Nat. Volatiles & Essent. Oils, 2022; 9(2): 255-261

NVEO
Natural Volatiles &
Essential Oils

Potent Medicinal Applications Of Essential Oil Of Hedychium Coronarium Koenig Species From The Konkan Region

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Abstract:

The rhizomes of Hedychium coronarium species were subjected to extraction of essential oil as secondary metabolites by the hydrodistillation method. A total of eleven components were revealed in the essential oil by GC/ GC-FID and GCHRMS analysis. Eucalyptol was the major component of oil along with α-terpinol and β-pinene. Out of four tested bacteria (two gram +ve and two gram –ve), the oil showed excellent antibacterial activity versus E. coli while the preeminent antifungal potency was found against C. albicans. The excellent antimalarial efficacy of oil was observed against Plasmodium falciparum. To the best, the presence of –(a) 1,4-cineole, (b) Bicyclo[2,2,1] heptan-3-one,6,6-dimethyl-2methylene,(c)7,11Dimethyldodeca-2,6,10-trien-1-ol(E,E) and (d)1-terpinol in Hedychium coronarium are reported first time for Konkan region in this study.

Keywords: Eucalyptol, Essential oil, Antimalarial activity.

1. Introduction:

Essential oils, the secondary metabolites of plants are found to be applicable in perfumery, pharmaceutical, and food industries because of their reported biological activities, innocuous way of action, and easy availability (1, 2). Essential oils (EOs) exhibit biological activity due to a single active component or due to the synergistic action of many compounds (3).

Hedychium coronarium Koenig, a perennial ornamental plant of the Zingiberaceae family is recognized by its other names like ginger lily, and garland flower. It is having the height of around 1.5 m with fleshy smelly rhizomes, which are reported to act as stimulants, tonics, and carminatives. In the Indian Ayurvedic system, the grounded rhizome of the species is utilized to treat fever (4). The Hedychium coronarium essential oil is reported to constitute terpenes as a major portion with eucalyptol and the β-pinene as key constituents (5). It is believed that oil yield along with compositions is wavered by terrestrial factors (6, 7). The study area belongs to the Konkan region of Maharashtra, a part of the western ghat region that has been declared as a world heritage center by UNESCO (8). Several research articles have focused on the antioxidant and antimicrobial efficacy of essential oil of this species (9, 10). However, the antimalarial activity of this species has not yet been studied extensively. Therefore, the present study was intended to study the chemical constituents and antibacterial, antifungal, and antimalarial activities of rhizome oil of this species from this area.

2. Experimental

2.1 Material collection: The plant understudy was gathered from the Goregaon-Raigad region (Lat. N 18° 9' 14.7456" Long. E73° 17' 52.872") in March 2019. The average height of the plant was 1-1.5m. The species was authenticated by Dr. Priyanka Ingle from B.S.I., Pune.

2.2 Extraction: The rhizomes of species were collected, washed, dried, and exposed for hydrodistillation for 8 hrs. using Clevenger's apparatus. Anhy. Sodium Sulphate was used to dehydrate the extracted oil and kept in the freezer till analysis. The percentage yield was calculated by volume to weight ratio (%v/w).

2.3 Characterization: GC**/**GC-HRMS as well as GC-FID analysis were conducted for analysis of rhizome oil. Two microliters of oil diluted in solvent was injected separately in GC**/**GC-HRMS and GC-FID instruments. Agilent technologies 7890 Gas chromatograph fortified with FID was used to record GC-FID, while Agilent technologies 7890 Gas chromatograph equipped with JEOL The Accu ToF GCV JMS-T100 GCV MS detector was employed to record GC/GCHRMS with He as carrier gas at 1.0 ml/min. The column used was EB5column (Length (30m) x diameter (0.25mm) x thickness (0.25µm) with column temperature 60° -280° C , programmed at 3° C/min .The scanning range was 45-650amu. The injector temp. was kept at 250° C, injection size 2µL primed in acetone, split ratio 1:10 and MS recorded at 70ev (EI).The characterization completed based on R.I value, Library search, by comparison with available literature (11).

2.4. In -vitro Antibacterial Study: Broth dilution method had been employed to evaluate MIC of extracted rhizome oil. Staphylococcus aureus, Staphylococcus pyogenus , the two gram +ve bacteria and Escherichia coli , Pseudomonas aeruginosa , the two gram negative bacteria , were tested versus rhizome oil with Ampicillin and Ciprofloxacin as reference drugs in experiments. In primary and secondary screening, series of dilutions of oil were prepared. The control plates were subcultured to grow the bacteria by evenly spreading on a suitable plate and kept at 37^0 C in incubator. The lowest concentration was taken as MIC (12).

2.5. In-vitro Antifungal Study: Agar dilution protocol was employed to test rhizome oil against some selected fungal pathogens viz. Aspergillus clavatus, Candida albicans and Aspergillus niger (12).

Stock solution of extracted rhizome oil made ready in DMSO, then combined in a quantified amount of molten dextrose agar to screen antifungal activities. To prepare inoculums, the stock of 100 mL of nutrient broth in 250 mL sterilized conical flasks incubated at 27^0C were used. The dilution which showed 99% inhibition was considered as MIC. Griseofulvin and Nystatin were the reference drugs used in the experiment. The errors were avoided by performing triplicate analysis.

2.6. In-vitro Antimalarial activity: An antimalarial potency of rhizome oil was assessed by using in-vitro micro technique (13). The Falciparum strain culture was kept in RPMI-1640 medium supported with glucose (1%), Sodium bicarbonate (0.23%), 25 mM HEPS and human serum (10%).

After d-sorbitol (5%) treatment, Plasmodium falciparum parasites were synchronized to get ring stage parasitized cells. JSB stain was used to find assay with initial ring stage parasitemia of hematocrit (3%) and 200 µl of RPMI-1640 medium to assess percent parasitemia with 50% RBCs (O^{+ve}). After incubation for 36 hours, thin blood smears from each well were set and JSB stain was used to stain it (14).

To record the maturation of the ring stage parasites into schizonts and trophozoites in presence of a varying concentration of rhizome oil, the slides were observed microscopically. The concentration inhibiting complete maturation into schizonts was considered as MIC.

3. Results and Discussion:

3.1 Chemical composition: The pale yellow-colored essential oil with 0.2 %(v/w) yield (1.2ml from 600g) was extracted through hydro distillation of rhizomes of the species. The rhizome oil subjected to the GC-FID and GC/GC-HRMS revealed a total of 11 components comprising 99.96 % of the oil (Table-1). The major constituents found in rhizome oil were eucalyptol (42.85%), α –terpineol (20.97%), β-Pinene(19.92%), 4-terpinol(8.63%) and β-linalool(3.19%). The rhizome oil constitutes 77.96% monoterpenes (oxygenated) and 20.96% monoterpenes (hydrocarbons). The following four chemical compounds extracted from rhizomes of Hedychium coronarium are reported first time-(a)1,4-Cineole (b) Bicyclo [2,2,1] heptan-3-one,6,6-dimethyl-2-methylene (c) 7,11-Dimethyldodeca-2,6,10-trien-1-ol(E,E) and (d)1-terpineol .

The comparative study of the percentage of chemical constituents of rhizome oil of species is reported in table no.1. Several researchers have reported results on Hedychium coronarium species, out of which Prakash et al. from northern India, Joy et al. and Sabulal et al. from south India, and Ray et al. from eastern India have been considered for comparison.

The percentage of eucalyptol was observed to be higher than the other three reports except for work reported by Sabulal and co-workers. (7). The percentage of α –terpineol and 4-terpinol were found highest in this sample than in the reported literature. The β-linalool, one of the important oxygenated hydrocarbons is also found in a higher percentage than in other reported work except by Prakash et al. from northern India (9). Fenchol was also found absent in all three reports except a report from Joy et al. (15).

Table 1: Percentage composition and comparison with the literature of Hedychium coronarium Essential oil

Sr. No.	Constituents	RI^1 values	RI^2 values	Test sample	Sabulal and co-workers (2007)	Joy and co-workers (2007)	Prakash and co-workers (2010)	Ray and co-workers (2018)
1.	α-Tricyclene	-	921	-	-	-	-	0.16
2.	α-Thujene	-	924	-	-	-	-	0.24
3.	α-Pinene	-	932	-	-	4.06	3.5	10.20
4.	**Camphene**	**943**	**946**	**1.04**	0.7	0.30	3.7	0.67
5.	Sabinene	-	969	-	tr	-	-	0.02
6.	**β-Pinene**	**970**	**974**	**19.92**	24.5	10.39	-	23.80
7.	Myrcene	-	988	-	1.2	-	tr	0.53
8.	α – Phellandrene	-	1002	-	2.7	-	0.7	0.72
9.	δ-3-carene	-	1008	-	-	-	-	-
10.	α –Terpinene	-	1016	-	0.6	-	-	0.22
11.	p-Cymene	-	1020	-	0.6	4.08		1.20
12.	Limonene	-	1024	-	2.6	2.14	20.3	1.23
13.	**Eucalyptol**	**1023**	**1026**	**42.85**	48.7	41.42	tr	40.59
14.	γ-terpinene	-	1054	-	1.4	-	8.9	1.94

15.	Terpinolene	-	1085	-	0.5	-	-	0.30
16.	**β-Linalool**	**1081**	**1095**	**3.19**	1.2	0.79	29.3	1.56
17.	Cis-p-Mentha-2,8-dien-1-ol	-	1133	-	-	-	-	0.27
18.	Trans-Pinacarveol	-	1135	-	-	0.96	-	0.17
19.	Camphor	-	1141	-	-	-	-	0.22
20.	Pinocarvene	-	1160	-	-	-	-	0.12
21.	Borneol	-	1160	-	-	2.04	2.1	0.68
22.	**Terpinen-4-ol**	**1175**	**1174**	**8.63**	3.1	3.55	0.5	2.72
23.	**α –Terpineol**	**1172**	**1186**	**20.97**	7.8	8.80	1.5	4.92
24.	**1,4-Cineole**	**1001**	**1008**	**0.37**	-	-	-	-
25.	α-Fenchyl acetate	-	1218	-	-	-	-	-
26.	Geraniol	-	1247	-	-	-	tr	0.02
27.	Carvacrol	-	1294	-	-	0.34	tr	0.14
28.	Eugenol	-	1352	-	-		-	0.43
29.	β-Caryophyllene	-	1420	-	-	-		0.09
30.	α -Humulene	-	1453	-	-		0.1	0.38
31.	**Fenchol**	**1100**	**1100**	**2.0**	-	0.22	-	-
32.	ar-Curcumene	-	1479	-	-	-	2.7	-
33.	Germacrene-D	-	1484	-	-	-	-	-
34.	β -Bisabolene	-	1505	-	-	-	0.1	-
35.	**Bicyclo[2,2,1] heptan-3-one,6,6-dimethyl-2-methylene**	**1114**	**1163**	**0.32**	-	-		-
37.	γ –Cadinene	-	1513	-	-	-	0.3	-
38.	**7,11-Dimethyldodeca-2,6,10-trien-1-ol(E,E)**	**1634**	**1581**	**0.35**	-	-		-
39.	Elemol	-	1554	-	-		-	0.17
40.	Cis-Nerolidol	-	1561	-	-	1.33	tr	-
41.	**1-Terpineol**	**1120**	**1137**	**0.32**	-	-		-
42.	10-epi-γ-Eudesmol	-	1622	-	-	-	-	4.64
43.	β-Eudesmol	-	1649	-	-	-	-	0.11
	Total (%)	-		**99.96**	98.3	93.31	98.8	99.84
	Monoterpenoid (%)	-		**98.92**	86.80	72.30	83.8	92.55

- Compounds not detected.

RI^1- Retention index values detected on EB5 column.

RI^2- Retention index values from literature (Adams 2017).

3.2. Antibacterial activities: The rhizome oil was tested by Broth dilution method. Table 2 shows the antibacterial activities of rhizome oil against E. coli, P. aeruginosa, the two gram –ve bacteria and S. aureus, S. pyogenus, the two-gram +ve bacteria. The presence of oxygenated monoterpenes (eucalyptol, α –Terpineol, 4-Terpinol, and β-linalool) enhanced the antibacterial activities versus gram +ve and a gram -ve bacteria (16). Rhizome oil exhibited superb antibacterial activity versus E. coli (MTCC-443) and moderate antibacterial activities against P. aeruginosa and S. aureus (MTCC-96) than the reference drug Ampicillin. This may be because of the presence of oxygenated monoterpenes in higher amounts.

Table-2: MICs of Rhizome oil against bacteria

Strains	MICs (µg/ml) Rhizome oil	MICs (µg/ml) Ampicillin (Standard)	MICs (µg/ml) Ciprofloxacin (Standard)
E. coli (MTCC-443)	62.5	100	25
P. aeruginosa (MTCC-1688)	100	100	25
S. aureus (MTCC-96)	100	250	50
S. pyogenus (MTCC-442)	125	100	50

3.3. Antifungal activities: An efficacy of rhizome oil versus C. albicans, A. niger and A. clavatus species is shown in table 3. Out of three fungal strains, oil exhibited admirable antifungal activity against the C. albicans (MTCC-227) with the reference drug Griseofulvin. This might be due to the presence of linalool in the oil sample (17).

Table-3: MICs of Rhizome oil versus fungi

Test pathogens	MICs (µg/ml) Rhizome oil	MICs (µg/ml) Greseofulvin (Standard)	MICs (µg/ml) Nystatin (Standard)
C. albicans (MTCC-227)	250	500	100
A. niger (MTCC-282)	1000	100	100
A. clavatus (MTCC-1323)	1000	100	100

3.3.Antimalarial activities: The antimalarial ability of rhizome oil was checked against Plasmodium falciparum with two important antimalarial drugs viz. Chloroquine and Quinine as reference drugs.

The result is tabulated in table 4. The oil has shown comparable antimalarial activity against the standards used.

Table-4: MIC of Rhizome oil against Plasmodium falciparum

Sr. No.	Test pathogens	Mean IC_{50} (µg/ml)
I.	Rhizome oil	0.85
II.	Chloroquine	0.020
III.	Quinine	0.268

Conclusion: The present study concludes that the chemical constituents of Hedychium coronarium rhizome oil are influenced by geographical and environmental factors. The monoterpenoid-rich essential oil exhibited excellent antibacterial and antifungal efficacy against E. coli and C. albicans respectively. Moreover, rhizome oil showed good antimalarial activity versus Plasmodium falciparum. The chemical compounds – (a)1,4-Cineole (b) Bicyclo [2,2,1] heptan-3-one,6,6-dimethyl-2-methylene (c)7,11-Dimethyldodeca-2,6,10-trien-1-ol(E,E) and (d)1-terpineol extracted from rhizomes of Hedychium coronarium species from Konkan region of India are first time reported in this study.

Acknowledgment: Authors convey thanks to SAIF- IIT Bombay, Microcare laboratory Gujarat for technical and biological activities support, and B. S. I., Pune for species authentication.

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