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Chemical composition and antibacterial activity of the rhizome oil of *Hedychium larsenii*

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The composition of essential oil from the rhizomes of *Hedychium larsenii* M. Dan & Sathish was examined by GC-FID and GC-MS techniques. 99% of the oil consisted of monoterpenoids. Sesquiterpenoids were present only in negligible quantities. Linalool and 1,8-cineole were identified as the major components. The oil showed moderate antibacterial activity against Gram-positive and Gram-negative bacteria.

Keywords: Hedychium larsenii (*Zingiberaceae*), rhizome, essential oil, GC, GC-MS, antibacterial activity

The genus *Hedychium* Koenig (*Zingiberaceae*) comprises about 80 species distributed from India to south-east Asia (1). Plants of the genus *Hedychium* are used in perfumery and in ethnomedicine. Aroma concentrate prepared from the flowers of *H. coronarium* is used in perfumery. According to ethnobotanical information, the roots of *H. acuminatum* is used for diarrhoea, snake bite and liver complaints, while the rhizome of *H. spicatum* is used for asthma, bronchitis, blood purification, gastric disorders and as antiemetic. Rhizone of *H. garcile* is used for chest pain (2).

Hedychium larsenii M. Dan & Sathish is a rhizomatous herb, with a leafy stem ca 60 cm tall. Inflorescences are ca 13 cm long, dense, cone-like, flowers are white, turning creamy with age and fragrant (3). Since there are no reports on the chemistry and antibacterical activity of the rhizome oil of *H. larsenii*, we thought it desirable to undertake the present study. Rhizomes of *H. larsenii*, brought from Manipur, India, were under cultivation in the Tropical Botanic Garden and Research Institute (TBGRI), Palode, South India (100 m).

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Rhizomes of the plant, harvested from the garden in June 2004, were used for the present investigation. A voucher specimen No. 54623 is deposited in TBGT.

EXPERIMENTAL

Sample preparation

Fresh rhizomes were cut into small pieces and hydrodistilled using a Clevenger apparatus with a water-cooled oil receiver. The oil (yield 0.14%, *V/m*) was transferred into a stoppered tube, dried over anhydrous sodium sulphate and stored in a refrigerator at 4 °C until analyzed.

GC-FID and GC-MS analyses

GC-FID analysis of the oil was carried out on a Nucon 5765 gas chromatograph (India) fitted with a SE-30 10% Chromosorb-W packed stainless steel column (2 m x 2 mm). Oven programme was: 80–150 °C: 8 °C min^{-1}, 150–230 °C: 5 °C min^{-1}, 230 °C (10 min); carrier gas: nitrogen, flow rate 40 mL min^{-1}; injector temperature 220 °C; detector temperature 250 °C. GC-MS analysis of the oil was performed by splitless injection of 1.0 μL of the oil on a Hewlett Packard 6890 (USA) gas chromatograph fitted with a cross-linked 5% PH ME siloxane HP-5 MS capillary column (30 m x 0.32 mm, 0.25 μm coating thickness), coupled with a model 5973 mass detector. GC-MS operation conditions were as follows: injector temperature 220 °C; transfer line 290 °C; oven temperature programme 60–246 °C: 3 °C min^{-1}; carrier gas: helium at 1.4 mL min^{-1}; mass spectra: Electron Impact (EI^+) mode: 70 eV, ion source temperature: 250 °C. Individual components were identified by Wiley 275.L database matching and by comparison of retention times and mass spectra of constituents with published data (4). Relative percentage of components was calculated from the peak area-percent report of volatiles from GC-FID data (Table I). Relative retention indices were calculated with reference to the retention times of standard hydrocarbons C_5-C_{30} (Aldrich, India) (5).

Table I. Chemical composition of the rhizome oil of Hedychium larsenii

Component	Retention time (min)	Concentration (%)	RRI
Camphene	3.23	0.24	968
β-Pinene	3.71	3.85	985
Myrcene	3.97	0.14	994
α-Phellandrene	4.23	0.11	1004
α-Terpinene	4.50	0.12	1015
p-Cymene	4.70	8.18	1023
Limonene	4.78	0.63	1026
1,8-Cineole	4.88	14.41	1030
γ-Terpinene	5.52	2.35	1056
Linalooloxide A	5.92	0.97	1072

Table I. continued

Component	Retention time (min)	Concentration (%)	RRI
Linalooloxide B	6.37	1.07	1089
Linalool	6.96	62.26	1106
Fenchyl alcohol	7.15	0.17	1110
Borneol	8.75	0.86	1146
Terpinene-4-ol	9.16	1.09	1155
α-Terpineol	9.67	2.48	1166
α-Selinene	20.73	0.31	1466
Selina-3,7 (11)-diene	21.98	0.21	1497
Juniper camphor	27.21	0.49	1700
Total:		99.94	

RRI – relative retention index

Antibacterial analysis

The rhizome oil of *H. larsenii* was tested for antibacterial activity by the disc agar diffusion method (6, 7). Gram-positive and Gram-negative bacterial strains which were obtained from the Institute of Microbial Technology, Chandigarh, India, were used for testing. The bacteria were grown on the Mueller-Hinton agar medium (pH 7.2–7.4). Microbial suspensions were then made from the agar plates using relevant broths. The agar

Table II. In vitro antibacterial activity of the rhizome oil of Hedychium larsenii

Tested bacteria	Diameter of inhibition zone (mm)[a]	
	Rhizome oil[b]	Streptomycin[c]
Gram-positive bacteria		
Bacillus cereus (MTCC 430)	9	24
Bacillus subtilis (MTCC 441)	9	22
Staphylococcus aureus (MTCC 96)	12	26
Gram-negative bacteria		
Escherichia coli (MTCC 443)	8	11
Klebsiella pneumoniae (MTCC 109)	–	18
Proteus vulgaris (MTCC 426)	14	18
Pseudomonas aeruginosa (MTCC 741)	10	9
Pseudomonas fluorescens (MTCC103)	10	16
Salmonella typhi (MTCC 733)	9	16
Serratia marcescens (MTCC 97)	9	20

[a] Experiments were done in triplicate and the results are mean values.
[b] Oil dilution, 1:1 in DMSO; 5 μL oil per disc.
[c] Streptomycin: 2 μg per disc.

media were poured into the plates to a uniform depth of 5 mm and allowed to solidify. The microbial suspensions were then streaked over the media surface using a sterile cotton swab to ensure confluent growth of the organism. 10 μL aliquots of the oil diluated 1:1 with DMSO were impregnated on Whatman No. 1 filter paper discs of 6 mm size. The discs were then aseptically applied to the surface of agar plates at well-spaced intervals. The plates were incubated at 36 °C for 24 h and the observed zones of inhibition were measured. Control discs impregnated with 10 μL of DMSO, inert solvent, and reference substance streptomycin, 2 μL of 1 mg mL^{-1} solution, were used alongside the test discs in each experiment (Table II).

RESULTS AND DISCUSSION

Hydrodistillation of fresh rhizomes of *H. larsenii* afforded a pleasant-smelling pale yellow oil in 0.14% (*V/m*) yield. Other physical properties were $d^{20} = 0.920$, $\alpha_D^{20} = -5.39°$. Table I shows the essential oil components whose percentage was determined by GC--FID and the identity was established by GC-MS, literature data and by relative retention indices.

The GC (Fig. 1) shows nineteen compounds accounting for 99.9% of the oil. 98.9% of the oil consisted of monoterpenes while sesquiterpenes accounted for only 1%. The constituents identified consisted of eight monoterpene hydrocarbons (camphene, β-pinene, myrcene, α-phellandrene, α-terpinene, *p*-cymene, limonene and γ-terpinene), one monoterpene ether (1,8-cineole), seven monoterpene alcohols (linalool, linalool oxide A and B, fenchyl alcohol, borneol, terpinene-4-ol and α-terpineol) and three sesquiterpenes [(α--selinene, selina-3,7(11)-diene and juniper camphor)]. 1,8-cineole was identified as the major constituent in some of the previously studied *Hedychium* species, such as *H. spicatum* (27–56%) (8), *H. coronarium* (47.4%) (9), *H. venustum* (18%) (9), *H. flavescens* (50.9%) (9) and *H. acuminatum* (76%) (10). However, the major constituent in *H. larsenii* was identified as linalool (62.3%), 1,8-cineole is the second major constituent (14.4%), followed by *p*-cymene (8.2%).

The oil was tested against Gram-positive and Gram-negative bacteria. The results presented in Table II show that 10 μl of the oil at 1:1 dilution with DMSO is moderately active against most of the tested bacteria as compared to 2 μg streptomycin per disc. The oil did not show any activity against the Gram-negative bacterium *Klebsiella pneumoniae*, but activity comparable to that of streptomycin against *Pseudomonas aeruginosa was observed.*

CONCLUSION

The study has helped in identifying *H. larsenii*, a hitherto uninvestigated aromatic plant, as a new natural source of linalool (62%). Preliminary antibacterial studies have shown that the oil has modest activity against Gram-positive and Gram-negative bacteria.

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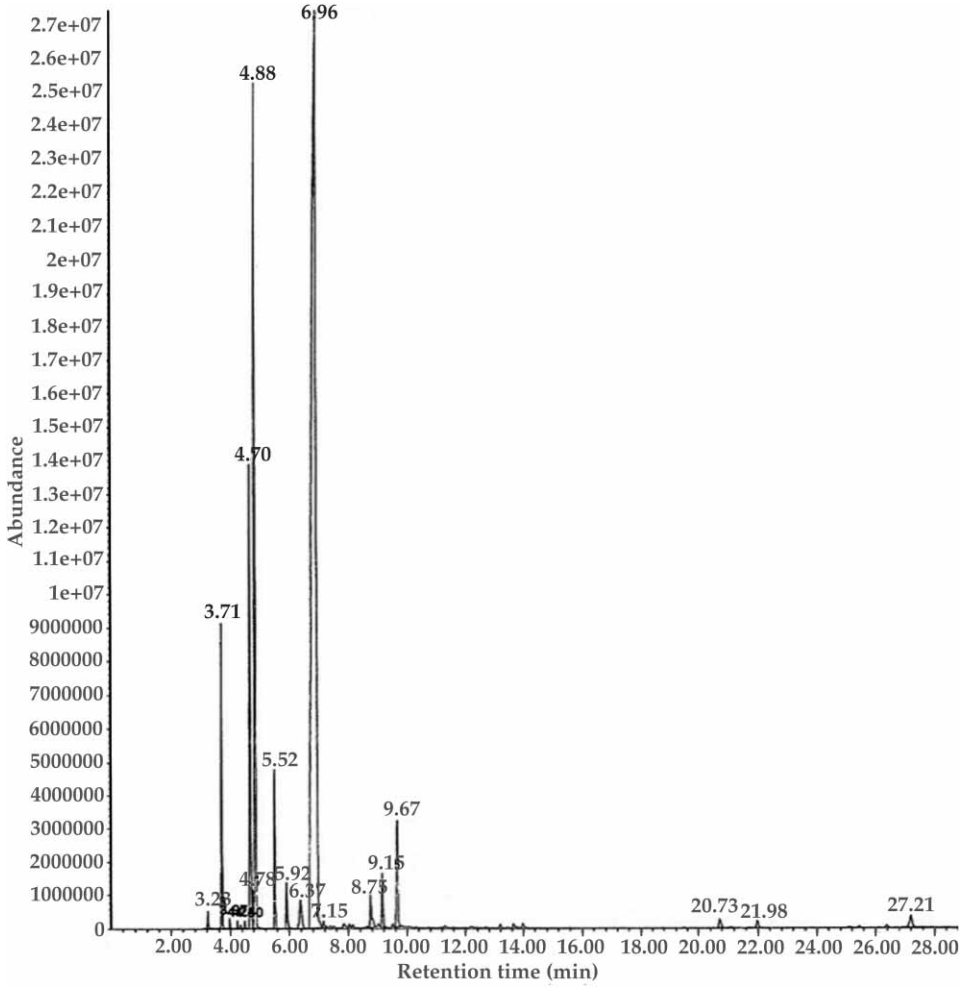

Fig. 1. GC of the rhizome oil of *Hedychium larsenii* on a cross linked 5% PHME siloxane HP-5 MS capillary column (30 x 0.32 mm i.d.). For peaks identification see Table I.

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S A Ž E T A K

Kemijski sastav i antibakterijsko djelovanje eteričnog ulja iz rizoma biljke *Hedychium larsenii*

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Kemijski sastav eteričnog ulja iz rizoma biljke *Hedychium larsenii* M. Dan & Sathish ispitivan je pomoću GC-FID i GC-MS. Najvažniji sastojci ulja bili su linalol i 1,8-cineol, a na monoterpene otpada 99%. Seskviterpeni su prisutni samo u zanemarivim količinama. Eterično ulje je pokazalo umjereno antibakterijsko djelovanje na Gram-pozitivne i Gram-negativne bakterije.

Ključne riječi: Hedychium larsenii (Zingiberaceae), eterično ulje, GC, GC-MS, antibakterijsko djelovanje

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