DOI 10.1007/s10600-022-03835-2
Chemistry of Natural Compounds, Vol. 58, No. 5, September, 2022

ESSENTIAL OIL COMPOSITIONS AND ANTIMICROBIAL ACTIVITY OF THE LEAVES AND RHIZOMES OF *Alpinia calcicola* FROM VIETNAM

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The lack of information on the chemical constituents and biological activity of the volatile and nonvolatile extracts of *Alpinia calcicola* Q. B. Nguyen & M. F. Newman (Zingiberaceae) attracted our interest; hence, we report here the result of an extensive study on the chemical constituents and antimicrobial activity of essential oils from the leaves and rhizomes of *A. calcicola*. This species is one of the newly described *Alpinia* plants from Vietnam [1].

The compositions and biological activities of essential oils from *Alpinia* plants grown in Vietnam [2–14] and other parts of the world [15, 16] have been reported. The results revealed the abundance of monoterpenes and sesquiterpenes compounds in the essential oils of majority of these *Alpinia* plants. Moreover, the essential oils from previously described *Alpinia* plants exhibited biological activities such as antimicrobial [4–6], larvicidal [4], and insecticidal [15, 16]. The aim of the present study is to report the chemical constituents and antimicrobial activity of essential oils from the leaves and rhizomes of *A. calcicola* collected in Vietnam for the first time. The leaves and rhizomes of *A. calcicola* were collected from Pu Mat National Park (GPS 19°05′15″N, 104°38′09″ E), Vietnam, at an elevation of 337 m. The collection was done in September 2020. Plants were identified by Dr. Le Thi Huong and a voucher specimen (LTH 925) was deposited in the plant specimen room, Vinh University, Vietnam. The leaves and rhizomes were separated from debris, stones, and other substances by handpicking to obtain 2.0 kg of each sample, which were subjected to separate hydrodistillation using a Clevenger-type apparatus, as described previously [2–14].

The chemical analysis of the essential oils was performed by using gas chromatography (GC) on an Agilent Technologies HP 7890 Plus Gas chromatograph equipped with FID and fitted with HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were as described previously [2–14]. An Agilent Technologies HP 7890N Plus Chromatograph fitted with capillary 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 this gas chromatography-mass spectrometry (GC-MS) experiment, under the same conditions as those used for the gas chromatography analysis as described above. The MS conditions were as follows: ionization voltage 70 eV; emission current 40 mA; acquisitions scan mass range 35–350 amu at a sampling rate of 1.0 scan/s. The identification of constituents from the GC/MS spectra of *A. calcicola* 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 checked with those of other essential oils of known composition [17].

The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay [4–6, 18]. Stock solutions of the oil were prepared in dimethyl sulfoxide (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 where 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. Streptomycin was used as the antibacterial standard whereas nystatin and cycloheximide were used as antifungal standards.

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TABLE 1. Chemical Constituents of Essential Oils from the Leaves and Rhizomes of *A. calcicola*, %

Compound [a]	RI[b]	Leaves	Rhizomes	Compound [a]	RI[b]	Leaves	Rhizomes
α-Pinene	939	2.5	2.5	γ-Cadinene	1528	2.5	2.9
Camphene	955	–	0.2	δ-Cadinene	1536	9.7	10.1
β-Pinene	984	14.4	15.3	*trans*-Cadina-1,4-diene	1546	0.6	–
Myrcene	992	0.2	0.2	α-Cadinene	1515	0.5	0.6
o-Cymene	1029	–	0.2	α-Calacorene	1560	2.2	–
Limonene	1034	0.2	0.4	Scapanol	1592	4.0	3.4
α-Terpineol	1197	–	0.4	Spathulenol	1595	0.5	0.2
Fenchyl acetate	1227	–	0.5	Caryophyllene oxide	1603	0.8	0.3
α-Cubebene	1359	0.2	–	β-Oplopenone	1627	0.7	–
α-Copaene	1388	1.5	0.7	Humulene epoxide II	1630	0.4	0.2
β-Bourbonene	1399	1.5	0.1	1,10-di-*epi*-Cubenol	1632	0.4	0.7
cis-β-Elemene	1409	0.9	0.7	Cadina-1,(10),4-dien-8α-ol	1643	1.0	–
β-Copaene	1410	0.3	–	1-*epi*-Cubenol	1645	1.0	1.2
β-Caryophyllene	1435	7.7	1.9	*epi*-α-Cubenol	1657	3.1	3.1
β-Gurjunene	1444	0.4	–	*epi*-α-Muurolol	1658	3.6	9.4
cis-Muurola-3,5-diene	1465	0.2	–	δ-Cadinol	1661	1.6	2.8
α-Humulene	1470	3.9	2.8	Eudesm-3-en-6-ol	1663	0.5	–
9-*epi*-(*E*)-Caryophyllene	1478	5.1	2.0	α-Cadinol	1671	7.0	19.1
trans-Cadina-1(16),4-diene	1486	0.3	–	*trans*-Calamine-10-ol	1688	–	0.6
γ-Muurolene	1489	2.7	1.5	Guiaol acetate	1712	–	2.3
cis-epoxy-4,10-Amorphane	1492	0.7	–	Total		92.4	92.7
Germacrene D	1497	4.4	2.5	Monoterpene hydrocarbons		17.3	18.8
β-Selinene	1503	0.4	–	Monoterpene, oxygenated		–	0.8
trans-Muurola-4(14),5-diene	1509	2.1	1.0	Sesquiterpene hydrocarbons		49.8	29.8
α-Muurolene	1512	3.2	3.0	Sesquiterpene, oxygenated		25.3	43.3

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

TABLE 2. Antimicrobial Activity of the Leaves and Rhizomes Essential Oil of *A. calcicola*

Microorganisms	MIC, μg/mL		IC_{50}, μg/mL	
	Leaves	Rhizomes	Leaves	Rhizomes
Enterococcus faecalis ATCC299212	9.22 ± 0.10	5.67 ± 0.00	16.0 ± 0.00	32.0 ± 0.11
Staphylococcus aureus ATCC25923	21.33 ± 0.01	5.67 ± 0.00	64.0 ± 0.00	16.0 ± 0.00
Bacillus cereus ATCC14579	21.67 ± 0.05	7.67 ± 0.00	64.0 ± 0.00	16.0 ± 0.12
Pseudomonas aeruginosa ATCC27853	5.67 ± 0.05	23.45 ± 0.05	16.0 ± 0.05	64.0 ± 0.01
*Candida albican*s ATCC10231	32.89 ± 1.50	33.67 ± 1.00	64.0 ± 0.00	64.0 ± 0.10

Escherichia coli ATCC25922 and *Salmonella enteric* ATCC13076 – no activity.

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. calcicola*, which completely inhibited the growth of the microorganisms. The IC_{50} values were determined by the percentage of microorganism-inhibited growth based on the turbidity measurement data of the EPOCH2C spectrophotometer (BioTeK Instruments, Highland Park Winooski, VT, USA) and Rawdata computer software (Belgium).

The hydrodistilled essential oils were colored light yellow. The obtained yields are consistent with values reported previously for the majority of the *Alpinia* essential oils, which varied from 0.10 to 0.40%. For example, the yields of the hydrodistilled essential oils of *A. napoensis* leaves, stems, and roots were 0.21%, 0.17%, and 0.25% respectively [2], whereas *A. napoensis* rhizome gave oil in a yield of 0.26% [4]. *A. globosa* and *A. tonkinensis* leaf essential oils from Vietnam were obtained in yields of 0.16% and 0.21%, respectively [5]. The yields for *A. malaccensis* fruits essential oil was 0.40% [6]. Essential oil from the dried rhizomes of *A. kwangsiensis* [15] grown in China was obtained in a yield of 0.16%. The main

classes of compounds identified in the leaves and rhizomes of *A. calcicola* were monoterpene hydrocarbons (17.3% and 18.8%), sesquiterpene hydrocarbons (49.8% and 29.8%), and oxygenated sesquiterpene (25.3% and 43.3%) respectively, as seen in Table 1.

The oxygenated monoterpenes were absent in the leaf oil and present in much lower amounts (0.8%) in the rhizome oil. The main constituents of *A. calcicola* leaf essential oil were β-pinene (14.4%), δ-cadinene (9.7%), β-caryophyllene (7.7%), α-cadinol (7.0%), and 9-*epi*-(*E*)-caryophyllene (5.1%), whereas α-cadinol (19.1%), β-pinene (15.3%), δ-cadinene (10.1%), and *epi*-α-muurolol (9.4%) were found in the rhizome essential oil. To our knowledge, this is the first report on the essential oil constituents of *A. calcicola*. It could be seen that terpene compounds predominate in the essential oils, as was previously reported for other *Alpinia* oil samples grown in Vietnam, such as *A. napoensis* [2, 4], *A. menghaiensis* [3], *A. maclurei* [3], *A. globosa* [5], and *A. tonkinensis* [5]. Moreover, terpenes were also the predominant compounds of other *Alpinia* essential oils reported from other parts of the world, which include *A. kwangsiensis* [15] and *A. zerumbet* [16]. A noteworthy observation is that the chemical identities of the various terpene compounds present in the *Alpinia* essential oils differed from one species to another. This is an indication of chemical variability in their compositional pattern.

The essential oils from the leaves and rhizomes of *A. calcicola* displayed antimicrobial activity against five of the seven tested microorganisms (Table 2). Essential oil from the leaves of *A. calcicola* was most active against *Pseudomonas aeruginosa* ATCC27853 and *Candida albicans* ATCC 10231, with minimum inhibitory concentration (MIC) values of 5.67 µg/mL and 32.89 µg/mL, respectively. The obtained IC_{50} value was 64.0 µg/mL. The rhizomes oil displayed the highest antimicrobial activity against *Enterococcus faecalis* ATCC299212 (MIC, 5.67 µg/mL), *Staphylococcus aureus* ATCC25923 (MIC, 5.67 µg/mL) and *Bacillus cereus* ATCC14579 (MIC, 7.67 µg/mL), with IC_{50} values of 32.0 µg/mL, 16.0 µg/mL, and 16.0 µg/mL, respectively. Both the leaf and rhizome oils exhibited no antimicrobial activity against *Escherichia coli* ATCC 25922, and *Salmonella enterica* ATCC13076. Overall, the studied essential oils exhibited good antimicrobial activity against the tested microorganisms with MIC values in the range 5.67–33.67 µg/mL. The reference compounds, namely, streptomycin for Gram-positive bacteria, exhibited activity with MIC values within the range 0.5 to 1.0 µg/mL, whereas cycloheximide used as an antifungal had MIC values within the range 1.2–3.7 µg/mL. Also, nystatin, an anticandidal compound displayed activity with MIC values within the range 0.8–2.3 µg/mL. It has been postulated [19] that substances with MIC values ≤ 100 µg/mL were considered to have good antimicrobial activity, whereas MIC values from 500–100 µg/mL are considered as a moderate activity. In addition, MIC values from 1000–500 µg/mL are said to be of weak activity whereas MIC values above 1000 µg/mL are considerably inactive. The IC_{50} values have also been considered to be within the range 10–120 µg/mL [4–6]. Accordingly, essential oils from leaves and rhizomes of *A. calcicola* possessed good activity against *E. faecalis*, *S. aureus*, *B. cereus*, *P. aeruginosa*, and *C. albicans*, because the MIC values ≤ 50 µg/mL. The results in this study are in agreement and comparable with data reported on the antimicrobial activity of essential oils of other *Alpinia* plants analyzed from Vietnam and other parts of the world, such as *A. napoensis* [4], *A. globosa* [5], *A. tonkinensis* [5], *A. malaccensis* [6], *A. zerumbet* [16], among others. The chemical constituents and antimicrobial activity of *A. calcicola* essential oils are being reported for the first time. It is believed that the constituents present in the studied essential oils might have influenced the observed antimicrobial activity of *A. calcicola*. A section of the chemical compounds identified in the essential oils were reported previously to possess antimicrobial activity [20, 21].

ACKNOWLEDGMENT

The authors are grateful to Mrs. Muslimat Buhari for the typesetting of the manuscript to our satisfaction.

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