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Composition and antimicrobial activity of essential oil from the fruits of *Amomum cannicarpum*

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Essential oil from the fruits of *Amomum cannicarpum* (Wight) Bentham ex Baker (*Zingiberaceae*) was hydrodistilled and characterized by gas chromatography-mass spectrometry (GC-MS). Major constituents of the oil were β-pinene (14.00%), elemol (10.45%) and α-cadinol (8.50%). Thirty-three (91.48%) out of forty-one constituents were identified by GC-MS and subsequent data analysis. Antimicrobial activity of the oil against Gram--positive and Gram-negative bacteria and some fungi, was determined by the disc diffusion assay. The oil showed good antibacterial activity against *Salmonella typhi*, *Pseudomonas aeruginosa* and *Proteus vulgaris* and very good antifungal activity against *Candida albicans* and *C. glabrata*.

Keywords: *Amomum cannicarpum* (*Zingiberaceae)*, essential oil, GC-MS, β-pinene, elemol, α-cadinol, antibacterial activity, antifungal activity

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Zingiberaceae is one of the essential oil bearing plant families. The genus *Amomum* in this family has over ninety species distributed in Africa, tropical Asia, Australia and the Pacific Islands (1, 2). These plants are mostly terrestrial, rhizomatous herbs. *Amomum* seeds are used as spices and their plant parts are used in traditional medicine for curing toothache, dysentery, diarrhoea, rheumatism, vomiting, dyspepsia and lung diseases (3, 4). *Amomum subulatum* or 'large cardamom' distributed in the eastern Himalayas is the most investigated *Amomum* species. The chemical composition of essential oils from the seeds of *A. subulatum* have been studied by various groups and the major constituent, 1,8-cineole, has been found to vary from 61 to 86% (5, 6). Also, oils from different parts of other *Amomum* species such as *A. thyrsoideum* (7), *A. longiligulare* (7), *A. villosum* (8), *A.*

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muricarpum (9), *A. kwangsiense* (10), *A. schmidtti* (11), *A. xanthioides* (12), *A. tsao-ko* (12), *A. linguiforme* (4, 13) and *A. testaceum* (14) were studied previously.

We have recently reported the chemical composition and antimicrobial activities of the rhizome and leaf oils of *Amomum cannicarpum* (15, 16). We have also studied the chemical compositions of the leaf oil of *A. muricatum* from the Western Ghats region in South India (17). β-pinene is the major constituent of these oils and this is in agreement with previous reports on other *Amomum* oils (7, 8, 10–14).

A. cannicarpum is a stout, gregarious herb growing up to 3.5 m. It is endemic to South India and is fairly common in its evergreen forests (1). This is the first report on the chemical composition and antimicrobial activity of the essential oil from the fruits of *A. cannicarpum*.

EXPERIMENTAL

Plant collection and oil isolation

Mature fruits of *A. cannicarpum* were collected from Cheruthoni, Idukki district in Kerala (India) in February 2005. A voucher specimen was deposited in the Herbarium of Tropical Botanic Garden and Research Institute (Pacha-Palode, Thiruvananthapuram, India). Hydrodistillation of the fresh fruits (959 g) using a Clevenger-type apparatus for three hours yielded 0.8 mL of a colourless, pleasant smelling oil.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the essential oil was carried out by splitless injection of 1.0 μL of the oil on a Hewlett Packard 6890 gas chromatograph (Hewlett Packard, USA) fitted with an HP-5 MS cross-linked 5% PH ME siloxane, 30 m × 0.32 mm × 0.25 μm capillary column, coupled with a model 5973 mass detector. GC-MS operation conditions: injector temperature 220 °C, transfer line 240 °C, oven temperature programme 60–243 °C (3 °C min^{-1}), carrier gas He 1.4 mL min^{-1}, mass spectra: electron impact (EI^+) mode 70 eV, ion source temperature 240 °C. Individual components were identified by Wiley 275.L database matching and by comparison of mass spectra with published data (18). Relative percentages of individual components in the oil (Table I) were calculated from their peak areas in the total ion chromatogram obtained from the GC-MS experiment. Relative retention indices (RRI) of constituents in Table I were determined on the above PH ME siloxane capillary column using C_5-C_{30} straight chain alkanes (Aldrich, India) as standards.

Bacterial and fungal strains

Gram-positive bacteria, *Staphylococcus aureus* (MTCC 96), *Bacillus cereus* (MTCC 430), *B. subtilis* (MTCC 441), Gram-negative bacteria, *Serratia marcescens* (MTCC 97), *Pseudomonas fluorescens* (MTCC 103), *P. aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (MTCC 109), *Proteus vulgaris* (MTCC 426), *Escherichia coli* (MTCC 443), *Salmonella typhi* (MTCC

733) and the fungi, *Candida albicans* (MTCC 227), *C. albicans* (MTCC 1637), *C. albicans* (MTCC 3017), *C. glabrata* (MTCC 3019) were obtained as the Microbial Type Culture Collection (MTCC) from the Institute of Microbial Technology, Chandigarh (India).

Table I. Chemical composition of the essential oil from the fruits of Amomum cannicarpum *determined by gas chromatography-mass spectrometry*

Constituent	RRI[a]	Concentration (%)
β-pinene	972	14.00
p-cymene	1022	3.88
Limonene	1025	3.41
1,8-Cineole	1027	0.48
trans-linalool oxide	1069	0.43
cis-linalool oxide	1086	0.70
Linalool	1102	3.60
α-pinene oxide	1105	0.67
α-campholenal	1123	0.70
trans-pinocarveol	1135	3.04
Pinocarvone	1158	1.19
Cryptone	1184	3.07
α-terpeniol	1190	1.51
Myrtenal	1193	2.07
Verbenone	1206	0.63
Cuminal	1235	1.01
Ascaridole	1252	1.33
α-amorphene	1472	0.56
10,11-Epoxy calamenene	1485	0.93
β-bisabolene	1504	1.22
Elemol	1548	10.45
E-nerolidol	1564	1.41
Widdrol	1592	2.04
Humulene epoxide II	1598	0.72
1,10-di-*epi*-cubenol	1605	0.79
1-*epi*-cubenol	1619	1.13
γ-eudesmol	1625	3.98
epi-α-muurolol	1637	4.74
β-eudesmol	1642	5.92
α-cadinol	1651	8.50
(z)-α-*trans*-bergamotol	1714	3.20
Oplopanone	1732	2.50
14-Hydroxy-α-muurolene	1781	1.66
Total		91.48

[a] Relative retention index

Antibacterial and antifungal activity

Antibacterial activity of essential oil from the fruits of *A. cannicarpum* was tested against the above-mentioned Gram-positive and Gram-negative bacteria by the disc agar diffusion method (19, 20). The bacteria were grown on Mueller-Hinton agar media (pH 7.3). Agar media were poured into the plates to a uniform depth of 5 mm and allowed to solidify. The microbial suspensions at 5×10^6 cfu mL^{-1} were streaked over the surface of media using a sterile cotton swab to ensure confluent growth of the organism. The discs used were Whatman No. 1 papers, 6 mm in diameter. 10-μL aliquots of the oil 33.3% (*V/V*) in DMSO, spotted on filter paper discs, which were then aseptically applied to the surface of agar plates at well-spaced intervals. The plates were incubated at 37 °C for 24 hours and the observed growth inhibition zones, including disc diameters were measured. Control discs impregnated with 10 μL of the solvent DMSO and streptomycin (2 μg per disc), reference for bacteria, were used alongside the test discs in each experiment (Table II).

The above-listed fungi were obtained as MTCC, cultured in modified Sabouraud's agar and suspensions at 5 x 10^6 cfu mL^{-1} were used. 10 μL of oil (33.3%, *V/V*) in DMSO, was impregnated on discs amplitude with 10 μL of DMSO and fluconazole (2 μg per disc) used as controls (Table II).

Table II. Antimicrobial and antifungal activity of the essential oil from the fruits of Amomum cannicarpum *determined by the disc diffusion assay*

Bacteria/fungi	Diameter of inhibition zone (mm)[a]	
	Oil[b]	Control[c]
Gram-positive bacteria		
Staphylococcus aureus	11	25
B. cereus	0	17
Bacillus subtilis	0	11
Gram-negative bacteria		
Serratia marcescens	7	16
Pseudomonas fluorescens	*10.3*	*20*
P. aeruginosa	12	11
Klebsiella pneumoniae	0	15
Proteus vulgaris	11	15
Escherichia coli	0	17
Salmonella typhi	10	0
Fungi		
Candida albicans	11.7	10
C. albicans	11	7
C. albicans	11.7	25
C. glabrata	11	9

[a] Experiments were done in triplicate and the results are mean values.
[b] 10 μL of oil (33.3%, *V/V*) in DMSO.
[c] Control – 10 μL streptomycin per disc for bacteria, and 10 μL fluconazole (2 μg per disc) for fungi.

RESULTS AND DISCUSSION

Thirty-three constituents out of forty-one, containing 91.48% of the essential oil from the fruits of *A. cannicarpum*, were identified by GC-MS (Table I). The gas chromatogram of the oil on a HP-5 MS capillary column is shown in Fig. 1. The percentage of oxygenated sesquiterpenes in the oil was 47.97%, followed by monoterpene hydrocarbons (21.29%) and oxygenated monoterpenes (20.43%). The percentage of sesquiterpene hydrocarbons in the oil was relatively low (1.78%). The major constituents of the oil were β-pinene (14.00%), elemol (10.45%) and α-cadinol (8.50%). Similar studies on essential oils from the rhizomes (15) and leaves (16) of *A. cannicarpum* and other *Amomum* species (7, 8, 10–14) showed that β-pinene is one of the major constituents in *Amomum* oils.

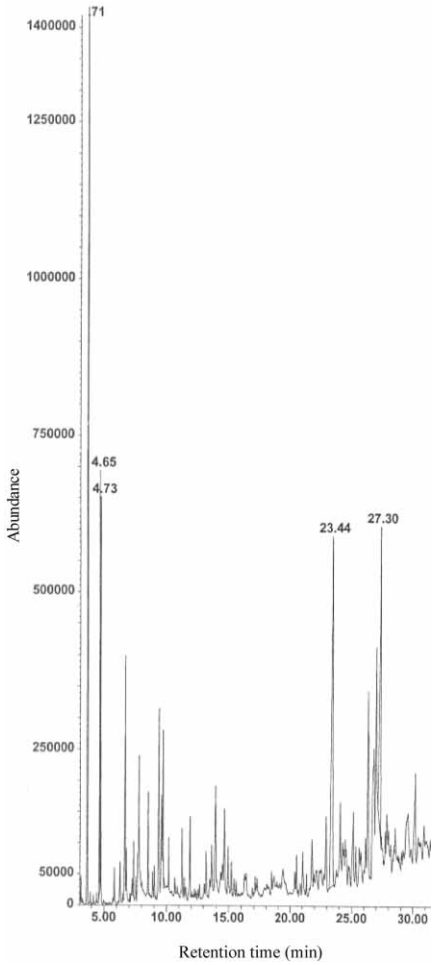

Fig. 1. Gas chromatogram of the essential oil from the fruits of *Amomum cannicarpum* on a HP-5 MS cross-linked 5% PH ME siloxane (30 m x 0.32 mm x 0.25 μm) capillary column.

Antibacterial and antifungal activities of the essential oil were tested by the disc diffusion assay. The oil at a 33.3% (*V*/*V*) concentration in dimethyl sulfoxide showed good activity against the Gram-negative bacteria *Salmonella typhi*, *Pseudomonas aeruginosa* and *Proteus vulgaris* in comparison with streptomycin at 2 μg per disc and against the fungi *Candida albicans* and *C. glabrata*, in comparison with the antifungal control, fluconazole at 2 μg per disc (Table II). No activity was observed against *Bacillus subtilis*, *B. cereus*, *Klebsiella pneumoniae* and *Escherichia coli*.

CONCLUSIONS

Chemical characterization and antimicrobial screening studies on plant-based essential oils could lead to a discovery of new natural antimicrobials. The present study is the first report on chemical characterization of the essential oil from the fruits of *A. cannicarpum*. The oil showed promising antimicrobial activity against, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Proteus vulgaris* and the fungi *Candida albicans* and *C. glabrata*.

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S A Ž E T A K

Sastav i antimikrobno djelovanje eteričnog ulja iz plodova biljke *Amomum cannicarpum*

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Eterično ulje iz plodova biljke *Amomum cannicarpum* (Wight) Bentham ex Baker (*Zingiberaceae*) dobiveno je destilacijom vodenom parom, a zatim je pomoću plinske kromatografije i masene spektrometrije (GC-MS) određen njegov sastav. Najvažniji sastojci u ulju bili su β-pinen (14,00%), elemol (10,45%) i α-kadinol (8,50%). Ukupno su od 41 sastojka identificirana 33 sastojka (91,5%). Disk-difuzijskom metodom određeno je antimikrobno djelovanje ulja na Gram-pozitivne i Gram-negativne bakterije i neke gljivice. Dosta snažno antimikrobno djelovanje zapaženo je na bakterije *Salmonella typhi*, *Pseudomonas aeruginosa* i *Proteus vulgaris* te na gljivice *Candida albicans* i *C. glabrata*.

Ključne riječi: Amomum cannicarpum (Zingiberaceae), eterično ulje, GC-MS, β-pinen, elemol, α-kadinol, antibakterijsko djelovanje, antimikotsko djelovanje

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