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Do N Dai
Faculty of Agriculture, Forestry and Fishery Nghean College of Economics, Ly Tu Trong, Vinh City Nghean Province, Vietnam

Le T Huong
Faculty of Biology, Vinh University, Le Duan, Vinh City, Nghean Province, Vietnam

Tran D Thang
Faculty of Chemistry, Vinh University Department, Le Duan, Vinh City, Nghean Province, Vietnam

Isiaka A Ogunwande
Natural Products Research Unit, Faculty of Science, Department of Chemistry, Lagos State University, PMB, LASU Post Office Ojo, Lagos, Nigeria

Correspondence:
Do N Dai
Faculty of Agriculture, Forestry and Fishery Nghean College of Economics, Ly Tu Trong, Vinh City Nghean Province, Vietnam

Chemical composition of essential oils of *Amomum villosum* Lour

Do N Dai, Le T Huong, Tran D Thang and Isiaka A Ogunwande

Abstract
The characterization of essential oils from the leaves and root barks of *Amomum villosum* Lour grown in two localities of Vietnam was performed by means of gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques. The classes of compounds identified in the oil samples were monoterpene hydrocarbons (74.0%-89.7%), oxygenated monoterpenes (1.7%-5.8%), sesquiterpene hydrocarbons (4.6%-12.0%) and oxygenated sesquiterpenes (0.6%-11.8%). The main constituents of the essential oils were the monoterpene hydrocarbons represented by β-pinene (34.7%-56.6%) and α-pinene (11.6%-22.1%).

Keywords: *Amomum villosum*, essential oil composition, monoterpenes, β-pinene, α-pinene

1. Introduction
In this paper, we present the chemical compositions of essential oils from the leaves and roots of *Amomum villosum* Lour grown in Vietnam, as part of our continued investigations into the volatile compounds of Vietnamese plants. A previous study revealed that *A. villosum* 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 [1]. *Amomum villosum* extract has the function of promoting the digestion function [2]. Both the roots and leaves extracts of *A. villosum* had antioxidant activities [3]. Extract of *A. villosum* could be used to treat growth retardation during adolescence [4]. The polysaccharides of *A. villosum* showed strong inhibitory activity on the growth of HepG2 cells, free radical scavenging activities *in vitro,* significantly prevented the formation of malondialdehyde and enhanced the activities of antioxidant enzymes in CCl_4-induced liver injury mice [5]. Extracts of *A. villosum* have exhibited a variety of biological activities such as inhibition of thromboxane synthesis, inhibition of platelet aggregation, inhibit the stomach enzymes to digest proteins, analgesic effect, anti-ulcer, promote intestinal movement in mice and enhances gastrointestinal aircraft [5], [6].
The phenolic compounds isolated from *A. villosum* includes 3-ethoxy-hydroxy benzoic acid, vanillic acid-1-beta-D-glucopyranosyl ester, isorhamnetin-3-beta-D-glucoside, flavanocoumarin and isoflavanocoumarin [6]. Two quercetin glycosides quercetin-3-O-alpha-L-rhamnoside and quercetin-3-O-beta-D-glucoside were isolated and identified from the plant [7]. Other phytochemical compounds isolated from *A. villosum* were quercetin, quercitroside, isoquercitroside, vanillic acid, 3,4-dihydroxy-benzoic acid, β-sitosterol, daucosterol, stigmasterol, ergosterol, ergosta-7,22dien-3β,5α,6β-triol, stearic acid, palmitic acid, typhonoside B and polygonin [8]. Bornyl acetate, (*E*)-*p*-hydroxycinnamic acid, (*E*)-*p*-carboxycinnamic acid and 3,3',4,4'-tetrahydroxybiphenyl were also isolated from the plant [9]. Some bioactive polysaccharides [10, 11], fatty acids and their esters [12] have been isolated from the plant. Ethyl octacosate, docosyl hexylate, stigmast-4-ene-1,3-dione, β-sitosterol and daucosterol were isolated and identified from the roots and rhizomes of *A. villosum* [13].
Previous study revealed that the main compounds of the leaf oil of A. *villosum* produced in Yunnan [14] were α-pinene (31.29%) and β-pinene (58.52%). The main compounds in the essential oil of dry fruits of *A. villosum* [15] were camphor (36.9%), camphene (13.9%), D-limonene (13.4%) and bornyl acetate (11.1%). The main chemical components in hot organic solvent extraction and microwave assisted extraction were found as acetic acid, bornyl acetate, camphor, borneol, copaene and spathulenol [16]. The main chemical constituent of seed essential oil of *A. villosum* [17] were identified as bornyl acetate (40.60%), borneol (14.30%), d-camphor (17.15%) and l-camphor (10.75%).

The chemical composition of the volatile oils of the new hybrid obtained by cross-breeding of *A. villosum* were bornyl acetate (30.54%), camphor (22.3%), limonene (8.28%), camphene (6.71%), β-caryophyllene (5.14%) [18] (Zhang *et al*., 2012). The abundance of bornyl acetate and camphor has been reported in the fruits volatiles of *A. villosum* [19]. Another investigation has reported high proportions of bornyl acetate, camphor, borneol and limonene in the essential oil of the plant [19]. The main compounds identified in the essential oils of the plant from China were α-pinene, camphene, β-pinene, β -myrcene, limonene, linalool, camphor, isoborneol, borneol and bornyl acetate. These 10 major components have been assumed to the main antioxidant components of the oil [20].

2. Materials and methods

2.1 Plant samples

Leaves and roots of *A. villosum* were collected from Vũ Quang National Park, Hà Tĩnh, Vietnam and Pù Mát National Park, Nghệ An, Vietnam, in August 2014. The plant samples were identified by Dr. Dai D.N. Voucher specimens LTH 442 and LTH 469 respectively were deposited at the Botany Museum, Vinh University, Vietnam. Plant samples were air-dried prior to extraction.

2.2 Hydrodistillation of essential oils

Briefly, 500 g 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 [21]. The volatile oils distilled over water 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.

2.3 Gas chromatography (GC) analysis of the oils

The GC analysis of essential oils was carried out using an Agilent Technologies HP 6890 Plus GC 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: column oven- 40 ºC, injection pot-250 ºC while the detector temperature was 260 ºC. Time programming: 40 ºC for 2 min, temperature and then raise to 220 ºC (and held isothermally for 10 min) at 4 ºC/min. The carrier gas used was H_2 at a flow rate of 1 mL/min. The split ratio was 10:1 while 1.0 μL of the essential oil 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.

2.4 Gas chromatography-Mass spectrometry (GC-MS) analysis of the oils.

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 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. 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 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 [22] 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.

3. Results & Discussion

The yields of essential oils were 0.30% and 0.25% (v/w, Vũ Quang samples; leaf and root respectively), 0.28% and 0.21% (v/w, Pù Mát sample; leaf and root respectively) calculated on a dry weight basis. All the oil samples were light yellow in coloration. Table 1 indicates the chemical constituents present in the oil, their percentages as well as retention indices on HP-5MS column. Monoterpene hydrocarbons (89.7% leaf and 66.5% root) represent the most abundant class of compound identified in Vũ Quang oil samples. However, the root oil contained a high proportion of sesquiterpene hydrocarbons (12.0%) and oxygenated derivatives (11.8%). β-Pinene (56.6% leaf and 34.7% root) and α-pinene (22.0% leaf and 11.6% root) are the main constituents of Vũ Quang oil samples. Also, monoterpene hydrocarbons (88.0%) represent the main class of compound found in the leaf oil obtained from Pù Mát forest reserve. However, in addition to an abundance of monoterpene hydrocarbons (74.0%), sesquiterpene hydrocarbons (10.3%) and oxygenated derivatives (7.8%) could also be found in the root oil. The main constituents of the leaf oil were β-pinene (53.6%) and α-pinene (22.1%). The abundance of α-pinene and β-pinene in the essential oils of the leaf was in agreement with previous study [19] on the leaf oil of the plant.

Previously essential oil from other *Amomum* plants grown in Vietnam were studied for their chemical constituents. In the leaf oil of *A. aculeatum*, limonene (20.8%), valencene (18.0%) and α-phellandrene (8.7%) occurred in higher proportions [23]. The leaf oil of *A. longiligulare* [24] comprised mainly of β-caryophyllene (26.6%), α-pinene (15.6%), viridiflorol (14.0%) and α-humulene (12.5%) while β-caryophyllene (37.4%), α-humulene (16.5%) and hexahydrofarnesyl acetone (10.0%) were the major compounds identified in the stem oil. The root oil was rich in camphene (15.7%) and hexadecanoic acid (10.0%). The major compounds in the leaf of *A. maximum* [25] were β-pinene (40.8%), β-elemene (10.9%) and α-pinene (9.7%), while the stems comprised β-pinene (20.4%), β-elemene (12.8%) and β-caryophyllene (10.3%). However, β-pinene (28.0%), α-pinene (15.0%) and β-phellandrene (11.6%) were the main constituents of the root oil. The compounds occurring in higher quantity in the leaf of *A. muricarpum* [25] were α-pinene (48.4%) and β-pinene (25.9%) while the stem comprised of were α-pinene (47.2%), δ-3-carene (9.4%) and β-pinene (9.2%). The authors reported abundance of α-pinene (54.7%) and β-pinene (14.3%) in the root with α-pinene (29.3%) and β-pinene (17.9%) making up the fruit. The flower oil presented a compositional pattern made up of α-pinene (24.1%), β-pinene (14.1%) and τ-muurolol (13.0%).

It could be seen that the monoterene hydrocarbons α-pinene and β-pinene were present only in the leaf oil of *A. villosum* while the oxygenated counterparts mostly camphor and bornyl acetate and derivatives were conspicuous in the other parts of the plant. Moreover quantitative amounts of α-pinene and β-pinene were also present in the essential oils of various parts of *A. muricarpum*. The potent anti-inflammatory, cytotoxicity and

inhibition of nitric oxide production effects of essential oils *A. villosum* have been attributed to the action of camphor, borneol and bornylacetate [20].

3.1 Tables

Table 1: Essential oil constituents of *A. villosum* [a]

Compounds [b]	RI [c]	RI [d]	VqL	VqR	PmL	PmR
α-Thujene	930	921	1.0	1.1	1.3	1.1
α-Pinene	939	932	22.0	11.6	22.1	14.0
Camphene	953	946	0.9	2.7	0.8	4.2
β-Pinene	980	976	56.6	34.7	53.6	41.6
β-Myrcene	990	988	1.5	1.3	1.3	1.3
α-Phellandrene	1006	1002	-	0.2	0.2	0.9
δ-3-Carene	1011	1008	0.2	0.4	1.4	4.8
α-Terpinene	1017	1014	0.5	2.2	0.7	1.1
p-Cymene	1024	1020	-	1.5	0.4	1.0
Limonene	1032	1024	3.8	4.4	4.2	-
(*Z*)-β-Ocimene	1043	1032	0.2	-	0.1	-
(*E*)-β-Ocimene	1052	1044	0.4	0.1	0.2	0.4
γ-Terpinene	1061	1056	0.8	3.5	1.2	2.0
α-Terpinolene	1090	1985	0.2	1.0	0.3	0.7
trans-Sabinene hydrate	1101	1098	0.1	-	0.2	-
allo-Ocimene	1128	1128	0.3	0.8	0.2	0.9
Camphor	1145	1141	-	-	-	0.5
trans-Verbenol	1153	1150	0.2	-	0.3	-
Pinocarvone	1165	1167	0.2	-	0.3	-
Borneol	1167	1165	-	0.1	-	0.3
Terpinen-4-ol	1177	1175	0.6	1.2	0.8	0.7
α-Terpineol	1189	1189	-	0.1	-	-
Methyl chavicol	1204	1202	0.6	0.4	-	-
Myrtenal	1209	1197	-	-	0.5	0.2
Fenchyl acetate [e]	1228	1229	-	1.3	-	1.9
Nerol	1234	1227	-	0.1	-	-
Dihydro-edulan I	1280	1276	1.3	-	-	-
Bornyl acetate	1289	1287	-	1.1	-	1.4
Bicycloelemene	1327	1337	1.3	1.5	0.6	2.0
α-Copaene	1377	1374	-	0.4	-	0.1
Methyl-(*E*)-cinnamate	1380	1376	-	-	0.3	0.6
β-Elemene	1391	1389	-	0.1	0.2	0.1
α-Gurjunene	1412	1409	-	0.2	-	0.2
β-Caryophyllene	1419	1417	2.0	2.4	1.2	1.5
Aromadendrene	1441	1439	0.5	1.4	-	2.0
α-Humulene	1454	1452	0.9	1.2	2.0	0.9
γ-Gurjunene	1477	1473	-	-	-	0.2
α-Amorphene	1485	1484	-	0.7	-	0.1
β-Selinene	1486	1486	-	-	-	0.2
Eudesma-4,11-diene	1490	1489	-	0.3	-	0.4
Zingiberene	1494	1493	-	0.3	-	0.2
Bicyclogermacrene	1500	1500	1.2	2.6	0.6	1.9
β-Bisabolene	1506	1505	-	0.2	-	0.2
(*E,E*)-α-Farnesene	1508	1505	0.2	-	-	-
δ-Cadinene	1525	1522	-	0.7	-	0.3
Tetradecamethyl-cycloheptasiloxane[f]	1526	1520	0.4	-	-	-
Germacrene B	1561	1550	0.3	-	-	-
(*E*)-Nerolidol	1563	1561	-	0.2	0.1	0.1
Spathulenol	1578	1577	0.2	2.7	0.2	2.2
Caryophyllene oxide	1583	1581	0.3	2.5	0.3	1.4
Viridiflorol	1593	1592	-	1.5	-	0.6
Isospathulenol	1640	1636	-	1.8	-	0.8
τ-Muurolol	1646	1640	0.1	-	-	0.5
β-Eudesmol	1651	1649	-	0.6	0.2	-
α-Cadinol	1654	1652	-	1.0	-	0.5
Vulgarol B	1688	1688	-	1.5	-	-
(*E,E*)-Farnesol	1718	1722	-	-	0.3	1.7
Total			98.6	94.8	96.4	97.9
Monoterpene hydrocarbons			89.7	66.5	88.0	74.0
Oxygenated monoterpenes			1.7	4.5	2.7	5.8
Sesquiterpene hydrocarbons			6.2	12.0	4.6	10.3
Oxygenated sesquiterpenes			0.6	11.8	1.1	7.8
Non-terpenes			0.4	-	-	-

[a] SD (±) were insignificant and excluded from the Table to avoid congestion; [b] Elution order on HP-5MS column; [c] Retention indices on HP-5MS column; [d] Literature retention indices; [e] correct isomer not identified; [f] tentative identification; - not identified; VqL-Vu Quang leaf; VqR- Vu Quang root; PmL- Pù Mát leaf; PmR- Pù Mát root

4. Conclusions

The chemical constituents of essential oils of *A. villosum* are being reported for the first time. Although monoterpenes and sesquiterpenes compound predominate, the compositional patterns was different from other *Amomum* plants grown in Vietnam or other parts of the world.

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6. References

1. Kim JY, Lee SH, Park J, Kim MY, Chang GT, Kim H. Effects of *Amomum villosum* on longitudinal bone growth in adolescent rats. Planta Medica, 2012; 78:69. DOI: 10.1055/s-0032-1320616.
2. Yin WY, Zeng G, Zheng WD, Rui L**.** Experimental study of *Amomum villosum* on improving digestion function of mice. Food Research and Development, 2008; 6(1):30-33
3. You XM, Li YZ, Liao YC, Li T, Hong Y, Zeng D. *et al*. Antioxidant activity of the extracts from *Amomum Villosum* roots and leaves. *Food Science and Technology*, 2012; (2):226-228.
4. Sun HL, Ji YK, Hocheol K, Seul KP, Cho YK, Sun YC, *et al*. *Amomum villosum* induces longitudinal bone growth in adolescent female rats. Journal of Traditional Chinese Medicine. 2012; 32(3):453-458.
5. Danyan Z, Shijie L, Xiong QP, Jiang CG, Lai XP. Extraction, characterization and biological activities of polysaccharides from *Amomum villosum*. Carbohydrate polymers, 2013; 95(1):114-122.
6. Chen C, Fu C, Ye WC, Zhou GX. Study on phenolic constituents of *Amomum villosum*. Journal of Chinese Medicinal Materials. 2012; 35(4):571-573.
7. Sun L, Yu JG, Zhou LD, Luo XZ, Din W, Yang SL. Two flavone glycosides from Chinese traditional medicine *Amomum villosum*. China Journal of Chinese Materia Medica, 2002; 27(1):36-38.
8. An XQ, Li ZZ, Shen LG, Si JY. Chemical constituents of *Amomum villosum* Lour. Natural Product Research and Development, 2011; 23(6):1021-1024.
9. Fu C, Chen C, Zhou GX, Ye WC. Chemical constituents from fruits of *Amomum villosum*. Chinese Traditional and Herbal Drugs, 2011; 42(12):2410-2412.
10. Yajuan Y, Xia L, Mianjie W, Jingping C, Shijie L, Man C, Danyan Z. Effect of extraction methods on property and bioactivity of water-soluble polysaccharides from *Amomum villosum*. Carbohydrate Polymers, 2015; 117(6):632-635.
11. Zhang JN, Xiong QP, Shijie L, Xia L, Chen JP, Man C *et al*. A comparison study on polysaccharides from novel hybrids of *Amomum villosum* and its female parent. International Journal of Biological Macromolecules. 2015; 81(1):396-399.
12. Liu SP, Chen ZL, Cao JY, Zhang XQ. Analysis of lipid-soluble components of *Amomum villosum* fruits by GC/MS. Journal of Chinese Medicinal Materials. 2011; 34(9):1376-1379.
13. Fan X, Du YC, Wei JX. Chemical constituents of roots, rhizomes and stems of *Amomum villosum* Lour. Zhongguo Zhong Yao Za Zhi, 1994; 19(12):734-736.
14. Pu F, Cu JQ, Zhang ZJ. The essential oil of *Amomum villosum* Lour. Journal of Essential Oil Research, 1989; 1(4):197-199.
15. Song GX, Deng CH, Wu D, Hu YM. Determination of volatile components of *Amomum villosum* Lour. by gas chromatography-mass spectrometry with head-space solid-phase microextraction. Journal of Fudan University. 2004; 31(4):237-246.
16. Wang L, Situ QW. Extraction and determination of volatile components in *Amomum villosum* Lour. Modern Food Science and Technology, 2010; 26(9):3347-350.
17. Lian JL, Wu GQ, Chen ZH. Study on the chemical constituents of the essential oil of *Amomum villosum* Lour seeds cultivated in Nanping. Journal of Fujian College of Forestry. 1987; 7(4):297-303.
18. Zhang DY, Zheng SY, Chen YL, Li SJ, Ouyang XN. Analysis of volatile oils extracted from Spring No. 1 by GC-MS: A new cultivar obtained by cross-breeding of *Amomum Villosum* Lour. Advanced Materials Research, 2012; (550-553):1837-1840.
19. Ding P, Du JF, Wei G, Liu JM, Xu HH. The comparative studies of volatile constituents in fruits of *Amomum villosum* and *Amomum thyrsodlum*. Chinese Pharmaceutical Journal. 2001; 36(4):235-237.
20. Xue X, Depo Y, Wang DM, Xu XJ, Zhu LP, Zhao ZM. Solidification of floating organic drop liquid-phase microextraction cell fishing with gas chromatography–mass spectrometry for screening bioactive components from *Amomum villosum Lour*. Biomedical Chromatography, 2015; 29(4):626-632.
21. Vietnamese Pharmacopoeia. Medical Publishing House, Hanoi, Vietnam, 1997.
22. NIST. Chemistry Web Book. Data from NIST Standard Reference Database 2011, 69.
23. Huong LT, Chau LTM, Thang TD, Ogunwande IA. Constituents of essential oils from the leaf of *Amomum aculeatum* Roxb. Journal of Essential Oil Bearing Plants. 2014; 17(6):1352-1355.
24. Chau LTM, Thang TD, Huong LT, Ogunwande IA. Constituents of essential oils from *Amomum longiligulare*. Chemistry of Natural Compounds, 2015; 51(6):1181-1183.
25. Huong LT, Dai DN, Thang TD, Bach TT, Ogunwande IA. Volatile constituents of *Amomum maximum* Roxb and *Amomum microcarpum* C. F. Liang & D. Fang: two Zingiberaceae grown in Vietnam. Natural Products Research, 2015; 19(15):1469-1472.