of each of the pulverized sample were carefully introduced into a 5 L flask and distilled water was added until it covers the sample completely. Hydrodistillation was carried out in an all glass Clevenger-type distillation unit for 3 h, according to established procedure (Vietnamese Pharmacopoeia, 1997), at normal pressure. The volatile oils distilled over water and were collected separately in the receiver arm of the apparatus into a

clean and previously weighed sample bottles. The oils were kept under refrigeration until the moment of analyses.

Analysis of the essential oils

Gas chromatography (GC) analyses of essential oils were carried on Agilent Technologies HP 6890 Plus Gas Chromatograph which was equipped with a flame ionization detector and HP-5MS column. The dimension of the column is 30 m x 0.25 mm (film thickness 0.25 μm). The GC operating parameters based on temperature programming were as follows oven temperature 40° C, injection port 250° C while the detector temperature was 260° C. Oven temperature programming: 40° C for 2 min, and then raise to 220° C (and held isothermally for 10 min) at 4° C/min. The carrier gas used was H₂ at a flow rate of 1 mL/min. The split ratio was 10:1 while 1.0 μL of the diluted essential oil in hexane was injected into the GC at inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. Retention indices (RI) value of each component was determined relative to the retention times of a homologous n-alkane series with linear interpolation on the HP-5MS column. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors.

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5MS 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) analyses, 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. 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 (NIST, 2011) 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 (Adams, 2007).

RESULTS AND DISCUSSION

The yield of essential oils were 0.21% (v/w, leaf), 0.15% (v/w, stem), 0.24% (v/w, root), 0.18% (v/w, fruit) and 0.30% (v/w, flower), calculated on a dry weight basis. Oil samples were light yellow (leaf and root), yellow (stem) and colourless (fruit and flower). Table 1 indicates the chemical constituents present in the oils, their percentages as well as retention indices on HP-5MS column. Monoterpenes hydrocarbons (59.9%) and the oxygenated counterparts (27.2%) represent the main classes of compounds present in the leaf oil. Sesquiterpenes are less common (ca.

5.7%). 1,8-Cineole (17.7%), γ -terpinene (13.3%) and β -pinene (11.4%) were the compounds occurring in higher quantity. There are significant amounts of α -phellandrene (8.5%), α -pinene (7.8%), α -terpinene (6.4%) and terpinen-4-ol (5.9%). On the other hand, monoterpenes hydrocarbons (71.9%) and oxygenated counterparts (13.6%) were the main classes of compounds identified in the stem, of which the main compounds were γ -terpinene (16.9%), β -pinene (16.4%), 1,8-cineole (11.2%) and α -terpinene (9.4%). α -Pinene (8.4%) and α -phellandrene (5.9%) were also present in sizeable quantity.

Table 1
Chemical constituents of *A. macruora* oils^a

Compounds ^b	RI ^c	RI ^d	MI ^e	L	S	R	Fr	Fl
α -Thujene	930	921	f	1.6	2.0	1.9	0.4	0.6
α -Pinene	939	932	g	7.8	8.4	6.5	5.8	4.5
Camphene	953	946	f	1.2	2.1	2.9	1.3	0.8
Sabinene	976	969	g	-	-	-	-	9.0
β -Pinene	980	976	g	11.4	16.4	12.5	11.1	8.8
β -Myrcene	990	988	g	1.3	1.6	1.4	1.3	1.1
α -Phellandrene	1006	1004	g	8.5	5.9	1.9	3.7	1.5
δ -3-Carene	1011	1008	f	-	-	0.2	-	-
α -Terpinene	1017	1014	g	6.4	9.4	8.5	3.2	4.7
<i>o</i> -Cymene	1024	1022	f	4.5	3.8	2.1	1.2	0.6
β -Phellandrene	1028		f	-	-	-	-	3.4
1,8-Cineole	1034	1032	g	17.7	11.2	8.7	11.1	5.5
(<i>E</i>)- β -Ocimene	1052	1044	f	0.6	1.0	0.7	0.6	0.6
γ -Terpinene	1061	1054	f	13.3	16.9	13.9	5.9	7.9
<i>cis</i> -Sabinene hydrate	1073	1065	f	0.1	-	-	-	-
α -Terpinolene	1090	1089	f	3.3	4.4	3.7	1.4	1.3
Linalool	1100	1095	f	1.6	0.3	0.4	-	0.5
Nonanal	1106	1100	f	-	-	-	0.3	-
Fenchyl alcohol	1120	1118	f	0.1	-	0.1	-	-
<i>allo</i> -Ocimene	1128	1128	f	-	-	0.1	-	-
Camphor	1145	1141	f	0.1	-	0.2	0.1	0.3
Borneol	1167	1167	f	0.5	0.1	0.5	0.1	-
Terpinen-4-ol	1177	1177	f	5.9	1.1	2.3	0.6	4.4
α -Terpineol	1189	1187	f	0.7	0.1	0.2	0.3	1.5
Myrtenal	1207	1195	f	-	-	0.1	-	-
<i>trans</i> -Piperitol	1209	1207	f	0.2	-	-	-	-
Fenchyl acetate	1222	1229	f	0.1	0.3	7.8	0.3	-
4-Phenyl-2-butanol ⁱ	1241	1243	f	2.2	-	-	-	-
Geraniol	1253	1249	f	-	-	0.4	0.3	3.5
Bornyl acetate	1289	1287	g	0.2	0.3	1.0	5.0	7.2
Bicycloelemene	1327	1337	f	-	-	0.5	-	1.5
Neryl acetate	1362	1359	f	-	-	-	0.1	-
α -Copaene	1377	1374	f	0.1	0.2	0.7	0.2	0.5

Geranyl acetate	1381	1379	f	-	-	-	0.1	-
β -Elemene	1391	1389	f	1.7	1.3	0.8	3.4	0.7
α -Gurjunene	1412	1409	f	1.1	1.0	1.5	1.2	-
β -Caryophyllene	1419	1417	g	1.4	2.4	2.3	18.6	12.6
Widdrene	1431	1432	f	-	-	-	-	0.7
γ -Elemene	1437	1434	f	-	0.1	0.4	0.3	3.6
α -Guaiene	1440	1437	f	0.4	-	-	-	1.8
Aromadendrene	1441	1439	f	-	0.2	0.1	0.3	0.6
α -Humulene	1454	1452	f	0.2	0.5	0.8	3.1	1.0
Selina-4(15),7(11)-diene	1475	1470	g	-	-	0.4	-	-
γ -Gurjunene	1477	1475	f	-	-	-	0.1	1.0
γ -Muurolene	1480	1478	f	-	-	-	0.2	-
Germacrene D	1485	1484	f	-	0.2	0.6	0.5	3.3
α -Amorphene	1485	1485	f	-	-	0.2	0.2	-
β -Selinene	1486	1488	f	-	0.4	0.6	1.1	-
α -Selinene	1493	1498	f	0.3	0.7	-	-	-
Bicyclogermacrene	1500	1500	g	-	-	0.9	0.7	0.9
α -Muurolene	1500	1500	f	-	-	0.1	-	-
β -Bisabolene	1506	1505	f	-	-	0.1	-	-
(<i>E,E</i>)- α -Farnesene	1508	1507	g	-	-	-	0.1	-
δ -Cadinene	1525	1522	f	0.2	0.4	0.7	0.5	-
(<i>E</i>)-Nerolidol	1563	1561	f	0.2	0.3	0.8	0.3	-
Spathulenol	1578	1577	f	-	-	0.3	-	0.7
Caryophyllene oxide	1583	1581	g	0.3	0.4	0.6	7.1	0.7
τ -Muurolol	1646	1640	f	-	-	1.0	-	-
α -Cadinol	1654	1652	f	-	0.2	-	0.8	-
(<i>E,E</i>)-Farnesol	1718	1722	f	-	-	0.4	0.5	0.4
Phytol	2125	2119	g	-	-	0.2	0.1	-
Total				95.2	93.6	92.0	93.5	97.7
Monoterpene hydrocarbons				59.9	71.9	56.3	35.9	44.8
Oxygenated monoterpenes				27.2	13.6	21.7	18.0	22.9
Sesquiterpene hydrocarbons				5.4	7.2	10.7	30.5	28.2
Oxygenated monoterpenes				0.5	0.9	3.1	8.7	1.8
Diterpenes				-	-	0.2	0.1	-
Non-terpenes				2.2	-	-	0.3	-

^a SD, values were insignificant and excluded from the Table to avoid congestion; ^b Elution order from HP-5MS column; ^c Retention indices on HP-5MS column; ^d Literature retention indices; ^e Mode of Identification; ^f Identification by mass spectra and GC retention indices; ^g Identification by mass spectra, column retention indices and co-injection with authentic compound; ⁱ tentative identification; - Not found; L leaf; S stem; r root; Fr fruit; Fl flower

The main classes of compounds in the root oil were monoterpene hydrocarbons (56.3%), oxygenated monoterpenes (21.7%) and sesquiterpene hydrocarbons (10.7%). Moreover, the main constituents of the oil were γ -terpinene (13.9%), β -pinene (12.5%), 1,8-cineole (8.7%), α -terpinene (8.5%), fenchyl acetate (7.8%) and α -pinene (6.5%). The authors have identified a large proportion of

monoterpene hydrocarbons (35.9%), sesquiterpene hydrocarbons (30.5%) and oxygenated monoterpenes (18.0%) in the fruit. The oil was rich in β -caryophyllene (18.1%), β -pinene (11.1%) and 1,8-cineole (11.1%). In addition, caryophyllene oxide (7.1%), γ -terpinene (5.9%), α -pinene (5.8%) and bornyl acetate (5.0%) were also present in significant quantity. Moreover, monoterpene hydrocarbons

(44.8%), oxygen containing monoterpenes (22.9%) and sesquiterpene hydrocarbons (18.2%) were prominent in the flower oil. The quantitatively significant constituents of the oil were β -caryophyllene (12.6%), sabinene (9.0%), β -pinene (8.8%), γ -terpinene (7.9%) and bornyl acetate (7.2%).

The authors are unaware of any previous study on the essential oils of *A. macruora*. The present report may represent the first of its kind. However, relative information is available on the compositions of volatiles from some other *Alpinia* species growing in Vietnam. For example, the fruit oil of *A. menghaiensis* (Dai et al., 2016) comprised mainly of monoterpenes represented by β -pinene (40.4%) and α -pinene (11.3%). The leaf oil of *A. polyantha* has its major compounds as camphor (16.1%), α -pinene (15.2%) and β -agarofuran (12.9%), while α -pinene (12.4%), β -cubebene (10.6%) and β -agarofuran (10.3%) were present in the stem (Huong et al., 2015a). However, while β -cubebene (12.6%) and fenchyl acetate (10.8%) were identified in the root, the fruit was rich in δ -cadinene (10.9%), β -caryophyllene (9.1%) and β -pinene (8.7%). 1,8-Cineole was the most significant compound of rhizomes of *A. henryi* (Giang et al., 2007) and *A. laosensis* (Dung et al., 2000). Sesquiterpenes were the dominant compounds in the essential oils of *A. chinensis*. These include β -bisabolene (47.9%) in the leaf (Dung et al., 1994a), caryophyllene oxide (13.2%) and β -bisabolene (10.4%) in the root (Leclercq et al., 1994) as well as (*E,E*)- α -farnesene (26.5%), α -humulene (22.3%), β -bisabolene (17.1%) and β -caryophyllene (13.1%) in the flower (Dung et al., 1994b). The composition of the flowers of *A. speciosa* (Dung et al., 1994c) consisted mainly of terpene hydrocarbons β -pinene (34.0%), α -pinene (14.8%) and β -caryophyllene (10.8%). A previous study reported abundance of α -pinene and β -pinene in the leaf oils of *A. malaccensis* (Huong et al., 2015b). In another study, while (*E,E*)-farnesol make up the oil of the seed of *A. breviligulata* (Dung, et al., 1994d), α -pinene and β -pinene occurred in the leaf oil (Dung et al., 1994f). The seed oil of *A. katsumadai* was dominated by myrcene, linalool and citronellol (Dung et al., 1990). Although ubiquitous terpenes predominate in the essential oils of other *Alpinia* plants and *A. macruora* from Vietnam (Dung et al., 1993; Dung et al., 1994e), some variation could be seen among the components of essential oils. The main compounds of other *Alpinia* essential oils from Vietnam such as myrcene, citronellol fenchyl acetate, β -cubebene, β -

agarofuran, (*E,E*)-farnesol, β -cubebene, δ -cadinene, β -bisabolene, α -humulene and caryophyllene oxide were either absent or present in lower amounts in *A. macruora*.

The chemical constituents of essential oils of *Alpinia* plants from some other parts of the world have been documented. The major constituents of *A. calcarata* from India were 1,8-cineole (35.9%) and β -fenchyl acetate (12.9%), while α -terpineol (15.1%), α -fenchyl acetate (12.5%), (*E*)-nerolidol (10.1%) and caryophyllene oxide (10.1%) were the main compounds of *A. smithiae* (Raji et al., 2013). 1,8-Cineole (17.4%) and humulene epoxide II (14.1%) are the main components of *A. allughas* from India which displayed antioxidant and antifungal activities (Sethi et al., 2015). An analysis from Malaysia reported the abundance of β -sesquiphellandrene (36.5%) and (*E*)-methyl cinnamate (78.2%) in the rhizomes of *A. aquatica* and *A. malaccensis* respectively (Sirat et al., 1995). The leaf oil of *A. purpurata* (Victório et al., 2010) from Brazil was rich in β -pinene (34.7%) and α -pinene (11.8%). Another report identified *trans*-caryophyllene (32.61%) in the leaf; 1,8-cineole (32.25%) and myrcene (13.63%) from the pseudostem; tetracosane (42.61%) in the rhizome and tetracosane (13.39%) from the fruit of *A. rafflesiana* grown in Malaysia (Jusoh et al., 2013). The major components of *A. galanga* (Suresh et al., 2016) were 1,8-cineole (32.9%) and α -terpineol (12.7%). The Korean specie of *A. kwangsiensis* (Wu et al., 2015) rhizome afforded oil whose main compounds were camphor (17.59%), eucalyptol (15.16%), β -pinene (11.15%) and α -pinene (10.50%). The major components of rhizome oil of *A. conchigera* from Malaysia (Sirat et al., 1995) include β -sesquiphellandrene (20.5%), β -bisabolene (12.1%) and 1,8-cineole (11.6%). The rhizome of *A. speciosa* collected from India (Akshaya et al., 2010) contained terpinen-4-ol (15.4%) and 1,8-cineole (11.1%). The main constituents of essential oils of *A. nutans* were sabinene (27.8%), 1,8-cineole (17.4%) and terpinen-4-ol (14.9%) in the aerial parts while terpinen-4-ol (25.1%), γ -terpinene (19.4%), sabinene (14.2%) and 1,8-cineole (10.8%) were present in the flower (Joshi et al., 2010).

Both inter-species and intra-species variation could be observed in the essential oils of *Alpinia* plants. For example, valencene (19.04%), calamenene (10.11%) and nootkatone (8.97%) were the main components of *A. oxyphylla* from China (Wang et al., 2014). In another report, myrtenal (10.25%) and α -citral (9.85%) occurred as the major

component in leaf oil of *A. oxyphylla* from another part of China (Feng *et al.*, 2012). Terpinen-7-ol (40.5%) and sabinene hydrate (15.4%) were identified in *A. zerumbet* from Brazil (Victório *et al.*, 2010), while, 4-terpineol (32.9%), 1,8-cineole (21.4%) and γ -terpinolene (10.0%) were the monoterpenes of *A. zerumbet* from another region of Brazil (Mendes *et al.*, 2015). In addition, the major components in the oil of *A. calcarata* from India (Suresh *et al.*, 2016) were α -fenchyl acetate (12.9%), cubenol (15.0 %) and 1,8-cineole (12.1 %) while another sample from India contained 1,8-cineole (35.9%) and β -fenchyl acetate (12.9%). Also, 1,8-Cineole (25.4%), β -pinene (15.1%) and camphor (15.3%) were identified in the leaf of *A. nigra* (Kanjilal *et al.*, 2010), while the rhizome comprised of 1,8-cineole (34%) and α -fenchyl acetate (13.1%). Moreover, fresh leaf of *A. nigra* from Bangladesh (Islam *et al.*, 2014) gave oil containing large amount of 1,5,9,9-tetramethyl-1,4,7-cycloundecatriene (24.92%), β -pinene (12.90%), 5-amino-6-(2-fluoroanilino)furazano[3,4-b] pyrazine (12.18%) and isocaryophyllene (10.76%).

It could be seen that the essential oils of *Alpinia* plants from all over the world exhibited chemical variability. In addition, the combination of the observed major compounds of the leaf, stem and root of *A. macruora* (γ -terpinene/ β -pinene/1,8-cineole, β -caryophyllene/ β -pinene/1,8-cineole in the fruit, as well as β -caryophyllene/sabinene in the flower, were not reported previously for any of the studied *Alpinia* essential oils from Vietnam and other parts of the world. It was well known that each plant parts contained different phytochemical composition. The variation between these results and those from other parts of the world may be due to the ecological and climatic differences between these regions; as well as the age of the plants and chemotype.

The observed compositional patterns may have contributed to the biological activities of *Alpinia* essential oils such as antimicrobial (Joshi *et al.*, 2010; Jusoh *et al.*, 2013), antifungal (Sethi *et al.*, 2015), insecticidal (Wu *et al.*, 2015), modulation of the activity of aminoglycoside antibiotics (Mendes *et al.*, 2015), cytotoxic (Wang *et al.*, 2014), antioxidant (Joshi *et al.*, 2010; Wang *et al.*, 2014; Sethi *et al.*, 2015) and enhances skin permeation (Feng *et al.*, 2012).

CONCLUSION

The compositions of the leaf, stem, root, fruit and flower oils of *A. macruora* from Vietnam were reported. The essential oils were characterized by high contents of γ -terpinene/ β -pinene/1,8-cineole in the leaf, stem and root, β -caryophyllene/ β -pinene/1,8-cineole in the fruit and β -caryophyllene/sabinene in the flower. In addition, a comparative analysis of the chemical compositions of the studied oil samples with data on essential oils of other *Alpinia* plants from Vietnam and other parts of the world revealed a high chemical variation.

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