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Caryophyllene-rich rhizome oil of *Zingiber nimmonii* from South India: Chemical characterization and antimicrobial activity

Baby Sabulal [a], Mathew Dan [b], Anil John J [a], Rajani Kurup [a], Nediyamparambu Sukumaran Pradeep [c], Renju Krishna Valsamma [c], Varughese George [a,*]

[a] *Phytochemistry Division, Tropical Botanic Garden and Research Institute, Pacha-Palode, Thiruvananthapuram 695 562, Kerala, India*
[b] *Horticulture and Garden Development Division, Tropical Botanic Garden and Research Institute, Pacha-Palode, Thiruvananthapuram 695 562, Kerala, India*
[c] *Microbiology Division, Tropical Botanic Garden and Research Institute, Pacha-Palode, Thiruvananthapuram 695 562, Kerala, India*

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Abstract

Volatile oil from the rhizomes of *Zingiber nimmonii* (J. Graham) Dalzell was isolated, characterized by analytical gas chromatography and gas chromatography-mass spectroscopy. Sixty-five constituents accounting for 97.5% of the oil were identified. *Z. nimmonii* rhizome oil is a unique caryophyllene-rich natural source with isomeric caryophyllenes, β-caryophyllene (42.2%) and α-humulene (α-caryophyllene, 27.7%), as its major constituents along with traces of isocaryophyllene. The rhizome oil contained 71.2% sesquiterpenes, 14.2% oxygenated sesquiterpenes, 8.9% monoterpenes, 1.9% oxygenated monoterpenes and 1.3% non-terpenoid constituents. The antimicrobial activity of the oil was tested against human and plant pathogenic bacteria and fungi. The oil showed significant inhibitory activity against the fungi, *Candida glabrata*, *C. albicans* and *Aspergillus niger* and the bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa*. No activity was observed against the fungus *Fusarium oxysporum*.

Keywords: *Zingiber nimmonii*; Zingiberaceae; Essential oil; Chemical composition; β-caryophyllene; α-humulene; Antimicrobial activity

1. Introduction

The genus *Zingiber* has about 85 species of aromatic herbs mostly distributed in East Asia and tropical Australia (Mabberley, 1990). The term '*Zingiber*' is derived from the Sanskrit word '*shringavera*', owing to their 'horn-shaped' rhizomes. *Zingiber* species are rich in volatile oils and are used in traditional medicine and as spices. *Z. officinale* Roscoe or 'ginger' has been used in Indian traditional medicine for relief from arthritis, rheumatism, sprains, muscular aches and pains, congestion, coughs, sinusitis, sore throats, diarrhoea, cramps, indigestion, loss of appetite, motion sickness, fever, flu, chills, etc. (Varier, 1996). The ethnomedical and pharmacological activities of *Z. officinale* have been reviewed by various authors (Lawrence, 1984; Mascolo et al., 1989; Afzal et al., 2001; Anonymous, 2003).

Volatile oils from the rhizomes of *Z. officinale* and *Z. zerumbet* from various sources have been characterized (Connell, 1970; Sakamura et al., 1986; Chane-Ming et al., 2003; Pino et al., 2004). Zingiberene and ar-curcumene are the major constituents in most of the rhizome oils of *Z. officinale*. They are known for insecticidal, repellent and insect feeding deterrent activities (Connell, 1970; Sakamura et al., 1986; Millar, 1998; Antonious and Kochhar, 2003; Chane-Ming et al., 2003; Pino et al., 2004). Zerumbone is the major component in rhizome oils of *Z. zerumbet* (Chane-Ming et al., 2003). It shows potential anti-inflammatory and chemopreventive activities (Kitayama et al., 1999, 2001; Murakami et al., 2002; Kirana et al., 2003; Nakamura et al., 2004). Rhizome oils from

* Corresponding author. Tel.: +91 472 2869628; fax: +91 472 2869646.
E-mail address: sabulal@gmail.com (B. Sabulal).

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other *Zingiber* species such as *Z. cassumunar* (Assam, India) (Bordoloi et al., 1999), *Z. wrayi* var. *halabala* (Narathiwat, Thailand) (Chairgulprasert et al., 2005), *Z. ottensii* (Phetchaburi, Thailand) (Thubthimthed et al., 2005), etc. have also been studied.

Zingiber nimmonii (J. Graham) Dalzell, an endemic species from the Western Ghats in South India, grows both at low and high altitudes, in moist areas under the shades of trees (Bhat, 1993; Sabu, 2003). Its rhizomes are fleshy with a yellowish cross-section and an occasional purple tinge. Here, we report the isolation, chemical characterization, antibacterial and antifungal activities of the previously uninvestigated rhizome oil of *Z. nimmonii*.

2. Results and discussion

Sixty-five constituents out of 81, comprising 97.5% of the rhizome oil of *Z. nimmonii* were characterized by GC-FID and GC-MS (Table 1). Isomeric sesquiterpenes, β-caryophyllene and α-humulene (α-caryophyllene), were the major constituents in the rhizome oil of *Z. nimmonii*. Sesquiterpenes and their oxygenated derivatives constituted 85.4% of the oil. Monoterpenes, oxygenated monoterpenes and other components constituted 8.9%, 1.9% and 1.3% of the oil, respectively.

The analyzed oil contained 42.2% β-caryophyllene, 27.7% α-humulene (α-caryophyllene), 0.03% isocaryophyllene and 1.7% caryophyllene oxide. The major constituent of the oil, β-caryophyllene, is a natural bicyclic sesquiterpene with a rare cyclobutane ring. It is usually found in nature as a mixture with α-humulene and isocaryophyllene (Budavari, 1996). β-caryophyllene is also found in oils of *Copaiba balsam* 53.3% (Gramosa and Silveira, 2005), clove (*Syzigium aromaticum*, bud oil 19.5%) (Srivastava et al., 2005) and in minor quantities in other oils. It is also one of the terpenoids contributing to the spiciness of black pepper (*Piper nigrum*) oil (24.2%) (Singh et al., 2004).

β-Caryophyllene is known for its anti-inflammatory and local anaesthetic activities (Tambe et al., 1996; Ghelardini et al., 2001). It is used in spice blends, citrus flavors, soaps, detergents, creams and lotions, and also in a variety of food products and beverages (Budavari, 1996; Skold et al., 2006). It has been reported as a volatile compound emitted by plants into the atmosphere in response to herbivore attack and due to change in abiotic factors (Gouinguene and Turlings, 2002) and as sequestered by the Australian green tree frog, *Litoria caerulea* White from its diet (Smith et al., 2004).

Sesquiterpenes isolated from rhizome oils of *Z. zerumbet* and *Z. officinale* have shown potential antitumour, anti-inflammatory and insect repellent activities. Zerumbone (2,6,9 humulatriene-8-one) and α-humulene, are the major constituents in the rhizome oils of *Z. zerumbet* (Chane-Ming et al., 2003). The α,β-unsaturated carbonyl group of zerumbone is a prerequisite for its antitumour activity (Murakami et al., 2002). α-Humulene, without the C-8 carbonyl group

Table 1
Chemical composition of the rhizome oil of *Zingiber nimmonii*

Constituent	RR_t	%
n-Nonane	898	t
Tricyclene	923	t
α-Thujene	927	t
α-Pinene[c]	936	t
Camphene	951	0.08
Sabinene	974	0.41
β-Pinene[c]	979	0.68
Myrcene[c]	991	4.18
δ-2-Carene	1003	0.06
α-Phellandrene[c]	1010	0.74
α-Terpinene[c]	1018	1.11
p-Cymene[c]	1025	0.64
o-Cymene	1028	0.19
Limonene[c]	1033	0.44
(E)-β-ocimene	1049	0.07
γ-Terpinene[c]	1058	0.10
Terpinolene	1088	0.13
trans-Sabinene hydrate	1104	0.09
2-Nonen-1-ol	1145	0.10
Camphor	1151	1.00
Camphene hydrate	1154	0.25
α-Phellandren-8-ol	1166	0.06
Borneol[c]	1172	0.08
Terpinen-4-ol	1182	0.22
α-Terpineol[c]	1195	0.07
Myrtenal	1203	0.10
n-Decanal	1207	0.09
t-Piperitol	1214	t
Bornyl acetate	1291	t
6-Tridecene	1307	t
α-Copaene	1384	t
β-Elemene	1399	0.14
Isocaryophyllene	1418	t
β-Caryophyllene[c]	1441	42.15
α-Humulene (α-caryophyllene)[c]	1474	27.68
γ-Muurolene	1486	0.15
2-Nonyn-1-ol	1501	0.07
α-Muurolene	1510	0.14
β-Bisabolene	1514	0.06
γ-Cadinene	1525	0.08
δ-Cadinene	1534	0.25
Zonarene	1537	0.25
10-*epi*-Cubebol	1545	1.42
Germacrene B	1554	0.23
Nerolidol[a]	1570	0.14
trans-Sesquisabinene hydrate	1590	0.55
Caryophyllene oxide[c]	1604	1.68
Globulol	1610	0.33
(Z)-Bisabol-11-ol	1616	t
TMCD[b]	1618	0.33
3-Octadecyne	1629	1.05
cis-Cadin-4-en-7-ol	1643	0.19
Epoxy-*allo*-alloaromadendrene	1650	0.68
τ-Muurolol	1657	2.06
α-Muurolol	1660	0.68
Cubenol	1664	0.09
α-Cadinol	1670	2.72
14-Hydroxy-9-*epi*-(E)-caryophyllene	1674	1.15
β-Bisabolol	1680	1.29
α-Bisabolol	1695	0.19
cis-Z-α-bisabolene epoxide	1704	0.08
(Z)-α-*trans*-bergamotol	1707	0.06
(2Z,6Z)-Farnesol	1723	0.10
(2E,6E)-Farnesol	1729	0.06

Table 1 (*continued*)

Constituent	RR_t	%
(2E,6Z)-Farnesol	1750	0.36
Total number of constituents		81
Number of constituents identified		65
% Identified		97.5%
Monoterpene hydrocarbons		8.87%
Oxygenated monoterpenes		1.91%
Sesquiterpene hydrocarbons		71.19%
Oxygenated sesquiterpenes		14.19%
Other constituents		1.34%

RR_t – relative retention time (calculated); t – trace, <0.05%.

[a] Correct isomer not identified.

[b] TMCD – 1,5,8,8-tetramethyl-cycloundeca-5,9-dien-1-ol. All oil constituents identified by (i) mass spectral database match, (ii) comparison of mass spectrum with literature data and (iii) RR_t.

[c] Constituents identified by (i), (ii), (iii) and (iv) co-injection.

Table 2
Antimicrobial activity of the rhizome oil of *Zingiber nimmonii*

Tested bacteria/fungi (MTCC No.)	Diameter of inhibition zone (mm)	
	Rhizome oil	Control[a]
Gram-positive bacteria		
Bacillus cereus (430)	9.5 ± 0.5	16.7 ± 0.8
B. subtilis (441)	11.3 ± 0.6	10.7 ± 0.3
Staphylococcus aureus Subsp. *aureus* (2940)	9.0 ± 0.0	24.8 ± 0.3
Gram-negative bacteria		
Serratia marcescens (97)	10.0 ± 0.0	16.0 ± 0.0
Pseudomonas fluorescens (103)	10.3 ± 0.6	20.2 ± 0.3
P. aeruginosa (741)	8.0 ± 0.0	11.0 ± 0.0
Klebsiella pneumoniae (109)	8.0 ± 0.0	15.0 ± 0.0
Proteus vulgaris (426)	7.7 ± 0.6	15.2 ± 0.3
Escherichia coli (443)	7.7 ± 0.6	17.0 ± 0.0
Salmonella typhi (733)	10.0 ± 0.0	Nil
Fungi		
Fusarium oxysporum (224)	Nil	10.0 ± 0.0
Aspergillus niger (1344)	9.0 ± 0.0	11.3 ± 0.6
Candida glabrata (1637)	12.0 ± 0.0	7.0 ± 0.0
C. albicans (3049)	9.0 ± 0.0	9.0 ± 0.0

Oil dilution – 1:2 in DMSO. DMSO did not show inhibition zones. Experiments were done in triplicate and results are mean values ± standard deviation.

[a] Control – streptomycin at 2 μg/disc for bacteria and fluconazole at 2 μg/disc for fungi.

in zerumbone, does not show antitumour activity (Murakami et al., 1999). Zingiberene and ar-curcumene in *Z. officinale* rhizome oil are known for their insecticidal properties (Millar, 1998; Antonious and Kochhar, 2003). *Z. nimmonii* rhizome oil varied from the above two most studied *Zingiber* oils in its major constituents, providing chemotaxonomic support to the identity of this species. The present data also imply that the cyclization of farnesyl pyrophosphate to α- and β-caryophyllenes, catalyzed by humulene and caryophyllene cyclases, are major biosynthetic steps in *Z. nimmonii* (Croteau and Gundy, 1984).

The antimicrobial activity of *Z. nimmonii* rhizome oil was tested against common pathogens, viz., three Gram-positive bacteria, seven Gram-negative bacteria and four fungi, by the disc diffusion technique and the results are shown in Table 2. The oil, at 1:2 dilution in dimethyl sulfoxide, showed significant activity against the fungi, *Candida glabrata*, *C. albicans* and *Aspergillus niger*, in comparison with the antifungal control, fluconazole (2 μg/disc). *Fusarium oxysporum* f. *Zingiberi* (Schlect.) Synd. & Hans. is known to cause the disease 'rhizome rot' or 'fusarium yellows' on *Zingiber officinale* rhizomes in Hawaii, India and in Queensland, Australia (Lawrence, 1984; Stirling, 2004). Even though the composition of *Z. nimmonii* rhizome oil in this study is different from that of *Z. officinale*, antifungal analysis did not result in any inhibition zones against *Fusarium oxysporum*.

The rhizome oil, 1:2 in dimethyl sulfoxide, showed good activity against the bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa*, in comparison with streptomycin at 2 μg/disc. The good activity of *Z. nimmonii* rhizome oil against *Bacillus subtilis* and *Aspergillus niger*, both food contaminants, makes it a promising antimicrobial for food preservation.

3. Conclusion

Zingiber nimmonii rhizome oil studied here is a unique natural product with 69.9% of isomeric caryophyllenes, viz., β-caryophyllene (42.2%) and α-caryophyllene (27.7%), along with traces of isocaryophyllene (0.03%) in it. The major constituents of the rhizome oil of *Z. nimmonii* varied from the rhizome oils of *Z. zerumbet* and *Z. officinale*, the most studied *Zingiber* oils. The oil showed significant activities against the human pathogenic fungi, *Candida glabrata*, *C. albicans* and *Aspergillus niger*, but no activity against the plant pathogen, *Fusarium oxysporum*.

4. Experimental

4.1. General

GC-FID analysis was carried out on Shimadzu GC 2010 with FID and GC-MS analyses on Shimadzu GC-MS-QP 2010 coupled with a QP-2010 mass detector and Hewlett Packard 6890 gas chromatograph coupled with a model 5973 mass detector. DB-5 (5% phenyl modified 95% dimethyl polysiloxane, non-polar, J&W Scientific, USA), HP-5 (cross-linked 5% phenylmethylsiloxane, non-polar, Hewlett Packard, USA) and BP-20 (polyethylene glycol, polar, SGE, Australia) were the capillary columns used for these analyses. C_5–C_{30} straight chain alkanes, 29850-6, Aldrich Chemical Company, USA were used as standards for the determination of relative retention times (RR_t). WILEY 139, 275 and NIST 107 libraries were used for individual spectral matching of constituents in the oil.

4.2. Plant material

Zingiber nimmonii rhizomes were collected from Ponmudi Hills at an altitude of 1100 m on 5th October 2005, identified by Dr. Mathew Dan, one of the authors. A voucher specimen, TBGT 54655, has been deposited at the Herbarium of Tropical Botanic Garden and Research Institute (TBGRI).

4.3. Isolation of volatile oil

Fresh rhizomes (650 g) were hydrodistilled on a Clevenger-type apparatus for 4 h to obtain 0.25 ml of pale yellow coloured, pleasant smelling oil at 0.04% (v/w) yield. The oil was stored at 4 °C until further analysis.

4.4. GC-FID analysis

GC-FID analyses were carried out by injection of 0.2 μl of *Z. nimmonii* rhizome oil onto a Shimadzu GC 2010 with FID, fitted with a DB-5 capillary column (30 m × 0.25 mm × 0.25 μm). GC operation conditions: injection mode – split; split ratio – 50; injector temperature – 250 °C; oven temperature programme – 60° to 260 °C (5 °C min^{-1}); carrier gas – helium at 1.5 ml min^{-1}. Relative percentages of individual components of the oil in Table 1 were obtained from the GC-FID peak area-percent report.

4.5. GC-MS analysis

GC-MS analysis of *Z. nimmonii* rhizome oil was performed by injection of 0.2 μl of the oil on a Shimadzu GC-MS-QP 2010 coupled with a model QP-2010 mass detector, fitted with a DB-5 (30 m × 0.25 mm × 0.25 μm) capillary column. GC-MS operation conditions: injection mode – split; split ratio – 50; injector temperature – 250 °C; transfer line – 260 °C; oven temperature programme – 60° to 260 °C (5 °C min^{-1}); carrier gas – helium at 1.5 ml min^{-1}. Mass spectra: Electron Impact (EI^+) mode 70 eV, ion source temperature 220 °C. RR_ts of constituents in Table 1 were determined on the DB-5 column using C_5–C_{30} straight chain alkanes as standards. Individual constituents of the oil were identified by WILEY and NIST database matching, by comparison of mass spectra with published data (Adams, 2001) and by comparison of their RR_ts (Davies, 1990; Adams, 2001). Identification of the major constituents (>4%) of the oil was confirmed by injection of 0.2 μl of the oil onto a BP-20 (30 m × 0.32 mm × 0.25 μm) capillary column under the same instrumental and operational conditions as above and by subsequent analysis of their spectra. Identification of major constituents was further confirmed by co-injection of the oil with authentic standards onto a Hewlett Packard 6890 gas chromatograph fitted with an HP-5 (30 m × 0.32 mm × 0.25 μm) capillary column, coupled with a model 5973 mass detector, under similar operational conditions as above (Table 1).

4.6. Bacterial and fungal strains

The bacteria and fungi selected for this study are mostly human and plant pathogens. Gram-positive bacteria, *Bacillus cereus* (MTCC 430); *B. subtilis* (MTCC 441), *Staphylococcus aureus* subsp. *aureus* (MTCC 2940); Gram-negative bacteria, *Serratia marcescens* (MTCC 97), *Pseudomonas fluorescens* (MTCC 103), *P. aeruginosa* (741), *Klebsiella pneumoniae* (MTCC 109), *Proteus vulgaris* (MTCC 426), *Escherichia coli* (MTCC 443), *Salmonella typhi* (MTCC 733) and the fungi, *Fusarium oxysporum* (MTCC 224), *Candida glabrata* (MTCC 1637) *C. albicans* (MTCC 3049) and *Aspergillus niger* (MTCC 1344) were obtained as Microbial Type Culture Collection (MTCC) from the Institute of Microbial Technology, Chandigarh, India.

4.7. Antibacterial activity

Antibacterial activity of *Z. nimmonii* rhizome oil was tested against the above Gram-positive and Gram-negative bacteria by the disc agar diffusion method (Berghe and Vlietinck, 1991; Cappuccino and Sherman, 1998). These bacteria were grown on Mueller–Hinton agar medium (pH 7.3). Agar media were poured into the plates to 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 the confluent growth of the organism. The discs used were Whatman No. 1 papers, 6 mm in diameter. 10 μl aliquots of the rhizome oil were diluted with two volumes of dimethyl sulfoxide (DMSO) and impregnated on filter paper discs, which were then aseptically applied to the surface of the agar plates at well-spaced intervals. The plates were incubated at 37 °C for 24 h and observed growth inhibition zones, including the diameter of the discs, were measured. Control discs impregnated with 10 μl of the solvent DMSO and streptomycin 2 μg/disc, reference for bacteria, were used alongside the test discs in each experiment (Table 2).

4.8. Antifungal activity

The antifungal analyses were carried out by the disc agar diffusion method as above. The above-mentioned fungi were obtained as MTCC, cultured in modified Sabouraud's agar and suspensions at 5×10^6 cfu ml^{-1} (*Candida*) and 5×10^5 conidia ml^{-1} (*F. oxysporum* and *A. niger*) were used. Oil dilution was 1:2 in DMSO, discs impregnated with 10 μl of DMSO and fluconazole (2 μg/disc) were used as controls alongside the test discs in each experiment (Table 2).

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