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CHEMICAL COMPOSITION OF ESSENTIAL OILS AND ANTIMICROBIAL ACTIVITY OF *Amomum cinnamomeum* FROM VIETNAM

Le T. Huong,[1*] Ly N. Sam,[2,3] Bui D. Thach,[2] Do N. Dai,[3,4] and Isiaka A. Ogunwande[5*]

The lack of information on the chemical constituents and biological activity of the volatile and non-volatile extracts of *Amomum cinnamomeum* Skornick., Luu & H.D.Tran, sp. nov. (Zingiberaceae) attracted our interest, hence we report herein the results of a study on the chemical constituents and antimicrobial activity of the essential oil from the rhizome of *A. cinnamomeum*.

The compositions and biological activities of essential oils from *Amomum* plants grown in Vietnam [1–4] and other parts of the world [5–8] have been reported previously. The results also indicated that monoterpenes and sesquiterpenes were the predominant compounds in the essential oils of a majority of these *Amomum* plants. In addition, essential oils from these *Amomum* plants exhibited biological activities, such as antimicrobial [1, 5–8] and larvicidal activity [3], among others. The aim of the present study is to examine the chemical constituents and antimicrobial activity of essential oils from the leaves and rhizomes of *A. cinnamomeum* grown in Vietnam for the first time and to determine its potential uses. Mature leaves and rhizomes of *A. cinnamomeum* were collected from Quang Ngai, Nghe An Province, Vietnam, in July 2019. The sample was identified by Dr. D. N. Dai. A voucher specimen, LNS 804, was deposited at the Botany Museum, Nghe An College of Economics, Vietnam. A total of 1 kg of the pulverized sample was used. The essential oil was obtained by hydrodistillation, which was carried out in a Clevenger-type distillation unit designed according to an established specification [9].

Gas chromatography (GC) was performed on an Agilent Technologies HP 7890 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 as described previously [2–9]. An Agilent Technologies HP 7890N Plus Chromatograph fitted with capillary an HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD was used for gas chromatography-mass spectrometry (GC-MS) under the same conditions as those used for gas chromatography as described above. 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. The identification of constituents from the GC/MS spectra of *A. cinnamomeum* was performed based on retention indices (RI) determined with reference to a homologous series of *n*-alkanes (C_4–C_{40}), under identical experimental conditions. The mass spectral (MS) fragmentation patterns were compared with those of other essential oils of known composition [10].

The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay [11]. Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO). Dilution series (2^{14}, 2^{13}, 2^{12}, 2^{11}, 2^{10}, 2^9, 2^7, 2^5, 2^3, and 2^1 μg/mL) were prepared in sterile distilled water inside the micro-test tubes, from which they were transferred separately to 96-well microtiter plates. Bacteria grown in double-strength Mueller–Hinton broth or double-strength tryptic soy broth, and fungi sustained in double-strength Sabouraud dextrose broth, were standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. DMSO was used as a negative control.

1) School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City 4300, Nghe An Province, Vietnam, e-mail: lehuong223@gmail.com; 2) Institute of Tropical Biology, Vietnam Academy of Science and Technology, 85-Tran Quoc Toan, 3 District, Ho Chi Minh City, Vietnam; 3) Graduate University of Science and Technology, Vietnam Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi 1000, Vietnam; 4) Faculty of Agriculture, Forestry and Fishery, Nghe An College of Economics, 51-Ly Tu Trong, Vinh City 4300, Nghe An Province, Vietnam; 5) Foresight Institute of Research and Translation, Ibadan, Nigeria, e-mail: isiakaogunwande@gmail.com. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2021, pp. 487–489. Original article submitted April 5, 2020.

TABLE 1. Constituents of Essential Oils from the Leaves and Rhizomes of *Amomum cinnamomeum*

Compound [a]	RI[b]	Leaf	Rhizome	Compounds [a]	RI[b]	Leaf	Rhizome
Tricyclene	928	–	0.2	γ-Muurolene	1490	0.9	–
α-Pinene	939	4.4	2.4	β-Chamigrene	1491	–	0.4
Camphene	955	0.4	12.3	*n*-Pentadecane	1499	0.6	–
Benzaldehyde	964	0.2	–	Germacrene D	1499	–	0.6
Sabinene	978	0.4	–	β-Selinene	1505	2.5	0.6
β-Pinene	984	35.8	1.5	γ-Amorphene	1509	0.5	–
Myrcene	992	0.4	0.4	α-Selinene	1514	1.1	0.3
n-Octanal	1003	0.2	–	β-Bisabolene	1518	0.3	–
α-Phellandrene	1110	–	0.3	*cis*-Dihydrogarofuran	1521	–	0.3
δ-3-Carene	1016	0.2	0.2	γ-Cadinene	1529	–	0.3
o-Cymene	1029	0.6	1.2	δ-Cadinene	1537	0.5	0.3
Limonene	1034	1.2	3.7	*cis*-Calamenene	1538	1.0	0.2
β-Phellandrene	1035	1.5	7.4	α-Calacorene	1559	0.2	–
(*Z*)-β-Ocimene	1037	0.8	0.3	Elemol	1562	–	0.5
(*E*)-β-Ocimene	1048	0.3	0.2	(*E*)-Nerolidol	1571	0.6	0.2
2-Octenal	1058	0.2	–	Germacrene B	1577	–	0.3
2-Nonanone	1091	0.2	–	10-*epi*-Dihydrogarofuran	1579	–	0.5
γ-Terpinene	1063	–	0.6	Spathulenol	1598	0.9	0.4
Terpinolene	1094	0.9	0.7	Axenol	1599	0.2	–
Linalool	1101	1.7	0.5	Caryophyllene oxide	1605	5.9	0.9
(*E*)-4,8-Dimethylnona-1,3,7-triene	1118	0.1	–	Copaborneol	1625	2.5	–
Isoborneol	1166	0.3	0.5	Guaiol	1612	–	4.3
p-Cymen-8-ol	1190	0.1	–	Rosifoliol	1621	–	1.6
α-Terpineol	1197	0.1	–	Humulene oxide II	1632	0.6	1.1
Fenchyl acetate	1228	0.3	13.7	1-*epi*-Cubenol	1648	–	0.2
Thymol methyl ether	1238	–	0.2	γ-Eudesmol	1649	–	0.4
Carvacrol methyl ether	1248	–	0.2	Caryophylla-3(15),7(14)-dien-6-ol	1659	0.3	–
Linalyl acetate	1256	–	0.2	*epi*-α-Cadinol	1657	0.6	–
2-(*E*)-Decanal	1264	0.5	–	α-Muurolol	1661	1.1	0.2
(*E*)-Cinnamaldehyde	1279	11.5	0.7	Eudesma-4(15),7-dien-1β-ol	1665	0.2	7.3
Isobornyl acetate	1293	0.2	4.4	α-Cadinol	1673	0.4	–
Bornyl acetate	1294	0.2	9.7	Neointermedol	1675	0.9	2.1
Terpinene-4-ol-acetate	1306	–	0.3	Intermedol	1681	0.3	–
α-Terpinyl acetate	1356	–	2.2	Bulnesol	1685	–	4.2
Isocamphanyl acetate	1379	–	0.4	14-Hydroxy-9-*epi*-(*E*)-caryophyllene	1688	0.5	1.3
α-Copaene	1389	4.7	0.5	Cadalene	1692	0.2	–
β-Copaene	1402	0.1	–	Zerumbone	1757	0.2	0.2
cis-β-Elemene	1403	0.1	0.4	γ-Bicyclohomofarnesal	1826	–	0.6
β-Caryophyllene	1438	2.2	0.9	Total		95.1	96.8
γ-Elemene	1443	–	0.3	Monoterpene hydrocarbons		46.9	31.9
trans-α-Bergamotene	1445	0.2	–	Oxygenated monoterpenes		14.5	32.6
(*E*)-Cinnamyl acetate	1449	0.1	0.1	Sesquiterpene hydrocarbons		15.3	6.6
α-Humulene	1471	0.5	0.5	Oxygenated sesquiterpenes		16.4	25.7
9-*epi*-(*E*)-caryophyllene	1478	1.0	0.2	Non-terpenes		2.0	–

[a] Elution order on HP-5MS column; [b] Retention indices on HP-5MS column; – not identified.

Streptomycin was used as the antibacterial standard, while nystatin and cycloheximide were used as antifungal standards. All experiments were performed in triplicate. After incubation at 37°C for 24 h, the MIC values were determined as the lowest concentration of essential oils of *A. cinnamomeum* that completely inhibited the growth of the microorganisms. The IC_{50} values were determined as the percentage inhibition of growth of microorganisms based on the turbidity measurement data of an EPOCH2C spectrophotometer (BioTeK Instruments, Inc Highland Park Winooski, VT, USA) and Rawdata computer software (Belgium).

TABLE 2. Antimicrobial Activity of the Leaf and Rhizome Essential Oils of *A. cinnamomeum*

Microorganism	MIC, µg/mL		IC_{50}, µg/mL	
	Leaf	Rhizome	Leaf	Rhizome
Enterococcus faecalis ATCC299212	16.0 ± 0.10	32.0 ± 0.00	4.98 ± 0.00	10.34 ± 0.12
Staphylococcus aureus ATCC25923	16.0 ± 0.00	32.0 ± 0.00	3.78 ± 0.00	15.98 ± 0.11
Bacillus cereus ATCC14579	16.0 ± 0.11	32.0 ± 0.00	5.78 ± 0.00	9.78 ± 0.20
*Candida albican*s ATCC10231	64.0 ± 0.50	32.0 ± 0.00	28.79 ± 0.00	9.79 ± 0.10

Both the leaf and rhizome oils exhibited no antimicrobial action against *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *Salmonella enterica* ATCC13076.

The average yields of the essential oils were 0.12% and 0.19% (v/w, ± 0.01), respectively, for the leaf and rhizome, calculated on a dry weight basis. Fifty-nine compounds each were identified from both oil samples. The major classes of compounds were monoterpene hydrocarbons (46.9 and 31.9%), oxygenated monoterpenes (14.5 and 32.6%), sesquiterpene hydrocarbons (15.3 and 6.6%), and oxygenated sesquiterpene (16.4 and 25.7%); see Table 1.

The main constituents of the leaves oil were β-pinene (35.8%), (*E*)-cinnamaldehyde (11.5%), and caryophyllene oxide (5.9%), while the rhizome oil was dominated by fenchyl acetate (13.7%), camphene (12.3%), bornyl acetate (9.7%), β-phellandrene (7.4%), and eudesma-4(15),7-dien-1β-ol (7.3%). This is the first report on the volatile constituents of *A. cinnamomeum*. Terpene compounds predominate in the essential oils, as was reported for other *Amomum* oil samples grown in Vietnam, such as *A. rubidium* [1, 2] *A. gagnepainii* [12], *A. repoense* [12], *A. longiligulare* [3], *A. villosum* [4], *A. aculeatum* [4], *A. maximum* [13], and *A. microcarpum* [9]. However, the identities of these terpene compounds differed from one species to another, thus exhibiting chemical variability in their compositional pattern [9].

The leaves oil of *A. cinnamomeum* displayed antimicrobial activity towards *Enterococcus faecalis* ATCC 299212 (MIC 16.0 µg/mL), *Staphylococcus aureus* ATCC 25923 (MIC 16.0 µg/mL), and *Bacillus cereus* ATCC 14579 (MIC 16.0 µg/mL). The oil also inhibited the growth of *Candida albicans* ATCC 10231, with MIC of 64.0 µg/mL. The median inhibitory concentrations (IC_{50}) against the tested microbes were evaluated as 4.98, 3.78, 5.78, and 28.79 µg/mL, respectively. However, the rhizome oil exhibited antimicrobial activity against the four microorganisms with MIC value of 32.0 µg/mL, while the IC_{50} values were estimated to be 10.34, 15.98, 9.78, and 9.79 µg/mL, respectively. The MIC and IC_{50} provided evidence that the leaf and rhizome oils of *A. cinnamomeum* showed potent antimicrobial activity against *E. faecalis*, *S. aureus*, *B. cereus*, and *C. albicans.* Both the leaf and stem oils exhibited no antimicrobial action against *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *Salmonella enterica* ATCC13076. Streptomycin displayed antimicrobial activity with MIC values in the range 0.28 to 3.20 µg/mL, while nystatin had an MIC value of 8.0 µg/mL, with cycloheximide showing activity at MIC of 3.20 µg/mL. This is the first report on the antimicrobial activity of essential oil of space *A. cinnamomeum.* The results in this study are comparable with data obtained on the antimicrobial action of other *Amomum* essential oil reported in the literature such as *A. rubidium* [1, 2], *A. subulatum* [5, 6], *A. cannicarpum* [14], *A. uliginosum* [15], *A. tsao-ko* [7], and *A. kravanh* [16]. The antimicrobial activities of the essential oil of *A. cinnamomeum* can be related to its main compounds or some synergy between the major and minor compounds. The present essential oil constituents, such as β-pinene [6], caryophyllene oxide [17], (*E*)-cinnamaldehyde [18], fenchyl acetate [19], and camphene [19], were previously reported to have significant broad-spectrum activity.

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