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Phytochemical Profiling and Biological Evaluation of *Hedychium coronarium* Essential Oil from the Konkan Province

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Abstract: *Hedychium coronarium*, a unique species from the Zingiberaceae family, is native to the Western Ghats of India. Although the essential oil of this plant has been studied in northern and southern India, extensive research has yet to explore its isolation and bioefficacy from the Konkan region. This study fills that gap by isolating the rhizome essential oil using steam distillation (SDREO) and analyzing its composition with GC/FID and GC/HRMS techniques. The results revealed that monoterpenoids dominate the oil, making up 41.07% of its chemical profile. The SDREO exhibited strong antibacterial activity against *E. coli* and *P. aeruginosa*, along with moderate antifungal effects against *Candida albicans*. Furthermore, the oil displayed impressive antioxidant activity in the DPPH assay, with gallic acid as a standard, and showed significant anti-inflammatory effects through the membrane stabilization method. These findings highlight the role of geographical factors and isolation methods employed in shaping the essential oil's composition, with notable differences observed compared to other reports from different regions.

Keywords: Essential oil, *Hedychium coronarium* species., Steam distilled Rhizome Essential Oil (SDREO), Konkan region

I. INTRODUCTION

Plants are an abundant treasure trove of bioactive compounds with remarkable medicinal properties. In India, more than 2,500 plant species have been explored, with around 150 of them being harnessed by biopharmaceutical companies for their therapeutic potential. As the world's largest producer of medicinal plants, India proudly holds the title of "the botanical garden of the world"(1). The Zingiberaceae family is a prominent source of medicinal plants, particularly those yielding essential oils. Thanks to their demonstrated biological activities, these oils have considerable potential for use in the pharmaceutical, perfumery, and food industries. The bioactive effects of these oils can result from a single active compound or the combined action of multiple components. The composition and biological properties of these oils are significantly shaped by environmental and ecological factors (2,3).

The species *Hedychium coronarium* is a perennial herb renowned for its medicinal properties and is found worldwide. Significant research on this species has been conducted in southern, northern, and eastern India (4,5,6). However, extensive research is lacking from the Konkan coast, aside from our previous studies (7,8,9). Since the composition of essential oils is heavily influenced by environmental and ecological factors, this study aims to explore *Hedychium coronarium* from the Konkan region, part of the Western Ghats, which has been designated as a UNESCO World Heritage site (10).

Although earlier research has addressed the composition and some bioactivities of this species, its anti-inflammatory potential has not yet been studied. Accordingly, this study aims to isolate, characterize, and assess various biological activities, with a particular focus on the unexplored anti-inflammatory effects of this species from this region.

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II. MATERIALS AND METHODS

2.1. Plant material: The fresh 250 g rhizomes of this species were gathered from the Goregaon-Raigad region (Lat. N 18.1525°, Long. E73.2942°). The species was validated by the B. S. I., Pune.

2.2 Isolation of oil:

Steam distillation: The plant rhizomes were thoroughly washed, dried, and placed into a steam distillation apparatus. Approximately one liter of distilled water was added, and the distillation process was carried out for about 4–5 hours. The extraction was conducted at a maintained temperature of 80–90°C. The essential oil obtained was collected in vials and subsequently dehydrated using anhydrous sodium sulfate.

2.3 Analysis of oil:

Gas Chromatography-Mass Spectrometry (GC and GC/MS) analysis: GC/HRMS analysis was performed by using Agilent technologies 7890 Gas chromatograph equipped with JEOL The Accu ToF GCV JMS-T100 GCV MS detector. Helium was the carrier gas used in GC with a flow rate of 1.0 ml/min. with HP5 column also called EB5column (30 m length x 0.25 mm diameter x 0.25 µm thickness) was fortified in GC during analysis. The mass spectra were recorded at 70 ev (EI) and the compositions of essential oil were recognized based on R. I., Library MS search (NIST), and by comparing MS with reported literature (8). Shimadzu QP-2010 Ultra Gas Chromatograph with HP5MS column (length 30m, film thickness:0.25 µm with max. temp. 250^0C) was used for GC analysis with Helium as carrier gas. The percentage composition was calculated by the Area normalization method and was compared with standards (11).

2.4. *In-vitro* Antimicrobial Study: The broth dilution method was employed to determine the minimum inhibitory concentration (MIC) of the extracted rhizome essential oil, using DMSO as the diluent. Two gram-positive bacteria — *Staphylococcus aureus* (MTCC 96) and *Streptococcus pyogenes* (MTCC 442) — along with two gram-negative bacteria — *Escherichia coli* (MTCC 443) and *Pseudomonas aeruginosa* (MTCC 1688) — were tested against the rhizome oil. Additionally, the agar dilution method was utilized to evaluate the antifungal activity of the rhizome oils against selected fungal strains, namely *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282), and *Aspergillus clavatus* (MTCC 1323) (12).

2.5. *In-vitro* Antioxidant activity: Two assays viz. DPPH and ABTS were employed to check the anti-oxidant efficacy of extracted rhizome essential oil following standard protocols (13,14).

2.6. *In-vitro* Anti-inflammatory Activities:

The anti-inflammatory efficacy of SDREO was evaluated against human RBCs. The three different methods viz. Heat-induced hemolysis**,** Inhibition of albumin denaturation**,** and Proteinase inhibitory action were employed with five different concentrations of test samples - 25, 50, 75, 100, 125, and 150µg/ml by following standard protocols (15,16,17).

III. RESULTS AND DISCUSSION

3.1 Constituent of oil: The average essential oil yield by steam distillation was 0.57 %. The yellow-colored essential oil was isolated from *H. coronarium*, constituting 09 components, which comprised 99.64 % essential oil (Table-1). The principal components were – Eucalyptol (29.32%), Retinal (23.05%), α-terpinol (11.75%), Aromadendrene oxide-2(10.99%), Lycopene (8.42%), etc.

The SDREO constitutes 41.07 % oxygenated monoterpenes, 10.99% oxygenated sesquiterpenes, 40.32% carotenoids, 6.00% triglycerides, and the remaining other compounds as tabulated in Table 1.

Several researchers have reported results on *Hedychium coronarium* species, out of which Prakash et al. (4), from northern India, Ray et al. (5), from eastern India, and Sabulal et al. (6) from south India, have been considered for comparison. The percentage of eucalyptol was observed to be lower than the other three reports. The percentage of α-terpineol was found to be higher in this sample than in the reported literature.

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Compared to our previous study (9), the SDREO contained two common components—Eucalyptol and α-Terpinol, though in lower concentrations. Notably, carotenoids were present in significant amounts in SDREO but were absent in HDREO. These findings indicate that the chemical composition differs both qualitatively and quantitatively from earlier reports, likely due to variations in the isolation techniques employed and environmental factors. Further, Sesquiterpene Oxygenated compounds and triglycerides were absent in the previous study.

Table 1. The composition of *H. coronarium* oil and comparison with the literature

Sr. No.	Components	RI^1	RI^2	By SD (%)	By HD (%) Nagore et al. (2022)
1.	Eucalyptol	1023	1026	29.32	42.85
2.	α-Terpinol	1172	1176	11.75	20.97
3.	2,4-di-t-Butyl phenol	1539	1542	1.26	-
4	Lycopene	3878	3882	8.42	-
5.	Retinal	2466	2466	23.05	-
6.	β-Carotene	3978	3978	2.23	-
7.	Rhodopin	4025	4035	6.62	-
8.	Aromadendrene oxide-2	2299	2299	10.99	-
9.	Docosanoic acid, 1,2,3-propanetriyl ester	7318	7325	6.00	-
	Total Chemical Components (% composition)	NA	NA	09 (99.64%)	11 (99.96%)
	Monoterpene Hydrocarbons	NA	NA	-	20.96 %
	Monoterpene Oxygenated (1,2)	NA	NA	41.07%	77.96%
	Sesquiterpene Hydrocarbons	NA	NA	-	-
	Sesquiterpene Oxygenated (8)	NA	NA	10.99%	-
	Carotenoids (4,5,6,7)	NA	NA	40.32	-
	Triglycerides (9)	NA	NA	6.00 %	-
	Others (3)	NA	NA	1.26%	1.08%

- Compounds undetected.
RI^1- Retention index values detected on HP5 column.
RI^2- Retention index values from literature (Adams 2007).
SD- Steam distillation
HD-Hydrodistillation.
NA-Not Applicable

3.2 *In-vitro* antibacterial activities:

The results are concised in Table 2. The SDREO exhibited excellent efficacies against *E. coli* and *P. aeruginosa* with ampicillin as a reference drug.

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The presence of oxygenated monoterpenes (eucalyptol & α-terpineol) enhanced the antibacterial activities versus gram +ve and a gram -ve bacteria (18). The similar results can be observed in our previous study (9). So, monoterpenes have major role to play in antibacterial activities.

Table 2. MIC of SDREO against selected bacteria

Test Pathogens	MICs of SDREO (µg/ml)	MICs (µg/ml) of Ampicillin (Standard)	MICs (µg/ml) of Ciprofloxacin (Standard)
E. coli (MTCC-443)	100	100	25
P. aeruginosa (MTCC-1688)	100	100	25
S. aureus (MTCC-96)	150	250	50
S. pyogenus (MTCC-442)	250	100	50

3.3. *In-vitro* antifungal activities: Table 3 summarizes the *in-vitro* potency of SDREO against selected fungal strains. Out of the three strains used, the oil displayed excellent antifungal activity versus *C. albicans* (MTCC-227) concerning the standard drug Griseofulvin. The synergistic action of monoterpenes and carotenoids could have suppressed the activities.

Table 3. MICs of SDREO against fungi

Test pathogens	MICs of SDREO (µg/ml)	MICs (µg/ml) of Griseofulvin (Standard)	MICs (µg/ml) of Nystatin (Standard)
C. albicans (MTCC-227)	500	500	100
A. niger (MTCC-282)	1000	100	100
A. clavatus (MTCC-1323)	1000	100	100

3.4. *In-vitro* antioxidant activities: The antioxidant efficacy of SDREO was evaluated by DPPH and ABTS assays. The result is tabulated in table 4. It seems that the oil showed moderate antioxidant activities in both assays. This could be because of synergy of main components.

Sr. No.	Test samples	Mean IC_{50} values (µg/ml)	
		DPPH assay	ABTS assay
1.	SDREO	61.74 ± 2.82	96.28 ± 1.26
3.	Standard (Gallic acid)	39.68 ± 0.89	53.14 ± 0.44

Table 4. IC_{50} values of SDREO in antioxidant assays

3.5 *In-vitro* anti-inflammatory activities: An anti-inflammatory potency of SDREO was assessed by three methods. The results are tabulated in table 5. The SDREO exhibited an amazing anti-inflammatory activity, best by the membrane stabilization and least by proteinase inhibition method.

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Table 5. Anti-inflammatory activities of SDREO

Sr. No.	Test sample	Methods used		
		Albumin denaturation (µg/ml)	Membrane stabilization (µg/ml)	Proteinase inhibition (µg/ml)
1.	SDREO	84.91 ± 1.90	83.86 ± 1.25	99.13 ± 1.85
3.	Standard (Diclofenac sodium)	75.84 ± 1.25	79.06 ± 1.02	81.28 ± 2.09

These exceptionally well anti-inflammatory efficacies of SDREO can be devoted to important anti-inflammatory agent i.e., eucalyptol, present in major amounts in the sample.

IV. CONCLUSIONS

This study demonstrates that the chemical composition of *H. coronarium* rhizome oil is strongly influenced by environmental factors and the extraction methods employed. The current findings show notable qualitative and quantitative differences compared to previously reported data. The high content of oxygenated monoterpenoids (41.07%) is likely responsible for the oil's potent antimicrobial, antioxidant, and remarkable anti-inflammatory activities.

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