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CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL FROM THE RHIZOMES OF *Curcuma pambrosima* GROWING IN VIETNAM

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

Curcuma pambrosima Skornick. & N.S. Ly is a rhizomatous herb that grows up to 80 cm tall. The aromatic and ovoid-lanceolate rhizome is externally light brown and internally cream white. The pseudostem is about 10–25 cm long, green, and composed of leaf sheaths and sheathed by 2–4 leafless glabrous sheaths [1]. The lack of information on the chemical constituents and biological activity of the volatile and non-volatile extracts has aroused our interest and hence the present study on the chemical constituents and antimicrobial activity of essential oil from the rhizome of *C. pambrosima*.

Mature rhizomes of *C. pambrosima* were collected from Hoa Dong Commune, Dong Hoa District, Phu Yen Province, Vietnam, in August 2017. The sample was identified by Dr. D. N. Dai. A voucher specimen, DND 757, was deposited at the Botany Museum, Nghe An College of Economics, Vietnam. A total of 1 kg of the pulverized sample was used. Essential oil was obtained by hydrodistillation, which was carried out in a Clevenger-type distillation unit designed according to an established specification [2] as described previously [3–5].

Gas chromatography (GC) analysis was performed on an Agilent Technologies HP 7890 Plus gas chromatograph equipped with a FID and fitted with HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technology). The analytical conditions were: carrier gas He (1 mL/min), injector temperature at 250°C, detector temperature 260°C, and column temperature programmed from 40°C (2 min hold) to 220°C (10 min hold) at 4°C/min. Samples were injected by splitting and the split ratio was 10:1. The volume of diluted oil in hexane (1:10) injected was 1.0 μL. Inlet pressure was 6.1 kPa. Each analysis was performed in triplicate. The relative amounts of individual components were determined on normalized percentages.

An Agilent Technologies HP 7890N Plus chromatograph fitted with a 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 the gas chromatography-mass spectrometry (GC-MS) experiment, under the same conditions as those used for gas chromatography analysis as described previously [3–5]. The GC 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 35–350 amu at a sampling rate of 1.0 scan/s. The identification of constituents from the GC/MS spectra of *C. pambrosima* 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. The mass spectral (MS) fragmentation patterns were checked against those of other essential oils of known composition [6] and with those in the literature as described previously [3–5].

The minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values were measured by the microdilution broth susceptibility assay [7, 8]. Stock solutions of the oil were prepared in dimethylsulfoxide (DMSO).

1) Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam, e-mail: daidn23@gmail.com; 2) Faculty of Agriculture, Forestry and Fishery, Nghe An College of Economics, 51-Ly Tu Trong, Vinh City, Nghe An Province, Vietnam; 3) School of Natural Science Education, Vinh University, 182 Le Duan, Vinh City, Nghe An Province, Vietnam; 4) Institute of Tropical Biology, Vietnam Academy of Science and Technology, 85 Tran Quoc Toan road, District 3, Hochiminh City; 5) Foresight Institute of Research and Translation, Ibadan, Nigeria, e-mail: isiakaogunwande@gmail.com. Published in *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2022, pp. 128–130. Original article submitted February 22, 2020.

TABLE 1. Constituents of Essential Oil of *Curcuma pambrosima*

Compound[a]	RI[b]	%	Compound[a]	RI[b]	%
Tricyclene	928	0.1	α-Humulene	1472	5.2
α-Pinene	938	2.9	Germacrene D	1498	1.8
Camphene	955	4.8	β-Selinene	1505	1.0
Sabinene	978	0.6	Viridiflorene	1512	0.9
β-Pinene	985	9.5	Bicyclogermacrene	1514	0.7
Myrcene	992	0.1	δ-Cadinene	1537	0.7
o-Cymene	1030	0.3	*cis*-Calamenene	1539	0.5
Limonene	1034	2.4	*trans*-Cadine-1,4-diene	1548	0.8
1,8-Cineole	1037	2.1	Elemol	1565	0.2
γ-Terpinene	1064	0.2	(*E*)-Nerolidol	1570	0.5
Linalool	1103	0.1	Spathulenol	1590	0.4
Nonanol	1103	0.1	Caryophyllene oxide	1605	0.5
trans-Sabinol	1150	0.2	Guaiol (Champacol)	1614	3.3
Camphor	1155	9.7	Humulene epoxide II	1632	1.4
Isoborneol	1169	12.1	1-*epi*-Cubenol	1647	1.1
Pinocarvone	1173	0.3	allyl-2,4-Diacetoxybenzene	1652	0.4
Borneol	1177	0.5	*epi*-α-Muurolol	1661	1.0
Terpinen-4-ol	1187	0.2	α-Cadinol	1674	0.1
Myrtenal	1205	0.4	*neo*-Intermedeol	1677	0.7
Carvone	1253	0.4	Bulnesol	1686	2.4
2-Undecanone	1295	0.2	γ-Bicyclohomofarnesal	1682	0.4
Chavicol acetate	1352	0.4	Total		90.3
α-Cubebene	1360	0.4	Monoterpene hydrocarbons		20.9
α-Copaene	1389	0.8	Oxygenated monoterpenes		26.4
cis-β-Elemene	1403	0.4	Sesquiterpene hydrocarbons		30.1
α-Gurjunene	1425	0.4	Oxygenated sesquiterpenes		11.6
β-Caryophyllene	1437	15.4	Non-terpenes		1.3
(*Z*)-β-Farnesene	1461	1.3	Color of the oil = Light yellow		

[a]Elution order on HP-5MS column; [b]Retention indices on HP-5MS column.

TABLE 2. Antimicrobial Activity of Rhizome Essential Oil of *C. pambrosima*, μg/mL

Microorganism	MIC[a]	IC_{50}	Microorganism	MIC[a]	IC_{50}
Enterococcus faecalis ATCC299212	64.0 ± 0.10	20.45 ± 0.12	*Bacillus cereus* ATCC14579	64.0 ± 0.12	26.78 ± 0.20
Staphylococcus aureus ATCC25923	256.0 ± 0.20	100.56 ± 0.11	*Candida albican*s ATCC10231	16.0 ± 0.10	6.78 ± 0.10

Escherichia coli ATCC25922, *Pseudomonas aeruginosa* ATCC27853, *Salmonella enteric* ATCC13076 – no activity.

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 micro-test tubes from where they were transferred separately to 96-well microtiter plates. Bacteria were grown in double-strength Mueller–Hinton broth or double-strength tryptic soy broth, and fungi were sustained in double-strength Sabouraud dextrose broth, standardized to 5×10^5 and 1×10^3 CFU/mL, respectively. The last row, containing only the serial dilutions of sample without microorganisms, was used as a negative control. Ampicillin and nystatin served as positive controls for bacteria and fungal respectively. All experiments were performed in triplicate. After incubation at 37°C for 24 h, the MIC values were determined as well, with the lowest concentration of agents completely inhibiting the growth of microorganisms. The IC_{50} values were determined as the percentage of microorganisms inhibiting growth based on the turbidity measurement data of an EPOCH2C spectrophotometer (BioTeK Instruments, United States) and Rawdata computer software (Belgium).

The average yield of the hydrodistilled essential oil from *C. pambrosima* was 0.18% (v/w, ± 0.01), calculated on dry weight basis. Forty-nine compounds consisting of monoterpene hydrocarbons (20.9%), oxygenated monoterpenes (26.4%),

sesquiterpene hydrocarbons (30.1%), oxygenated sesquiterpene (11.6%), and non-terpene (1.3%) compounds were identified from the GC-MS spectra (Table 1). The main constituents of the oil were β-caryophyllene (15.4%), isoborneol (12.1%), camphor (9.7%), and β-pinene (9.5%). This is the first report on the volatile constituents of *C. pambrosima.*

Like previously analyzed *Curcuma* oil samples from Vietnam and elsewhere, terpene compounds predominate in the essential oil. However, the identities of these terpenoids differ from one sample to another. For example, camphor (25.83%) and germacrone (8.00%) were the major components of the rhizome of *C. singularis* [9], while curcumol (29.5%) and neocurdione (28.2%) predominate in *C. cochinchinensis* [5] grown in Vietnam. In addition, the oil of *C. aeruginosa* from Malaysia contained an abundance of camphor (29.39%) and germacrone (21.21%), while germacrone (15.76%), β-pinene (9.97%) and camphor (9.96%) were found in *C. glans*, with α-terpinolene (24.6%) and *p*-cymene (12.17%) occurring as major constituents of *Curcuma* cf. *xanthorrhiza* [10]. The chemical constituents of *C. aeruginosa* from Vietnam [4] consist mainly of β-pinene (21.9%), neocurdione (16.1%), and curcumol (15.2%). The main constituents of *C. piererrana* from Vietnam were isoborneol and camphor, while the composition of *C. nankunshanensis* oil from China was made up of curdione and germacrone [11]. From the above it can be seen that monoterpenes predominate in *C. piererrana* and *Curcuma* cf. *xanthorrhiza*, while a mixture of monoterpene and sesquiterpene can be seen prominent in *C. aeruginosa* and *C. glans*. Sesquiterpene compounds are major components of *C. nankunshanensis* [12] and *C. cochinchinensis* [5]. There are variations in the contents of *Curcuma* species depending on origin [12]. The variations in the terpene contents of the various studied *Curcuma* oils can be due to several factors such as nature of the plant, time of collection, environmental and climatic conditions, pH, etc. [13].

The gram-positive bacteria and yeast were more sensitive to the essential oil than the gram-negative bacteria. The rhizome essential oil of *C. pambrosima* displayed the strongest activity against *Candida albicans* (ATCC10231) with MIC of 16.0 µg/mL. In addition, strong antimicrobial activity was recorded against *Enterococcus faecalis* (ATCC299212) and *Bacillus cereus* (ATCC14579) with MIC of 64.0 µg/mL (Table 2). However, the oil displayed moderate activity towards *Staphylococcus aureus* (ATCC25923) with MIC of 256.0 µg/mL. The median inhibitory concentrations (IC_{50}) against the tested microbes were evaluated as 20.45, 100.56, 26.78, and 6.78 µg/mL, respectively. The MIC and IC_{50} provided evidence that the rhizome oil of *C. pambrosima* exhibited promising antimicrobial activity against *C. albicans*, *E. faecalis*, *B. cereus*, and *S. aurues*, respectively. The oil, however, did not display any antimicrobial activity towards *Escherichia coli* ATCC25922, *Pseudomonas aeruginosa* ATCC27853, and *Salmonella enterica* ATCC13076. Ampicillin exhibited MIC values in the range 0.32 to 2.56 µg/mL, while nystatin had MIC values of about 8.0 µg/mL.

This is the first report on the antimicrobial activity of essential oil of *C. pambrosima.* A comparison of the antimicrobial data with the literature on the antimicrobial activity of other *Curcuma* oils indicated that *C. pambrosima* oil showed activity greater against *C. albicans* than *C. aeruginosa* [10] and also displayed stronger activity against *B. cereus* than *C. singularis* [9] and *C. aeruginosa* [10] oil samples. However, the activity of *C. pambrosima* oil towards *S. aurues* was lower than those of *C. aeruginosa* [10] and *C. longa* [14]. The oil of *C. manga* [15], however, exhibited stronger and greater antimicrobial actions against both *E. faecalis* and *B. cereus* than *C. pambrosima. C. zedoaria* [16] essential oil was effective against *P. aeruginosa* in contrast to *C. pambrosima* oil. The antimicrobial action of *C. pambrosima* oil may be attributed to terpenes, particularly β-caryophyllene, isoborneol, camphor, and β-pinene present therein [17]. For example, the antibacterial activity of β-caryophyllene against *S. aureus* was reported recently [18].

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