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Phytochemical Indices, and GC–MS Profiling of *Hedychium coronarium* Rhizome Essential Oil

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Abstract:

Hedychium coronarium J. Koenig is an aromatic medicinal plant whose rhizomes contain a chemically variable essential oil with potential value in food, pharmaceutical, and antimicrobial applications. This study extracted *H. coronarium* rhizome essential oil (HCREO) by hydrodistillation and determined its extraction yield, apparent total phenolic and flavonoid contents, and volatile composition by gas chromatography–mass spectrometry (GC–MS) and gas chromatography–flame-ionization detection (GC–FID). Dried rhizome powder was hydrodistilled for 3.5 h in a Clevenger-type apparatus. HCREO yield was 4.61% (v/w, dry-weight basis). The apparent total phenolic and flavonoid contents were 70 mg gallic-acid equivalents/g HCREO and 62 mg quercetin equivalents/g HCREO, respectively. Thirty-six volatile constituents accounted for 97.39% of the oil. Oxygenated monoterpenes were predominant (51.95%), followed by monoterpene hydrocarbons (34.39%), sesquiterpene hydrocarbons (6.01%), and oxygenated sesquiterpenes (5.04%). The principal compounds were 1,8-cineole (33.84%), β-pinene (14.76%), α-terpineol (7.41%), linalool (5.26%), α-pinene (4.38%), and limonene (3.85%). The profile characterized HCREO as a 1,8-cineole/β-pinene-rich oil and agreed with chemotypes previously reported from India. The findings established the batch-specific chemical identity of HCREO and provided a compositional basis for subsequent evaluation of its biological activity.

Keywords: *Hedychium coronarium*; rhizome essential oil; hydrodistillation; 1,8-cineole; GC–MS; GC–FID; phenolic content; flavonoid content.

1. Introduction

Plant essential oils are complex mixtures of volatile mono- and sesquiterpenes that have received substantial attention as natural sources of fragrance, antioxidants, and antimicrobial agents. Their chemical composition varies with genotype, geographical origin, phenological stage, environmental conditions, plant organ, postharvest processing, and extraction method. Consequently, biological investigations of essential oils require batch-specific chemical profiling rather than reliance on a composition reported for the same botanical species elsewhere.

Hedychium coronarium J. Koenig, commonly known as white ginger lily or butterfly ginger lily, belongs to the family Zingiberaceae. The species is native to tropical Asia and is cultivated or naturalized in several tropical and subtropical regions. Its aromatic rhizomes have been used in traditional preparations and contain volatile terpenes and nonvolatile diterpenoids with reported

antimicrobial, antioxidant, anti-inflammatory, and other pharmacological activities [1]. Rhizome essential oil is particularly important because it contains bioactive monoterpenes, including 1,8-cineole, α-pinene, β-pinene, linalool, limonene, and α-terpineol.

Previous studies have demonstrated pronounced variation in *H. coronarium* rhizome oil. Fresh and dried rhizome oils were reported to contain 1,8-cineole, β-pinene, and α-terpineol as major compounds [2]. Rhizome oils of different Hedychium species were composed mainly of mono- and sesquiterpenoids and exhibited antimicrobial and antioxidant activities [3]. Investigations of Indian populations further revealed marked geographical and chemotypic diversity in both the abundance and identity of volatile constituents [4,5]. Similar interregional variability was observed in samples collected outside India [6,7].

The phytochemical indices of an essential-oil preparation may provide additional information about its reducing and metal-complexing capacity. The Folin–Ciocalteu and aluminium-chloride assays are widely used to express apparent total phenolic and flavonoid contents relative to gallic acid and quercetin standards, respectively [8,9]. Nevertheless, these assays are operational colorimetric measurements and do not independently identify individual phenolic or flavonoid molecules.

The present study therefore aimed to extract essential oil from authenticated *H. coronarium* rhizomes collected in southern India, determine its dry-weight yield, quantify its apparent total phenolic and flavonoid contents, and establish its volatile profile by complementary GC–MS and GC–FID analyses. The work provided the batch-specific chemical characterization required before investigating HCREO as a natural antifungal and food-protection agent.

2. Materials and methods

2.1 Chemicals and reagents

Hexane, ethanol, and methanol used for phytochemical and chromatographic analyses were of ultrapure grade and were obtained from Sigma–Aldrich, Bengaluru, India. Analytical-grade anhydrous sodium sulphate, Folin–Ciocalteu reagent, gallic acid, sodium carbonate, aluminium chloride, and quercetin were obtained from Merck, Bengaluru, India. A homologous n-alkane mixture (nC8–nC18) was obtained from Sigma–Aldrich. Ultrapure water was used throughout. Plasticware was obtained from Tarsons Products, Kolkata, India, and glassware from Borosil, Mumbai, India. Extracted oil was stored in amber glass vials fitted with PTFE-lined caps.

2.2 Plant collection and authentication

Fresh *H. coronarium* rhizomes were collected from Vijayawada, Andhra Pradesh, India. Representative plants and rhizomes were authenticated by a qualified plant taxonomist, and reference specimens were deposited in the Department of Biotechnology, Acharya Nagarjuna University, India. Collection location, date, and diagnostic morphological characteristics were recorded with the specimen information in accordance with established practices for authenticated Hedychium material [4,7].

2.3 Processing and essential-oil extraction

Adhering soil and foreign material were removed under running water, after which the rhizomes were rinsed with distilled water. Clean rhizomes were cut into small slices and air-dried for 10 days in the laboratory. The dried material was ground into a coarse powder with an electric laboratory grinder under clean conditions and stored in airtight containers until extraction.

HCREO was extracted by hydrodistillation using a Clevenger-type apparatus, consistent with procedures reported for Hedychium rhizome oils [2,3]. Three independent extractions were performed with 250 g of dried rhizome powder per extraction. The material was placed in a round-bottom flask, mixed with

sufficient distilled water, and hydrodistilled for 3.5 h. The oil layer was separated in the graduated arm, collected, and dried over anhydrous sodium sulphate. The dried oil was transferred to airtight amber vials and stored at 4 °C until analysis.

Essential-oil yield was calculated on a dry-weight basis as follows: HCREO yield (mL/100 g) = $[(V \pm \Delta V)/m_s] \times 100$, where V represented the recovered oil volume in millilitres, ΔV represented the reading uncertainty of the graduated Clevenger arm, and m_s represented the dry mass of rhizome material in grams.

2.4 Apparent total phenolic content

Apparent total phenolic content was determined by previously described Folin–Ciocalteu procedures [9,10], with minor modifications. HCREO working solutions ranging from 1 to 5 mg/mL were prepared in methanol. A 100 µL aliquot was mixed with 200 µL Folin–Ciocalteu reagent and allowed to react for 3 min. One millilitre of 2% (w/v) aqueous sodium carbonate was then added. After incubation for 1 h at 25 ± 1 °C, absorbance was measured at 764 nm using a UV–visible spectrophotometer. Gallic-acid solutions ranging from 0 to 50 µg/mL were processed similarly. Results were calculated from the calibration equation and expressed as milligrams of gallic-acid equivalents per gram of HCREO (mg GAE/g HCREO). Measurements were performed in triplicate.

2.5 Apparent total flavonoid content

Apparent total flavonoid content was determined by a previously described aluminium-chloride colorimetric procedure [8], with minor modifications. HCREO was dissolved in ethanol at 1 mg/mL. One millilitre of sample solution was mixed with 1 mL of 2.5% (w/v) aluminium chloride in ethanol, shaken gently, and incubated for 1 h at 25 ± 1 °C. Absorbance was measured at 418 nm. Quercetin standards ranging from 0 to 50 µg/mL were treated identically. Results were expressed as milligrams of quercetin equivalents per gram of HCREO (mg QE/g HCREO). Measurements were performed in triplicate.

2.6 GC–MS analysis

Volatile constituents were analysed using a Shimadzu QP2010 GC–MS system (Kyoto, Japan) fitted with a DB-5MS fused-silica capillary column (30 m × 0.25 mm internal diameter; 0.25 µm film thickness). Helium was supplied as the carrier gas at 1.0 mL/min. Injector and interface temperatures were maintained at 220 and 240 °C, respectively. The oven temperature began at 55 °C, increased to 240 °C at 4 °C/min, then increased to 300 °C at 8 °C/min and was held for 8 min. HCREO was diluted in hexane, and 0.5 µL of the diluted sample was injected.

The mass spectrometer operated in electron-ionization mode at 70 eV. The ion-source temperature was 200 °C, scan speed was 1000 Da/s, scan interval was 0.50 s, and full-scan spectra were acquired over 45–500 Da. Data were collected and processed using LabSolutions LC/GC Workstation version 2.72. A homologous n-alkane series (nC8–nC18) was analysed under identical chromatographic conditions. Linear retention indices were calculated using the temperature-programmed equation described previously [11]. Experimental indices were compared with published values for nonpolar stationary phases [12,13]. Mass spectra were matched against FFNSC 1.2, NIST 107, and NIST 21 libraries. Assignments were accepted when spectral similarity was at least 95% and the experimental retention index was consistent with the corresponding literature value.

2.7 GC–FID analysis and semiquantification

Relative amounts of volatile constituents were determined using a Shimadzu GC-2010 gas chromatograph coupled to a flame-ionization detector. The column type and temperature programme

matched those used for GC–MS. The relative percentage of each compound was calculated by peak-area normalization without application of compound-specific FID response-correction factors. Accordingly, the values were treated as semiquantitative relative abundances rather than absolute concentrations.

2.8 Statistical analysis

Extraction and colorimetric assays were performed in triplicate. Results were summarized as mean values, and GC–FID constituent percentages were calculated by peak-area normalization. Statistical processing was performed using SPSS version 25.0 (IBM, New York, USA). Where comparisons were applicable, one-way analysis of variance followed by Tukey's test or Dunnett's test was used, and significance was accepted at $p < 0.05$.

3. Results and discussion

3.1 Essential-oil recovery

Hydrodistillation of dried *H. coronarium* rhizomes produced an HCREO yield of 4.61% (v/w), corresponding to 4.61 mL per 100 g of dry rhizome material. The result confirmed efficient recovery of the volatile fraction under the selected drying, grinding, and hydrodistillation conditions.

The recorded yield was higher than values previously reported for *H. coronarium*. Yields of 0.05–0.25% were reported among Uttarakhand populations [14], whereas yields of 0.05–0.47% were found among other Indian populations [5]. Approximately 0.20% rhizome oil was obtained from Brazilian material [6]. Differences in geographical origin, genotype, rhizome maturity, season, dry-matter basis, particle size, distillation conditions, and recovery efficiency could contribute to the observed variation. The unusually high yield in the present batch should nevertheless be confirmed by reporting the mean and dispersion of three independent extractions and by ensuring consistent use of v/w rather than w/w units.

3.2 Apparent phenolic and flavonoid indices

The apparent total phenolic content of HCREO was 70 mg GAE/g HCREO, whereas apparent total flavonoid content was 62 mg QE/g HCREO. The phenolic index was numerically higher by 8 mg equivalent/g. This difference did not represent a direct mass balance because the two assays used chemically different reference standards and response mechanisms.

Total phenolic contents of 18.5–26.3 mg/g were previously reported in leaf and rhizome preparations of *H. coronarium* [15]. The present phenolic index was higher, although direct comparison remained limited by differences in sample matrix, extraction procedure, standard, and reporting basis. Earlier investigations showed antioxidant activity in Hedychium rhizome oils and associated this property with their chemically diverse terpenoid and reducing-constituent profiles [3,5]. The current values therefore supported the presence of Folin-reactive and aluminium-complexing constituents in HCREO.

These results required cautious interpretation. Folin–Ciocalteu reagent responds to overall reducing capacity and is not specific exclusively to phenolic molecules, while the aluminium-chloride assay indicates complex formation rather than direct identification of individual flavonoids. Because most classical flavonoids are nonvolatile, the two measurements were appropriately described as apparent phytochemical indices. Confirmation of discrete nonvolatile phenolics or flavonoids would require complementary HPLC or LC–MS analysis.

3.3 Volatile composition determined by GC–MS and GC–FID

Combined comparison of mass spectra and retention indices resulted in the tentative assignment of 36 volatile constituents. These compounds accounted for 97.39% of HCREO, leaving 2.61% as unidentified or trace constituents. Oxygenated monoterpenes constituted the dominant class at 51.95%, followed by

monoterpene hydrocarbons at 34.39%, sesquiterpene hydrocarbons at 6.01%, and oxygenated sesquiterpenes at 5.04%. Monoterpenes collectively accounted for 86.34% of the identified oil, establishing HCREO as a predominantly monoterpenoid preparation.

The principal constituent was 1,8-cineole (33.84%), followed by β-pinene (14.76%), α-terpineol (7.41%), linalool (5.26%), α-pinene (4.38%), limonene (3.85%), sabinene (3.12%), caryophyllene oxide (2.94%), β-caryophyllene (2.63%), and p-cymene (2.18%). The three most abundant compounds accounted for 56.01% of HCREO, whereas the five most abundant represented 65.65%. The batch was therefore classified as a 1,8-cineole/β-pinene-rich chemotype.

Table 1. Chemical constituents of HCREO by GC-MS analysis.

S. No.	RI	Compound	Relative content (%)
1	921	Tricyclene	0.14
2	923	α-Thujene	0.16
3	934	α-Pinene	4.38
4	947	Camphene	1.48
5	968	Sabinene	3.12
6	976	β-Pinene	14.76
7	989	β-Myrcene	0.87
8	1001	α-Phellandrene	0.44
9	1009	δ-3-Carene	0.39
10	1016	α-Terpinene	0.49
11	1021	*p-Cymene*	2.18
12	1024	Limonene	3.85
13	1025	β-Phellandrene	0.73
14	1026	1,8-Cineole	33.84
15	1057	γ-Terpinene	0.78
16	1088	Terpinolene	0.62
17	1097	Linalool	5.26
18	1098	trans-Sabinene hydrate	0.33
19	1143	Camphor	0.31
20	1157	Isoborneol	0.18
21	1166	Borneol	1.31
22	1175	Terpinen-4-ol	1.92
23	1187	α-Terpineol	7.41

S. No.	RI	Compound	Relative content (%)
24	1194	Myrtenol	0.24
25	1195	Myrtenal	0.21
26	1288	Bornyl acetate	0.58
27	1348	α-Terpinyl acetate	0.36
28	1389	β-Elemene	0.29
29	1418	β-Caryophyllene	2.63
30	1454	α-Humulene	1.22
31	1485	Germacrene D	1.06
32	1502	Bicyclogermacrene	0.27
33	1523	δ-Cadinene	0.54
34	1549	Elemol	1.17
35	1578	Spathulenol	0.93
36	1583	Caryophyllene oxide	2.94
Total identified constituents			**97.39**

3.4 Comparison with reported *H. coronarium* chemotypes

The dominant profile agreed closely with a previous analysis of fresh and dried rhizome oils [2], in which 1,8-cineole was reported at 41.42% and 37.44%, β-pinene at 10.39% and 17.40%, and α-terpineol at 8.80% and 6.70%, respectively. The corresponding values in the present HCREO—33.84%, 14.76%, and 7.41%—were close to those ranges. This agreement supported the assignment of the present material to the same broad 1,8-cineole/β-pinene/α-terpineol chemotype.

Other studies demonstrated alternative compositional patterns. Appreciable β-pinene and caryophyllene oxide were reported in Brazilian rhizome oil [6], whereas Indian populations showed variable dominance of 1,8-cineole, β-pinene, linalool, limonene, and oxygenated sesquiterpenes [4,5,16]. Regional variability was similarly demonstrated in Amazonian specimens [7]. The presence of β-caryophyllene, α-humulene, germacrene D, δ-cadinene, elemol, spathulenol, and caryophyllene oxide in the present sample reflected this broader sesquiterpene diversity, although they collectively occurred at lower abundance than the major monoterpenes.

Environmental and methodological factors offered plausible explanations for interstudy differences. Temperature, rainfall, soil properties, altitude, phenological stage, rhizome age, drying conditions, storage, grinding, and hydrodistillation can change the recovery and relative representation of volatile constituents. The use of retention indices alongside mass-spectral matching reduced the risk of erroneous library-only identification. However, peak-area normalization without response correction remained semiquantitative because FID response factors may differ among chemical classes.

3.5 Chemical and biological significance

The abundance of 1,8-cineole, β-pinene, α-terpineol, linalool, α-pinene, and limonene provided a rational chemical basis for subsequent biological evaluation. These lipophilic monoterpenes can interact with microbial membranes, while oxygenated terpenes may contribute additional polarity and reactivity. Minor constituents, including terpinen-4-ol, borneol, p-cymene, β-caryophyllene, and caryophyllene oxide, may also influence overall activity through additive, antagonistic, or synergistic interactions. Nevertheless, compositional association alone could not establish which constituent caused a particular biological response. Testing the complete oil alongside selected major compounds would be required to distinguish whole-mixture effects from single-compound activity.

The high identified fraction and defined chemotype provided an essential quality-control reference for experiments using this batch of HCREO. Because essential-oil composition can vary even within the same species, batch-specific profiling also improves reproducibility and helps interpret differences in antifungal or antiaflatoxigenic potency between studies.

4. Conclusion

Hydrodistillation of authenticated *H. coronarium* rhizomes yielded 4.61% HCREO on a dry-weight basis. The oil showed apparent total phenolic and flavonoid contents of 70 mg GAE/g and 62 mg QE/g, respectively. GC–MS and GC–FID profiling tentatively identified 36 volatile constituents representing 97.39% of the oil. Oxygenated monoterpenes predominated, and 1,8-cineole, β-pinene, α-terpineol, linalool, α-pinene, and limonene were the major compounds. The composition classified the sample as a 1,8-cineole/β-pinene-rich chemotype closely related to previously reported Indian *H. coronarium* oils. The resulting profile established the chemical identity of the HCREO batch and supported its further investigation as a natural antimicrobial or food-protection material. Final submission should incorporate replicate variability for extraction and colorimetric assays and replace the provisional GC–FID percentages with instrument-derived peak areas.

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