Research Paper

Chemical Composition, Antibacterial and Antioxidant Properties of Essential Oil from the Rhizomes of *Hedychium forrestii* var. *palaniense* Sanoj and M. Sabu

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The essential oil constituents of rhizomes of *Hedychium forrestii* var. *palaniense* were analyzed by gas chromatography and gas chromatography-mass spectrometry. A total of 26 constituents comprising 68.20% of the oils were identified. The volatile fraction was characterized by monoterpene hydrocarbons (42.31%), oxygenated monoterpenes (38.46%), sesquiterpene hydrocarbons (15.38%) and oxygenated sesquiterpenes (3.85%). The predominant constituents identified were β-pinene (18.30%), β-linalool (17.80%), 1,8-cineole (12.00%), and 4-terpineol (5.50%). Sesquiterpenoids were present only in negligible quantities. The antibacterial activities of the essential oil was evaluated against ten bacteria and *Pseudomonas aeruginosa* was the most susceptible organism with a zone of inhibition of 42.16±0.76 mm. Additionally, the essential oil showed promising radical scavenging and electron donating activity.

Key words: *Hedychium forrestii* var. *palaniense*, Zingiberaceae, β-pinene, β-linalool, 1,8-cineole, DPPH

The genus *Hedychium* J. Koenig comprises about 80 species, distributed from India to South East Asia[1]. This is considered to be the largest genus of Zingiberaceae in India with about 45 taxa[2]. They are generally used as ornamentals and are also used for their industrial (paper manufacture and perfumery) or medicinal properties[3-5].

Composition of essential oils from *Hedychium* species is characterized by the abundance of monoterpenes with major percentage of 1,8-cineole and pinene derivatives[6]. Essential oils from *H. spicatum*, *H. aurantiacum*, *H. coronarium* and *H. ellipticum* exhibited good antioxidant potentials by quenching 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and moderate to good Fe^{2+} chelating activity and could afford protection against oxidative damage[7]. Flower and leaf oils of *H. gardnerianum* showed good antimicrobial activity against *Staphylococcus aureus* and *S. epidermidis*[8]. The rhizome essential oil as well as solvent extracts of *H. flavescens*, *H. venustum*, *H. ellipticum*, *H. aurantiacum* and *H. coronarium* showed a broad antimicrobial spectrum[7,9-11]. Essential oil of *H. spicatum* is reported to possess antimicrobial and antioxidant activity[7,12]. Therefore, *Hedychium* species not only have ornamental value but also have medicinal and industrial value.

Hedychium forrestii var. *palaniense*, a recently reported taxon, is known only from four localities of Pulney Hills, Tamil Nadu, at an altitude of 1300-1600 m. It prefers to grow in grassy slopes and open rock cliffs as patches of 15-20 mature individuals[13]. There are no previous reports of the essential oil composition and its biological properties of this new taxon. Therefore the present study aimed to analyze the essential oil composition, antibacterial and antioxidant potential of the essential oil isolated from rhizomes of this least studied taxon.

MATERIALS AND METHODS

The rhizomes of *H. forrestii* var. *palaniense* were collected from Perumalmalai (1520 m) region of Pulney Hills, Tamilnadu in the month of May, 2014.

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Accepted 03 July 2016
Revised 24 February 2016
Received 15 November 2015
Indian J Pharm Sci 2016;78(4):452-457

Voucher specimen (RHT65200) has been deposited in The Rapinat Herbarium (RHT), St. Joseph's College, Tiruchirappalli, Tamilnadu.

Chemicals:

2,2-diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid (AA) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Potassium ferricyanide, trichloroacetic acid (TCA), and ferric chloride were purchased from Merck Chemical Supplies (Damstadt, Germany). All the chemicals used including the solvents, were of analytical grade.

Essential oil extraction:

Air dried rhizomes (25 g) of the plant were hydrodistilled for 3 h using a Clevenger-type apparatus. The distilled oil was dried over anhydrous sodium sulfate, and stored in tightly closed vials at 4° for analysis. The essential oil content was determined as percentage on dry weight basis as an average of three independent extractions.

Characterization of essential oil:

Gas chromatography-mass spectrometry (GC/MS) analysis was performed using Shimadzu GC-2010 gas chromatograph (GC-FID) equipped with QP 2010 mass spectrometer (MS). Approximately 0.1 µl of pure oil sample was subjected to GC and GC/MS analysis. The column used was DB-5. The oven temperature was programmed as follows; 70° for 5 min and then increased to 110° at the rate of 5°/min, then up to 200° at the rate of 3°/min and again up to 220° at the rate of 5°/min, at which the column was maintained for 5 min; injector temperature of 250°. Helium was used as carrier gas at a flow rate of 1 ml/min. Identification of the individual components was made by matching their recorded mass spectra and linear retention indices with the library (NIST and Wiley) provided by the instrument software, online database (http://www.flavornet.org) and by comparing their calculated retention indices with literature value[14].

Test microorganisms:

The bacterial strains used in the study were three Gram positive bacteria namely, *Streptococcus haemolyticus* (MTCC442), *Bacillus cereus* (MTCC430) and *Staphylococcus aureus* (MTCC87) and seven Gram negative, namely, *Vibrio parahaemolyticus* (MTCC451), *Vibrio cholerae* (MTCC3904), *Salmonella paratyphi* (MTCC735), *Enterobacter aerogens* (MTCC111), *Escherichia coli* (MTCC433), *Klebsiella pneumoniae* (MTCC3384), and *Pseudomonas aeruginosa* (MTCC741). All the tested strains are reference strains, and were collected from Microbial Type Culture Collection (MTCC) and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

Antibacterial activity test:

The antibacterial activity of essential oil was carried out by disc diffusion method[15], recommended by Clinical and Laboratory Standards Institute (CLSI), using 25 µl of standardized suspension of test bacteria (1.5×10^8 CFU/ml) spread on Mueller-Hinton agar (MHA, pH 7.3±0.1) plates. The discs (6 mm in diameter) were impregnated with 20 µl of essential oil, followed by air-drying and were placed on seeded agar plates. Amoxicillin (30 µg/disc) was used as positive control to determine the sensitivity of bacterial strain. The plates were incubated at 37° for 24 h. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

DPPH radical scavenging assay:

The free radical scavenging activity of essential oil was measured using the stable DPPH radical, according to the method of Blois[16] with minor modification. Briefly, 0.1 mM solution of DPPH in methanol was prepared and this solution (1 ml) was added to essential oil in methanol (3 ml) at concentrations ranging from 10-50 µl/ml. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation: %DPPH radical activity = $((A_0-A_1)/A_0)\times100$, where A_0 and A_1 are absorbance of the control and of the sample, respectively. Ascorbic acid was used for comparison.

Determination of reducing power:

The reducing power of the essential oil was measured by making use of the method described by Yen and Duh[17] with some modifications. Various concentrations (10, 20, 30, 40 and 50 µl/ml) of essential oil in methanol was taken separately and mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6). The dilute sample was then mixed with 5.0 ml of 1% potassium ferricyanide and the mixture was incubated at 50° for 20 min. 5.0 ml of 10% trichloroacetic acid was added to the mixture and was centrifuged at 3000 rpm for 10 min. 5.0 ml of the supernatant solution was mixed with 5.0 ml of distilled water and 1.0 ml of ferric chloride (1%).

The absorbance was measured spectrophotometrically at 700 nm (Shimadzu, UV-150-02). Ascorbic acid standard was used for comparison.

RESULTS AND DISCUSSION

The dried rhizomes (25 g) were hydrodistilled to obtain a pleasant smelling, pale yellow colored oil at a yield of 1.2% v/w. A GC of the volatile components of the rhizome is shown in fig. 1. The GC indicates the presence of more than 50 compounds, of which 26 were identified.

Table 1 lists the volatile components identified and those 26 constituents accounted for 68.20% of the oil hydrodistilled. The analyzed oil contained monoterpene hydrocarbons (42.31%), oxygenated monoterpenes (38.46%), sesquiterpene hydrocarbons (15.38%) and oxygenated sesquiterpenes (3.85%). The majority of the essential oil components were monoterpenes and the major constituents were β-pinene (18.30%), β-linalool (17.80%), 1,8-cineole (12.0%), 4-terpineol (5.50%), α-pinene (4.9%), γ-terpinene (1.90%), borneol (1.60%), α-terpineol (1.60%) and camphene (1.40%). The sesquiterpene compounds were present in least concentration and germacrene B (0.20%) was the leading one.

The present study showed that qualitative and quantitative differences were found among the oils of *H. forrestii* var. *palaniense* Sanoj and M Sabu and *H. forrestii* Diels which is a close relative of the taxon studied[18]. Sakhanokho *et al.*[18] reported that the major components in the essential oil from dried rhizomes of *H. forrestii* were characterized by linalool (56%), β-pinene (14%) and 1,8-cineole (10.1%) and found that the oil differs quantitatively from those of *H. forrestii* var. *palaniense*. It is interesting to see that, though the oils of both taxa contained monoterpenes (87.5% in *H. forrestii*) and sesquiterpenes (8.33% in *H. forrestii*), hydrocarbon sesquiterpenes were not detected in *H. forrestii*. However, a diterpene was found in the rhizome essential oil of *H. forrestii*[18].

Monoterpene constituents detected in the present study such as 4-thujanol, fenchol, p-menth-2-en-1-ol, camphor, pinocarvone and bornyl acetate were absent in the earlier reports[18]. At the same time the monoterpenes detected in the essential oil of *H. forrestii* like, sabinene, myrcene, limonene, cymene, trans-linalool oxide and cis-linalool oxide were not found in the present study. Earlier reports also showed that major essential oil constituents are common among various species of *Hedychium*[9,18-21] whereas minor components, which reveal the specific differences between taxa. Finally, we suggest that the differences in essential oil composition, both qualitative and quantitative, may depend on factors such as genotype, season and environment.

The results of the antibacterial studies of the essential oil obtained by the disc diffusion technique are given in Table 2. It was interesting to see that all the bacteria tested were susceptible and the essential oil showed hopeful antibacterial activity. A concentration of 20 µl of essential oil showed nearly equal and effective inhibition against all the strains used in the present study. The most susceptible bacterium tested was *P. aeruginosa* with a zone of inhibition of 42.16±0.76 mm. The results also showed that the essential oil was found to be more effective antibacterial agent than the standard antibiotic used in the study. Moreover, the

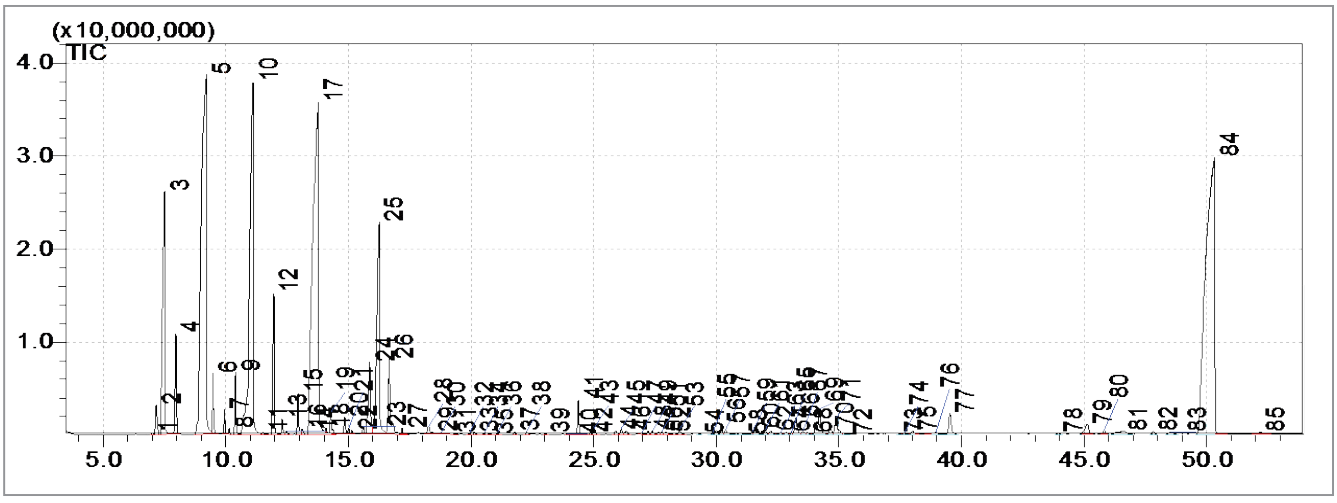

Fig. 1: GC-MS chromatogram of oil.
Total ion chromatogram (TIC) of the volatile compounds from dried rhizomes of *Hedychium forrestii* var. *palaniense*.

TABLE 1: CHEMICAL COMPOSITION OF ESSENTIAL OIL EXTRACTED FROM DRIED RHIZOMES

Component	RI_a	RI_b	Composition (%)
α-thujene	936	938	0.5
α-pinene	938	939	4.9
Camphene	953	953	1.4
β-pinene	994	994	18.3
α-phellandrene	1007	1006	0.3
3-careen	1009	1009	tr
α-terpinene	1011	1012	0.8
1,8-cineole	1029	1030	12
cis-β-ocimene	1036	1038	tr
4-thujanol	1072	1073	0.2
γ-terpinene	1074	1074	1.9
α-terpinolene	1086	1086	0.5
Linalool	1098	1100	17.8
Fenchol	1139	1139	tr
L-camphor	1140	1139	0.3
p-menth-2-en-1-ol	1145	1145	0.1
Pinocarvone	1158	1160	0.1
Borneol	1163	1162	1.6
4-terpineol	1180	1179	5.5
α-terpineol	1192	1195	1.6
Bornyl acetate	1294	1287	tr
δ-elemene	1338	1335	tr
β-caryophyllene	1466	1467	0.1
β-sesquiphellandrene	1558	1560	0.1
Germacrene B	1562	1562	0.2
Caryophyllene oxide	1574	1573	tr

RI_a and RI_b: Experimental and reference retention indices; tr-trace <0.1%

TABLE 2: ANTIBACTERIAL ACTIVITY OF EXTRACTED ESSENTIAL OIL FROM RHIZOMES

Species	Zone of inhibition (mm)	
	Essential oil	Amoxicillin
Vibrio parahaemolyticus (-)	36.33±0.57	32.33±0.57
Bacillus cereus (+)	36.17±0.28	34.00±0.50
Enterobacter aerogens (-)	34.20±0.23	32.17±0.28
Salmonella paratyphi (-)	34.33±0.60	32.67±0.57
Vibrio cholerae (-)	37.83±0.80	36.33±0.60
Staphylococcus aureus (+)	37.83±0.76	36.17±0.28
Escherichia coli (-)	36.00±0.86	30.33±0.30
Streptococcus haemolyticus (+)	37.16±0.77	34.16±0.29
Klebsiella pneumoniae (-)	38.20±0.28	32.33±0.57
Pseudomonas aeruginosa (-)	42.16±0.76	32.17±0.29

Values are presented as mean±SD of triplicate experiments; (+) Gram positive, (-) Gram negative

essential oil inhibit the growth of both Gram-positive and Gram-negative bacteria, consequently it may be used as a broad-spectrum natural antibacterial agent.

Monoterpenes are good antibacterial agents[22] in particular the oxygenated compounds[23]. The major components in the essential oil of *H. forrestii* var. *palaniense* were β-pinene, linalool, 1,8-cineole and 4-terpineol, which were studied for their antibacterial properties previously. The earlier studies showed that β-pinene, linalool and 4-terpineol were found to be possess good antibacterial properties[22-24] whereas 1,8-cineole was least effective[23,25]. However, recent studies showed that 1,8-cineole alone or in combination with other essential oils showed good antibacterial activity[10,26] and this might be due to synergism[27]. Essential oil of *H. forrestii* var. *palaniense* showed a promising antibacterial activity and it might be attributed not only by the major constituents but also by the action of minor components. Hence, essential oil from rhizomes of this plant can be used in the antibacterial formulations, which in turn broadens its applications in the medicinal and industrial fields.

The antioxidant potential of the essential oil obtained from *H. forrestii* var. *palaniense* was studied by analyzing the radical scavenging capacity and electron donating ability of the constituents in the essential oil. The DPPH radical scavenging activities of the essential oil and standard antioxidative compound (ascorbic acid) are shown in fig. 2. The result showed a dose-dependent inhibition of DPPH radical by both oil and the standard and the scavenging capacity of the essential oil was significant when compared with that of standard. Concentration at which the oil decreased DPPH radical by 50% (IC_{50} values) was 34.0 µl/ml. Correspondingly, IC_{50} value for ascorbic acid, used as standard, was 30.50 µg/ml. The reducing power of a sample was related to its electron transfer ability and might, therefore, served as an indicator of its potential antioxidant activity. The reducing power of the essential oil and ascorbic acid increased with the concentration (fig. 3). The oil exhibited significantly higher activity than the standard ascorbic acid. The results of the present study suggest that essential oil isolated from *H. forrestii* var. *palaniense* has promising radical scavenging ability and potent reducing power. Antioxidant properties of essential oils such as lipid peroxidation, scavenging of free radicals, chelating metal ions, and reducing power are often come from their monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes[28,29], which means that the strong antioxidant activity of essential oil from the rhizomes of *H. forrestii* var. *palaniense* may be related to the sum of the effects of constituents in the essential oil. Therefore, the high antioxidant activity of this essential oil strengthens their application for

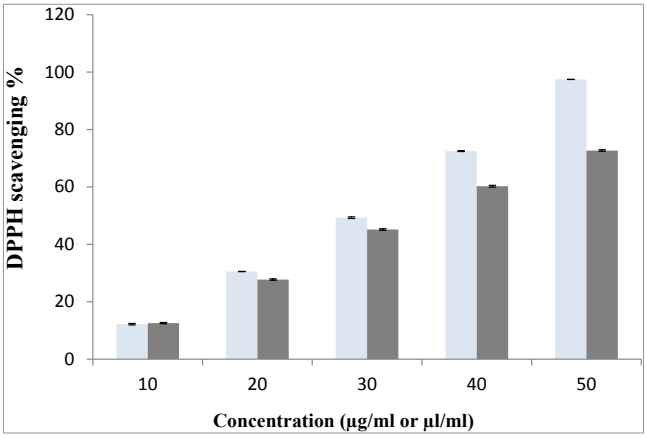

Fig. 2: DPPH radical scavenging activity.
DPPH radical scavenging activity of essential oil (µl/ml, ▪) isolated from dried rhizomes of *H. forrestii* var. *palaniense* and reference compound ascorbic acid (µg/ml, ▫). Values are mean±SD of triplicate experiments.

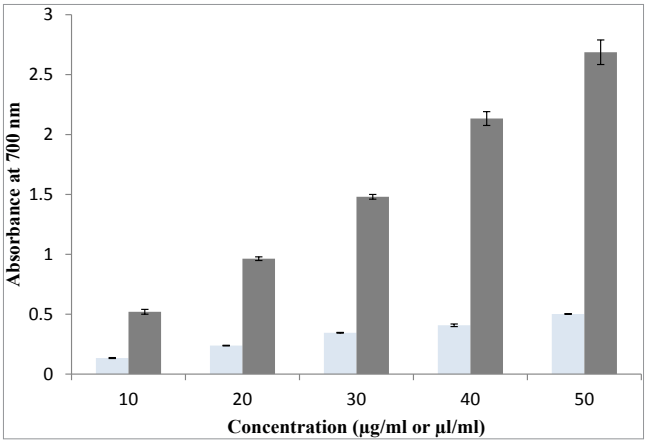

Fig. 3: Reducing power.
Reducing power of essential oil (µl/ml, ▪) isolated from dried rhizomes of *H. forrestii* var. *palaniense* and reference compound ascorbic acid (µg/ml, ▫). Values are mean±SD of triplicate experiments.

possible use as natural antioxidants.

Financial support and sponsorship:

Nil.

Conflicts of interest:

There are no conflicts of interest.

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