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Short Communication

Chemical constituents of essential oil from the stem of *Amomum villosum* Lour

Do N. Dai[1,✉], Le T. Huong[2], Tran D. Thang[3], Isiaka A. Ogunwande[4,✉] and Olanrewaju I. Eresanya[4]

[1]Faculty of Agriculture, Forestry and Fishery, Nghe An College of Economics, 51-Ly Tu Trong, Vinh City, Nghe An Province, Vietnam
[2]Faculty of Biology, Vinh University, 182-Le Duan, Vinh City, Nghe An Province, Vietnam
[3]Faculty of Chemistry, Vinh University, 182-Le Duan, Vinh City, Nghe An Province, Vietnam
[4]Natural Products Research Unit, Department of Chemistry, Faculty of Science, Lagos State University, Badagry Expressway Ojo, P. M. B. 0001, LASU Post Office, Ojo, Lagos, Nigeria

ABSTRACT

The chemical constituents of essential oil of *Amomum villosum* Lour stem grown in Vietnam was investigated by gas chromatography in combination with (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The essential oil was obtained by hydrodistillation. The yield of the essential oil was 0.21% (v/w) calculated on a dry weight basis. The GC/MS analysis of the essential oil revealed the identification of 36 compounds in which β-pinene (48.1%), α-pinene (16.9%) and methyl chavicol (7.0%) were the main constituents. The present result is the first of its kind aimed at the characterization of the chemical composition essential oil from the stem of *A. villosum* Lour.

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1. Introduction

Amomum villosum Lour., is an evergreen plant in the ginger family, growing in the shade of the tree, 1.5 to 3 meters high. The plant with a specific characteristic that flowers spread on the ground can bear fruit, while flowers on the branches cannot. The flowers bloom in March and April and its color is like white jade. It fruits in June and July. The fruit is up to 2 cm long, reddish-brown and covered by small spines and contain strongly aromatic seeds (Zhou, 1993). In Vietnamese traditional medicine, the fruits are used to treat indigestion, diarrhoea, flatulence, toothache, and as febrifuge and antiseptic. The seeds are antibacterial and stomachic and are also used in the treatment of dyspepsia, colic, flatulence, diarrhoea, vomiting and oedema. They are an ingredient in a formula used to treat threatened abortions. The powdered seed is applied to carious patches on the teeth to address toothache (Liu et al., 2006).

Extracts from *A. villosum* Lour are known to possess remarkable antioxidant activities (You et al., 2012; Zhang et al., 2013) and increase the longitudinal bone growth by stimulation of the chondrocyte hypertrophy and chondrogenesis, through regulation of IGF-1 and BMP signaling in the growth plate (Lee et al., 2012). Some of the compounds previously isolated from *A. villosum* Lour include 3-ethoxy-hydroxy benzoic acid,vanillic acid-1-β-D-glucopyranosyl ester, isorhamnetin-3-β-D-glucoside, flavanocoumarin and isoflavanocoumarin (Chen et al., 2012), quercetin-3-*O*-α-L-rhamnoside and quercetin-3-*O*-β-D-glucoside (Sun et al., 2002), ergosta-7,22dien-3β,5α,6β-triol, stearic acid, palmitic acid, typhonoside B and polygonin (An et al., 2011), (*E*)-*p*-hydroxycinnamic acid, (*E*)-*p*-carboxycinnamic acid and 3,3',4,4'-tetrahydroxybiphenyl (Fu et al., 2011).

Previous studies from Vietnam (Dai et al., 2016) and China (Pu et al., 1989) shows the main compounds of *A. villosum* Lour leaf oil to be α-pinene and β-pinene and in varying quantities. However, bornyl acetate, camphor and limonene featured as the significant compounds in the fruits and seed oils (Lian et al., 1987; Song et al., 2004; Wang and Situ, 2010; Zhang et al., 2012). The main compounds in the essential oil of dry fruits of

✉ Corresponding authors: Do N. Dai and Isiaka A. Ogunwande
Tel: +234-8059929304; Fax: +234-8059929304
E-mail address: daidn23@gmail.com; isiaka.ogunwande@lasu.edu.ng

A. villosum (Song et al., 2004) were camphor (36.9%), camphene (13.9%), D-limonene (13.4%) and bornyl acetate (11.1%), while bornyl acetate (40.6%), borneol (14.3%), D-camphor (17.2%) and L-camphor (10.8%) were identified in the seed (Wang and Situ, 2010). Lian et al. (1987) reported an abundance of bornyl acetate (30.5%), camphor (22.3%) and limonene (8.3%) in the fruit essential oil. Ma et al. (2007) described high contents bornyl acetate (62.3%) and camphor (18.4%) in the essential oil of *A. villosum* Lour. Bornyl acetate was identified as the main compound in the essential oil of different cultivars of *A. villosum* Lour (Ning et al., 2010). Previous result has shown the high contents of 1,8-cineole, isoborneol and α-terpineol in the fruits, while sabinene, myrtenol, α-humulene and bicyclogermacrene dominated in the leaf oil (Xing et al., 2012).

The main goal of the present paper was to characterize the volatile constituents of the stem of *A. villosum* Lour for the first time. The findings of this work may be important in order to clarify the similarities or differences in the chemical constituents of the various part of *A. villosum* Lour.

2. Experimental

2.1. Collection of plant sample

Samples from the stems of *A. villosum* Lour were collected in mid-day from Pù Mát, National Park (19.000ºN, 104.450ºE) Nghệ An, Vietnam, in August 2014. Dr. Dai performed the botanical identification of the plant sample. A voucher specimen LTH 469 was deposited at the Botany Museum, Vinh University, Vietnam.

2.2. Preparation of plant samples

Prior to hydrodistillation, the plant sample was air-dried under laboratory shade (21 ºC) for few two weeks to reduce the moisture contents. In addition, sediments and other unwanted materials were separated from the

Table 1

Volatile constituents of the stem of *Amomum villosum* Lour.

Compounds[a]	C.C	RI[b]	RI[c]	MI	Percent composition
α-Thujene	Mh	930	921	RI, MS	1.6 ± 0.01
α-Pinene	Mh	939	932	RI, MS, CoI	16.9 ± 0.01
Camphene	Mh	953	946	RI, MS	0.9 ± 0.00
β-Pinene	Mh	980	978	RI, MS, CoI	48.1 ± 0.01
β-Myrcene	Mh	990	988	RI, MS	1.9 ± 0.01
α-Phellandrene	Mh	1006	1004	‘’	0.2 ± 0.01
δ-3-Carene	Mh	1001	1008	‘’	1.3 ± 0.01
α-Terpinene	Mh	1017	1014	‘’	2.1 ± 0.01
o-Cymene	Mh	1024	1024	RI, MS, CoI	1.3 ± 0.01
Limonene	Mh	1032	1030	‘’	3.9 ± 0.01
(*Z*)-β-Ocimene	Mh	1043	1034	‘’	0.1 ± 0.01
(*E*)-β-Ocimene	Mh	1052	1044	‘’	0.1 ± 0.00
γ-Terpinene	Mh	1061	1056	‘’	3.4 ± 0.01
trans-Sabinene hydrate	Mo	1075	1075	RI, MS, CoI	0.1 ± 0.01
α-Terpinolene	Mh	1090	1089	RI, MS	1.1 ± 0.01
Linalool	Mo	1100	1100	‘’	0.1 ± 0.00
Pinocarvone	Mo	1165	1165	‘’	0.2 ± 0.00
Terpinen-4-ol	Mo	1177	1177	‘’	1.6 ± 0.00
α-Terpineol	Mo	1189	1187	‘’	0.1 ± 0.01
Methyl chavicol	Mo	1204	1196	RI, MS, CoI	7.0 ± 0.01
Fenchyl acetate	Mo	1228	1128	RI, MS	0.1 ± 0.00
Safrole	Mo	1287	1285	‘’	0.1 ± 0.00
Bornyl acetate	Mo	1289	1287	‘’	0.1 ± 0.00
Bicycloelemene	Sh	1327	1337	RI, MS, CoI	0.9 ± 0.01
Cyperene	Sh	1399	1398	‘’	0.4 ± 0.00
β-Caryophyllene	Sh	1419	1417	‘’	1.3 ± 0.01
Aromadendrene	Sh	1441	1439	‘’	0.1 ± 0.01
α-Humulene	Sh	1454	1452	‘’	0.3 ± 0.01
Bicyclogermacrene	Sh	1500	1500	RI, MS, CoI	1.0 ± 0.01
δ-Cadinene	Sh	1525	1522	‘’	0.1 ± 0.01
Germacrene B	Sh	1561	1550	‘’	0.2 ± 0.01
(*E*)-Nerolidol	So	1563	1561	RI, MS	0.3 ± 0.01
Spathulenol	So	1578	1577	‘’	0.6 ± 0.01
Caryophyllene oxide	So	1583	1581	‘’	0.4± 0.01
α-Cedrol	So	1601	1600	RS, MS, CoI	0.3 ± 0.01
τ-Muurolol	So	1646	1646	‘’	0.3 ± 0.01
Total					**98.5** ± 0.01
Monoterpene hydrocarbons					**82.9** ± 0.01
Oxygenated monoterpenes					**9.4** ± 0.01
Sesquiterpene hydrocarbons					**4.3** ± 0.01
Oxygenated sesquiterpenes					**1.9** ± 0.01

[a]Elution order on an HP-5MS column; [b]Retention indices on an HP-5MS column; [c]Literature retention indices; C.C. Class of compounds; Mh, Monoterpene hydrocarbons; Mo: Oxygenated monoterpenes; Sh: Sesquiterpene hydrocarbons; Oxygenated sesquiterpenes; RI, MS, CoI, Identification by mass spectra, GC retention indices, comparison with literature data and co-injection with authentic compounds; RI, MS, Identification by mass spectra, GC retention indices and comparison with literature data

samples. Afterwards, samples were pulverized to coarse powder using a locally made grinder.

2.3. Hydrodistillation of the essential oil

In this process, 500 g of air-dried and pulverized stem of *A. villosum* Lour were introduced into a 5 L flask and distilled water (5 L) was added to cover the sample completely. Hydrodistillation was carried out with a Clevenger-type distillation unit designed according to the specification (Vietnamese Pharmacopoeia, 1997). The distillation time was 3 h and conducted at normal pressure (760 mmHg). The volatile oil distilled over water, was collected separately into clean weighed sample bottles and kept under refrigeration (4 ºC) until the moment of analysis. The oil was kept under refrigeration (4 ºC) until the moment of analyses.

2.4. Gas chromatography analysis of the oil

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 6890 Plus Gas chromatograph equipped with an FID and fitted with an HP-5MS column (30 m × 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 ºC, detector temperature 260 ºC, column temperature programmed from 40 ºC (2 min hold) to 220 ºC (10 min hold) at 4 ºC/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. Each analysis was performed in triplicate. The relative amounts of individual components were calculated based on the GC peak area (FID response) without using correction factors as previously described (Dai et al., 2016).

2.4.1. Gas chromatography-mass spectrometry analysis of the oil

An Agilent Technologies HP 6890N Plus Chromatograph fitted with a fused silica capillary HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 µm) and interfaced with mass spectrometer HP 5973 MSD, was used for this experiment, under the same conditions as those used for gas chromatography analysis as described previously (Dai et al., 2016). 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 under a sampling rate of 1.0 scan/s.

2.4.2. Identification of the components of the essential oil

The identification of components was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C_4-C_{40}) under identical experimental conditions. In some cases, co-injection with known compounds or standards (Sigma-Aldrich, St. Louis, MO, USA) under the same GC conditions was employed. The mass spectral (MS) fragmentation patterns were checked with those of other essential oils of known composition (NIST, 2011).

2.5. Statistical Analysis

The mean and standard deviation of three experiments were determined. Statistical analysis of the differences between mean values obtained for experimental groups were calculated using Microsoft excel program, 2003.

3. Results and Discussion

3.1. Chemical composition of the *A. villosum* Lour essential oil

The essential oil was obtained in a yield of 0.21% (±0.01, v/w), calculated on a dry weight basis. The hydrodistillation process afforded a light yellow colour essential oil. The volatile compounds were displayed in Table 1, along with their percentages and retention indices calculated on an HP-5MS column. Thirty-six compounds representing 98.5% of the total volatile compounds were identified in the stem essential oil. This consisted of 14 monoterpene hydrocarbons (82.9%), 9 oxygenated monoterpenes (9.4%), 8 sesquiterpene hydrocarbons (4.3%) and 5 oxygenated sesquiterpenes (1.9%). The main compounds identified in the essential oil were β-pinene (48.1%) and α-pinene (16.9%). Other notable compounds were methyl chavicol (7.0%), limonene (3.9%), γ-terpinene (3.4%) and α-terpinene (2.1%).

The abundance of α-pinene and β-pinene in the essential oil is a noteworthy observation. In fact, α-pinene, δ-3-carene and β-pinene were the main compounds in the stem oil of *A. microcarpum* (Huong et al., 2015), while the same compounds along with β-elemene and β-caryophyllene were identified in *A. maximum* (Huong et al., 2015). However, β-caryophyllene, α-humulene and hexahydrofarnesyl acetone were the major compounds identified in the stem oil of *A. longiligulare* (Chau et al., 2015).

3.2. Chemical composition of the *Amomum* essential oils in similar reports

There is a report describing the volatile composition of various parts of other species of *Amomum* plants grown in Vietnam. Essential oil obtained by hydrodistillation of leaf of *A. aculeatum* (Huong et al., 2014) grown in Vietnam contained limonene and valencene. Accordingly, the main compounds of *A. gagnepainii* were farnesyl acetate, zerumbone and β-caryophyllene, while β-pinene, (*E*)-β-ocimene, γ-terpinene and α-pinene were present in *A. repoense*

(Huong et al., 2018).

4. Concluding remarks

The composition of essential oil from the stem of *A. villosum* Lour is being reported for the first time. Ubiquitous monoterpene compounds predominate in the essential oil. The main component of the essential oil was found to be similar and also different from other *Amomum* plants grown in Vietnam or other parts of the world. These observations may be due to some factors such as environmental and climatic conditions at the point of collection, time of collection as well age and nature of the plant.

Conflict of interest

The authors declare that there is no conflict of interest.

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