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Composition, antibacterial and anti-oxidant potentials of the essential oil of *Hedychium matthewii*

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Abstract

Essential oils are known for their medicinal value since time immemorial and continue to be of vital importance until the present day. The present study describes the composition, antibacterial and anti-oxidant potential of rhizome oil of *Hedychium matthewii*. Thirty-five constituents of the oil were identified to account for 82.7%, of which 85.7% was monoterpes and the rest were sesquiterpnes. Most of the major constituents were alcohols and linalool was the prominent one (45.7%). The antibacterial assay showed the bactericidal effect of the essential oil and the most susceptible organism was *Streptococcus hemolyticus* with a zone of inhibition of 33.8 ± 1.7 mm. The reducing power and nitric oxide scavenging activity of the essential oil was far exceeding the reference compound ascorbic acid and it could be the sign of the potential anti -oxidant power of the oil isolated. The present study also revealed the prospective of *H. matthewii* as a new natural source of linalool, which has medicinal and various industrial applications.

Introduction

The genus *Hedychium*, an ornamental and ethnomedicinal plant comprises about 90 species, mostly distributed from India to South-East Asia (Raj et al., 2005; Thomas et al., 2015). *Hedychium* species are a good source of various essential oil compounds having medicinal and other industrial applications (Sakhanokho et al., 2013). The essential oil obtained from leaves of *H. coronarium* constituted β-pinene as the major compound (Raj et al., 2013), whereas β-ocimene and linalool as the major constituents in its flower oil (Báez et al., 2011) and 1,8 cineole as the major compound in rhizome oil (Joy et al., 2007).

Leaf and flower essential oils of *H. gardnarianum* reported the presence of α and β-pinenes as the major components (Medeiros et al., 2003). *H. flavum* was characterized by 1,8 cineole and linalool as major compounds in the essential oil obtained from the inflorescence (Báez et al., 2011). Rhizome oil of *H. larsenii* was dominated by linalool (Raj et al., 2005), whereas leaf and inflorescence oil dominated by arcurcumin and *p*-cymene, respectively (Raj et al., 2013). It has been reported that rhizome oil of *H. thyrsiforme*, *H. flavum* and *H. bousigonianum* were dominated by 1,8 cineole, whereas linalool was the major constituent in *H. forrestii* and *H. coccineum*. β-Pinene was also found to be the major rhizome oil component in *H. elatum* and *H. flavescens* (Sakhanokho et al., 2013).

Essential oils obtained from *H. spicatum*, *H. aurantiacum*, *H. coronarium* and *H. ellipticum* exhibited good antioxidant potentials by quenching DPPH radicals and moderate to good Fe^{2+} chelating activity and may perhaps afford protection against oxidative damage (Joshi et al., 2008). Flower and leaf oils of *H. gardnerianum* showed good antimicrobial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* (Medeiros et al., 2003). The rhizome essential

oil and solvent extracts of *H. flavescens*, *H. venustum*, *H. ellipticum*, *H. aurantiacum* and *H. coronarium* showed a broad spectrum antimicrobial activity (Joshi et al., 2008; Joy et al., 2007; Sabulal et al., 2007). Essential oil of *H. spicatum* is reported to possess antimicrobial and anti-oxidant activity (Bisht et al., 2006; Joshi et al., 2008). Therefore, *Hedychium* species not only have ornamental value but also have medicinal and industrial value.

Hedychium matthewii, a recently reported taxon, is known only from Idukki, Kerala (Thomas et al., 2015). There are no reports available on the essential oil composition and their biological property of this species. Therefore, the present study aimed at analyses the composition, antibacterial and anti-oxidant potential of the essential oil isolated from rhizomes of this least studied taxon.

Materials and Methods

Plant material

The rhizomes of *H. matthewii* were collected from two populations at Idukki and brought to the laboratory and washed to remove adhering soil and dust particles. The rhizomes were cut into pieces and dried at room temperature. A voucher specimen (RHT65200) deposited in The Rapinat Herbarium (RHT), St. Joseph's College, Tiruchirappalli, Tamilnadu.

Chemicals

1, 1- Diphenyl-2-picrylhydrazyl (DPPH), sodium nitrite, sulfanilamide and ascorbic acid (AA) were purchased from Sigma chemical co. (USA). Potassium ferricyanide, trichloroacetic acid (TCA), sodium nitroprusside, α-napthyl-ethylenediamine and ferric chloride were purchased from Merck chemical supplies (Germany). All the chemicals used including the solvents, were of analytical grade.

Essential oil extraction

The dried rhizomes (25 g) were ground and hydro-distilled for 3 hours using a Clevenger-type apparatus. The distillate was dried over anhydrous sodium sulfate and stored in tightly closed vials at 4°C for analysis. The essential oil content was determined as a percentage on dry weight basis as an average of three independent extractions of each sample.

Characterization and identification of essential oil constituents

GC/MS analyses were 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 gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) analysis. The column used was DB-5. The oven temperature was programmed as follows; 70°C for 5 min and then increased to 110°C at the rate of 5°C/min, then up to 200°C at the rate of 3°C/min and again up to 220°C at the rate of 5°C/min, at which the column was maintained for 5 min; injector temperature of 250° C. 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://webbook.nist.gov/chemistry; http://www.flavornet.org) and by comparing their calculated retention indices with literature value (Adams, 2009).

Test microorganisms

The bacterial strains used in the study were three gram positive, namely, *Streptococcus haemolyticus* (MTCC442), *Bacillus cereus* (MTCC430) and *Staphylococcus aureus* (MTCC87) and nine gram negative, namely, *Vibrio parahaemolyticus* (MTCC451), *Vibrio cholerae* (MTCC3904), *Salmonella paratyphi* (MTCC735), *Enterobacter aerogens* (MTCC111), *Escherichia coli* (MTCC433), *Klebsiella pneumoniae* (MTCC3384), *Proteus vulgaris* (MTCC426), *Salmonella typhi* (MTCC733) and *Pseudomonas aeruginosa* (MTCC741). All the tested strains are reference strains, and were collected from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Sector 39-A, Chandigarh 160036, India.

Antibacterial activity test

The antibacterial activity of essential oil was carried out by disc diffusion method (Bauer, 1966), 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°C for 24 hours. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

Determination of reducing power

The reducing power of the essential oil was measured by making use of the method described by Yen and Duh (1993) with some modifications. Various concentrations (10, 20, 30, 40 and 50 µL/mL) of essential oil in methanol were 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°C 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). The ascorbic acid standard was used for comparison.

DPPH radical scavenging activity

The free radical scavenging activity of essential oil was measured using the stable DPPH radical, according to the method of Blois (Mani and Thomas, 2014) with minor modifications. 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 well 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 formula:

$$(\%) = ((A_0 - A_1)/A_0) \times 100 \quad (1)$$

Where, A_0 and A_1 are absorbance of the control and of the sample, respectively. Ascorbic acid was used as reference.

Nitric oxide (NO) scavenging activity

Nitric oxide scavenging effect was determined according to Griess Illosvoy reaction (Johnson, 1964) with slight modification. The reaction mixture contained: 10 mM SNP in 0.5 M phosphate buffer (pH 7.4) and various doses (10–50 µL/mL) of the essential oil in a final volume of 3 mL. After incubation for 60 min at 37° C, Griess reagent (0.1% α-napthyl-ethylenediamine in distilled water and 1% sulphanilamide in 5% H_3PO_4) was added. The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with α-napthyl-ethylenediamine was measured spectrophotometrically at 540 nm. Ascorbic acid was used as a positive control. Nitric oxide scavenging ability (%) was calculated by using the equation (1).

Statistical analysis

Experimental results were expressed as the mean ± standard deviation (SD) of the number of experiments indicated in the legends. Statistical significance was tested using a one way analysis of variance (ANOVA) followed by an LSD test, where $p<0.05$ was considered significant.

Results

Essential oil characterization

The dried rhizomes were hydrodistilled to obtain a pleasant smelling, pale yellow oil at a yield of 2.6%. The gas chromatogram (GC) of the rhizome essential oil of *H. matthewii* is shown in Figure 1. The GC profile indicates the presence of more than 50 compounds, of which 35 were identified by comparing and matching the mass spectra and GC retention index (RI) of the compounds with those of reference.

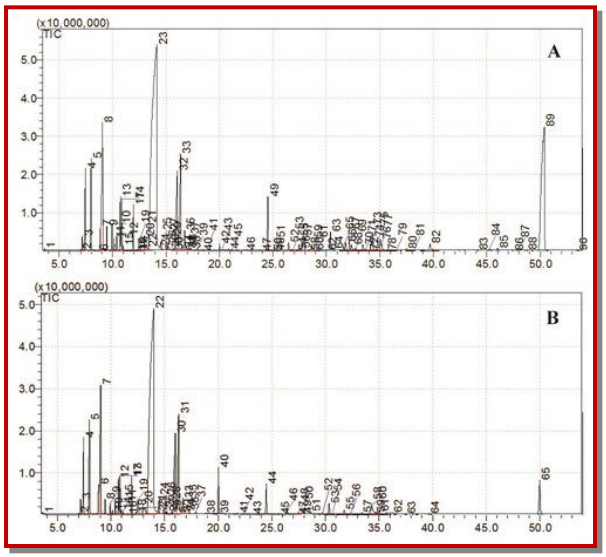

Figure 1: Total ion chromatogram (TIC) of the volatile compounds from the dried rhizomes of two populations (A and B) of *H. matthewii*

Table I depicts the volatile components indentified and those 35 compounds accounting for 82.7% of the oil hydrodistilled from the dried rhizome. 85.7% of the oil consisted of monoterpenes whereas rests (14.3%) accounted for sesquiterpenes. The constituents indentified consisted of fourteen monoterpene hydrocarbons (40%), sixteen oxygenated monoterpenes (45.7%), two sesquiterpene hydrocarbons (5.7%) and three oxygenated sesquiterpenes (8.6%). The analyzed oil contained esters, oxides, alcohols, phenols, aldehydes and ketones. The majority of essential oil constituents were found in the monoterpene class where the most prominent compounds were linalool (45.6%) followed by β-pinene (6.5%), borneol (6.3%), 4-terpineol (5.6%), camphene (3.3%) and α-pinene (2.5%).

Antibacterial assay

The results of the antibacterial studies, of the essential oil obtained, by the disc diffusion technique are given in Table II. By analyzing the antibacterial activity of essential oil, we observed that all the bacteria tested were susceptible and the essential oil showed hopeful antibacterial activity. A volume 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 *Streptococcus haemolyticus* with a zone of inhibition of 33.8 ± 1.7 mm. The bacteria such as *S. haemolyticus* and *Vibrio cholerae* were more susceptible towards essential oil than

Table I

Chemical composition of the essential oil from *H. matthewii*

SL. No.	Component	RI_a	RI_b	Composition (%)
1	α-Thujene	930	931	0.4 ± 0.1
2	α-Pinene	938	939	2.5 ± 0.5
3	Camphene	953	953	3.3 ± 0.9
4	Sabinene	972	972	1.2 ± 0.5
5	β-Pinene	981	980	6.5 ± 0.8
6	β-Myrcene	992	992	0.5 ± 0.04
7	α-Phellandrene	1007	1005	0.5 ± 0.2
8	3-Careen	1111	1011	0.2 ± 0.1
9	α-Terpinene	1012	1012	0.4 ± 0.05
10	o-Cymene	1026	1027	1.4 ± 0.1
11	D-Limonene	1031	1031	1.2 ± 0.03
12	1,8-Cineole	1033	1033	0.1 ± 0.02
13	trans-β-Ocimene	1050	1050	0.1 ± 0.1
14	γ-Terpinene	1059	1059	1.2 ± 0.1
15	α-Terpinolene	1063	1063	0.5 ± 0.2
16	trans-Linalool oxide	1065	1065	0.7 ± 0.5
17	Linalool	1098	1098	45.6 ± 3.9
18	Fenchol	1112	1112	0.02 ± 0.0
19	*p*-Menth-2-en-1-ol	1120	1121	0.2 ± 0.05
20	L-Camphor	1140	1139	0.1 ± 0.01
21	Borneol	1163	1162	6.3 ± 1.7
22	4-Terpineol	1180	1180	5.6 ± 1.5
23	α-Terpineol	1189	1189	0.7 ± 0.1
24	Myrtenal	1190	1190	0.2 ± 0.03
25	α-Phellandrene epoxide	1192	1192	0.1 ± 0.1
26	Piperitol	1199	1198	0.2 ± 0.1
27	Bornyl fomate	1232	1232	0.1 ± 0.01
28	Bornyl acetate	1283	1283	1.1 ± 1.0
29	Thymol	1284	1284	0.1 ± 0.02
30	α-Terpinyl acetate	1352	1352	0.03 ± 0.0
31	β-Caryophyllene	1418	1418	0.1 ± 0.1
32	α-Cedrene	1433	1433	0.2 ± 0.1
33	Caryophyllene oxide	1583	1583	0.1 ± 0.1
34	Guaiol	1597	1597	0.5 ± 0.2
35	10-epi-γ-Eudesmol	1619	1619	0.5 ± 0.2

Values are mean ± SD of two samples (n = 2); RI_a and RI_b: Experimental and reference retention indices

antibiotic tested. The results also showed that the essential oil was found to be effective antibacterial agent as the standard antibiotic used in the study. Moreover, the essential oil inhibited the growth of both gram positive and gram negative bacteria; consequently it may be used as a broad spectrum natural antibacterial agent.

Table II

Antibacterial activity of the essential oil from *H. matthewii*

Species	Zone of inhibition (mm)	
	Essential oil	Amoxicillin
Gram positive		
Bacillus cereus	19.3 ± 0.9	28.3 ± 0.6
Staphylococcus aureus	29.2 ± 0.8	30.2 ± 0.3
Streptococcus hemolyticus	33.8 ± 1.7	31.5 ± 0.9
Gram negative		
Enterobacter aerogens	20.6 ± 1.5	27.8 ± 0.3
Salmonella paratyphi	23.8 ± 1.2	30.5 ± 0.5
Vibrio cholerae	30.6 ± 1.04	27.8 ± 0.3
Salmonella typhii	28.0 ± 1.7	34.3 ± 0.8
Escherichia coli	21.0 ± 1.0	30.5 ± 0.9
Vibrio parahaemolyticus	26.3 ± 1.5	32.3 ± 0.6
Proteus vulgaris	28.2 ± 1.7	32.2 ± 0.3
Klebsiella pneumoniae	24.2 ± 1.04	26.0 ± 0.9
Pseudomonas aeruginosa	26.2 ± 1.6	28.3 ± 0.6

Values are presented as mean ± SD of triplicate experiments

Anti-oxidant assay

Reducing power: The reducing power of a compound or extract was related to its electron transfer ability and might, therefore, served as an indicator of its potential anti-oxidant activity. The reducing power of the essential oil and ascorbic acid increased with the concentration (Figure 2). The oil exhibited significantly higher activity than the standard ascorbic acid. The results of the present study suggest that essential oil isolated from *H. matthewii* has potent reducing power and promising anti-oxidant activity.

DPPH radical scavenging

DPPH radical scavenging assay, commonly used for analyzing the radical scavenging ability of a compound or extract, is simple and highly sensitive (Miguel, 2010). The anti-oxidant potential of the essential oil obtained from *H. matthewii* was studied by analyzing the radical scavenging capacity and electron donating ability of the constituents in the essential oil. The DPPH radical scavenging activity of the essential oil and standard anti -oxidative compound (ascorbic acid) is shown in Table III. The result showed a dose-dependent inhibition of DPHH radical by both oil and the standard and there was no significant difference exists between the

Table III

DPPH radical and nitric oxide scavenging activityof rhizome oil of *H. matthewii*

	Concentration (µL/mL)				
	10	20	30	40	50
DPPH radical					
Ascorbic acid (µg/mL)	12.6 ± 0.1[a]	30.4 ± 0.1[a]	49.2 ± 0.1[a]	72.5 ± 0.1[a]	97.4 ± 0.1[a]
Essential oil (µL/mL)	10.8 ± 0.1[a]	27.5 ± 0.2[a]	46.7 ± 0.1[a]	70.1 ± 0.2[a]	91.7 ± 0.1[a]
Nitric oxide					
Ascorbic acid (µg/mL)	10.3 ± 0.2[a]	21.2 ± 0.1[a]	32.6 ± 0.1[a]	41.1 ± 0.2[a]	52.2 ± 0.1[a]
Essential oil (µL/mL)	16.8 ± 0.1[b]	35.3 ± 0.1[b]	54.1 ± 0.1[b]	72.8 ± 0.1[b]	94.3 ± 0.3[b]

Values are mean ± SD of two samples analyzed individually in triplicate. Superscripts with the same letters within each column are not significantly different at $p<0.05$

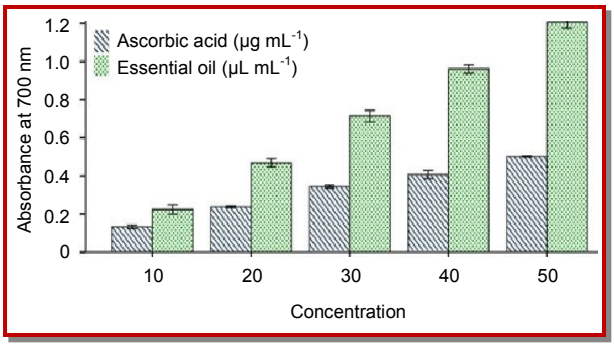

Figure 2: Reducing power of rhizome oil of *H. matthewii* and reference compound ascorbic acid (Values are mean ± SD of two samples analyzed individually in triplicate)

scavenging activity of the oil and the reference compound ascorbic acid. Concentration at which the oil decreased DPPH radical by 50% (IC_{50} value) was 31.5 µL/mL. Correspondingly, IC_{50} value for ascorbic acid, used as a standard, was 30.5 µg/mL.

Nitric oxide (NO) scavenging activity

Anti-oxidant activity of the essential oil was further determined by inhibition of nitric oxide radicals is shown in Table III. The nitric oxide scavenging capacity of extracts and the reference compound ascorbic acid was increased with increasing concentration. The results of the study showed that a significant difference ($p<0.05$) was observed between the two experimental conditions. A total of 50% (IC_{50}) of the nitric oxide radicals was scavenged by essential oil at a concentration of 27.5 µL/mL, whereas that of the ascorbate was 48 µg/mL. The experimental analysis showed that, the essential oil of *H. matthewii* had good nitric oxide scavenging effect.

Discussion

The antibacterial activities of essential oils are well-known. Recently, the antibacterial activity of essential oils of *Buddleja asiatica, Caesalpinia bonducella, Ginkgo biloba* and *Ligustrum lucidum* are reported (Bajpai et al, 2015; Bajpai et al, 2016; Khan et al., 2015; Shukla et al, 2016). Previous studies showed that multiplication of pathogenic *Salmonella* spp., *Escherichia coli* (O157:H7), *Listeria monocytogenes, Helicobacter pylori* and *Mycoplasma pneumoniae* were inhibited by essential oils (Burt, 2004). Monoterpenes are good antibacterial and antifungal agent (Jirovetz et al., 2005; Soković et al., 2010) in particular the oxygenated compounds (Kotan et al., 2007). The major component in the essential oil of *H. matthewii* was linalool an oxygenated monoterpene, followed by 4-terpineol, β-pinene, camphene, borneol and α-pinene, together constitute 73% of the essential oil isolated. The earlier studies showed that linalool, β-pinene and 4-terpineol were found to be possessing good antibacterial properties against several microorganisms (Barel et al., 1991; Kotan et al., 2007; Park et al., 2012; Soković et al., 2010). Essential oil of *H. matthewii* showed a promising antibacterial activity might be attributed to these major constituents. Hence, essential oil from rhizomes of this plant can be used in the antibacterial formulations, which in turn broadens its applications in the pharmaceutical and other industries. Moreover, the broad-spectrum antibacterial activity of the essential oil of *H. matthewii* may perhaps due to the liposolubility of its constituents. It is a good indication of the antiseptic power of the essential oil isolated (Marino et al., 1999; Porter and Wilkins, 1999).

The anti-oxidant activity of essential oils is another biological property of great interest because they may have various industrial applications (Aruoma, 1998; Kamatou and Viljoen, 2010; Maestri et al., 2006). Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the two major classes of free radicals which oxidize various biomolecules and in turn lead to cellular damages and being responsible for diverse diseases even though they have important role in host defence mechanisms (Miguel, 2010). Again, the damaging effects are being increased by generating the peroxinitrite anion ($ONOO^-$) after reacting with the superoxide anion radicals (Gomes et al., 2008; Kostka, 1995; Miyasaka and Hirata, 1997; Nagano, 1999). Therefore, molecules or agents which can inhibit the generation of NO have potential applications in the

pharmaceutical industry.

Present investigation showed that the essential oil isolated from *H. matthewii* is a potent inhibitor and/or scavenger of DPPH and NO radicals. Linalool, a monoterpenoid possessing biological properties such as antibacterial and anti-oxidant activities (Liu et al., 2012), might be the major active molecule present in the oil of *H. matthewii*. Earlier studies showed that, linalool found to be a potent scavenger of DPPH radical, inhibit the production of NO radical and also showed bactericidal activities (Liu et al., 2012; Peana et al., 2006).

Anti-oxidant properties of essential oils such as reducing power, scavenging of free radicals, lipid peroxidation, chelating metal ions etc. are often come from their monoterpene hydrocarbons, oxygenated monoterpenes and sesquiterpenes (Loizzo et al., 2010; Tepe et al., 2005), hence the strong anti-oxidant activity of essential oil isolated from the rhizomes of *H. matthewii* may not only be related to linalool but also the sum of the effects of constituents in the essential oil. Therefore, the high anti-oxidant activity of this essential oil strengthens their application for possible use as natural anti-oxidants.

Conclusion

Analysis of the essential oil isolated from the dried rhizomes of *H. matthewii* revealed the presence of good amounts of monoterpenes especially oxygenated compounds. The antimicrobial and anti-oxidant properties of the oil recommend its use in pharmaceutical and other industrial products. The study helped in identifying, *H. matthewii*, a hitherto unexplored aromatic plant, as a novel natural source of linalool (45%).

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Conflict of Interest

All authors have completed the ICMJE uniform disclosure form and declare no support from any organization for the submitted work.

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