Studies on the Rhizome Oils from Four *Hedychium* Species of South India: A Chemotaxonomic Approach

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Abstract

The genus *Hedychium* J. König (Zingiberaceae) with about 80 species has only four members in south India, viz., *H. coronarium* J. König, *H. flavescens* Carey ex Roscoe, *H. spicatum* var. *acuminatum* (Roscoe) Wall., and the endemic, *H. venustum* Wight. The chemical compositions of essential oils from the rhizomes of these four *Hedychium* species and their morphologies have been compared. The rhizome oils were characterized by analytical gas chromatography and gas chromatography-mass spectroscopy. The number of identified constituents in the rhizome oils of *H. coronarium, H. flavescens, H. spicatum* var. *acuminatum* and *H. venustum* were 24 (99.7%), 27 (98.8%), 41 (98.9%) and 57 (99.1%) respectively. 1,8-cineole, β-pinene and linalool constituted 70-75% of these rhizome oils. 1,8-cineole was the single major constituent in the rhizome oils of *H. coronarium* (48.7%), *H. venustum* (45.4%), and *H. spicatum* var. *acuminatum* (44.3%). β-pinene (43.6%) was the major component in the rhizome oil of *H. flavescens*. The percentages of sesquiterpenes in these oils were *H. venustum* (24.0%), *H. spicatum* var. *acuminatum* (22.1%), *H. coronarium* (3.1%) and *H. flavescens* (1.3%). Oil yields from the rhizomes of *H. venustum*, *H. spicatum* var. *acuminatum* and *H. coronarium* were comparable (0.13-0.16%), but that from the rhizomes of *H. flavescens* was substantially low (0.05%). *H. venustum* and *H. spicatum* var. *acuminatum* are morphologically similar and significantly different from *H. flavescens*. The chemical data on essential oils are in good agreement with the relative morphological features of these four *Hedychium* species and thus chemotaxonomically significant.

Introduction

The genus *Hedychium* J. König (Zingiberaceae) represents the spectacular

'Ginger lilies' comprising over 80 species distributed in India, China and South East Asia. In India it has about 50 species with about 17 endemics (Sabu, 2000). The genus is represented in south India by only four species, viz., *H. coronarium* J. König, *H. flavescens* Carey ex Roscoe, *H. spicatum* var. *acuminatum* (Roscoe) Wall. and *H. venustum* Wight, of which *H. venustum* is endemic to this region. *H. coronarium* is widely cultivated as a garden plant and the rest are restricted to hilly slopes at altitudes ranging from 1000 to 1800 m. All the species have fleshy, branched rhizomes characteristically aromatic due to the presence of essential oils. Essential oils are complex, volatile, aromatic, heterogeneous mixtures containing mostly mono- and sesquiterpenes and their derivatives. Inter-relationships of these four South Indian taxa of *Hedychium* were established by correlating their morphological characters and the percentages and distribution of mono- and sesquiterpenes in their rhizome oils.

Materials and Methods

Flowering specimens and rhizomes of *Hedychium flavescens, H. spicatum* var. *acuminatum* and *H. venustum* were collected from established populations in natural habitats at Devikolam, Munnar (Idukki District) and Ponmudi (Thiruvananthapuram District), Kerala State in South India. *H. coronarium* was collected from an established population in Tropical Botanic Garden and Research Institute (TBGRI), Thiruvananthapuram. All specimens were collected in September of 2004, and their vouchers (Mathew 54620, 54609, 54622 and 54624, respectively) were deposited in the Herbarium of TBGRI (TBGT). Seventeen morphological characters from each species were compared (Table 1), of which quantitative characters are the mean values of six measurements taken from different shoots within a clump. The measurements of leaves (length, breadth and area) were determined from middle leaves of different shoots.

Essential oils of these four taxa were isolated from fresh rhizomes by hydrodistillation in a Clevenger-type apparatus for 3 hrs. Chemical constituents of these oils were analysed by analytical gas chromatography (GC-FID) and gas chromatography-mass spectroscopy (GC-MS) as described in Sabulal *et al* (2007). GC-FID of rhizome oils were carried out on a Nucon 5765 gas chromatograph with a SE-30 10% Chromosorb-W packed stainless steel column (2 m x 2 mm). Oven programme consisted of 60°C (5 min), 60°-260°C (5°C/min), 260°C (10 min); carrier gas – nitrogen, flow rate 40 ml/min; injector temperature 240°C; detector temperature 240°C. GC-MS analysis of these oils were performed by splitless injection of 1.0 µl of each oil on a Helwett Packard 6890 gas chromatograph fitted with a

Table 1. Comparison of morphological characters of four species of *Hedychium*. See text for the species names.

Characters	***H. cor.***	***H. fla.***	***H. spi. ac.***	***H. ven.***
Shoot length (cm)	120 - 180	200- 260	100-140	100- 180
Leaf length (cm)	40-50	35-45	24-30	35-40
Leaf breadth (cm)	8-12	10-14	6-8	10-12
Leaf area (cm^2)*	206	258	187	210
Ligule length (cm)*	3.5	3.0	0.94	1.6
Petiole length (cm)*	1.0	1.5	1.0	3.5
Spike length (cm)*	10.3	15.6	19	23
Spike nature	Dense	Dense	Lax	Lax
Peduncle length (cm)*	1.5	1.5	2.0	2.0
Bract length (cm)*	6.0	5.0	2.3	2.5
Bract breadth (cm)*	3.1	2.5	1.0	1.0
Corolla lobe colour	White	Yellow	Yellow	Yellow
Staminode colour	White	Yellow	White	White
Lip length (cm)*	6.0	5.0	2.5	2.5
Lip shape	Obovate	Obovate	Oblanceolate	Oblanceolate
Lip colour	White	Yellow	White	White
Stamen colour	White	Yellow	Red	Red

*Values are mean of six measurements

cross-linked 5% PH ME siloxane HP-5 MS capillary column, 30 m x 0.32 mm, 0.25 µ coating thickness, coupled with a model 5973 mass detector. GC-MS operation conditions: injector temperature - 220°C; transfer line - 240°C; oven temperature programme - 60° to 243°C (3°C/min); carrier gas -He at 1.4 ml/min. Mass spectra: Electron Impact (EI^+) mode 70 eV, ion source temperature 240°C. Individual components were identified by Wiley 275.L database matching, comparison of mass spectra with published data and by comparison of their relative retention times. Morphological inter-relationship of the four species was established by calculating the Simple Similarity Coefficient (Sr.) (Table 2) using the following formula proposed by Sokal & Sneath (1963). The Sr. of chemical interrelationship was also calculated using the same formula (Table 3).

$$Sr = \frac{\text{common characters of two species}}{\text{common characters of two species} + \text{characters specific to one species} + \text{characters specific to other species}} \times 100$$

Results and Discussion

Morphological interrelationship based on Simple Similarity Coefficient of these four *Hedychium* species showed a maximum relationship between *H. spicatum* var. *acuminatum* and *H. venustum* with Sr. 36% (Table 2). The chemical inter-relationships between these four taxa were also determined based on the constituents identified from their rhizome oils by GC-MS analysis (Sabulal *et al*, 2007) (Table 3). *H. flavescens* is morphologically and chemically distinct from all others and showed maximum relationship to *H. coronarium* with Sr. 21% and 50% (Tables 2 & 3). The percentage of oil yields was calculated based on fresh weight of rhizomes. The oil yield was highest in *H. venustum* (0.16%) and lowest in *H. flavescens* (0.05%). The oil yields in *H. spicatum* var. *acuminatum* and *H. coronarium* were 0.13% and 0.13%, respectively.

Table 2. Simple Similarity Coefficient on morphological data between four species of *Hedychium* in South India. See text for the species names.

	H. cor.	*H. fla.*	*H. spi. ac.*	*H. ven.*
H. cor.	100	21	10	10
H. fla.		100	10	3
H. spi. ac.			100	36
H. ven.				100

Table 3. Simple Similarity Coefficient on chemical data between four species of *Hedychium* in South India. See text for the species names.

	H. cor.	*H. fla.*	*H. spi. ac.*	*H. ven.*
H. cor.	100	50	33	29
H. fla.		100	28	24
H. spi. ac.			100	66
H. ven.				100

Fifty-seven constituents (99.1%) were identified in the rhizome oil of *H. venustum*, forty-one constituents (99.2%) from *H. spicatum* var. *acuminatum*, twenty- five constituents (98.2%) from *H. flavescens*, and twenty-three constituents (97.5%) from *H. coronarium*. Thirteen constituents were found common in all the four species. The major constituents in these species were the monoterpenes 1,8-cineole, β-pinene and linalool, constituting 70-75% of these oils (Sabulal *et al*, 2007). 1,8-Cineole is the major constituent in three species, while β-pinene is the major one in *H. flavescens* (Fig. 1) showing the divergence of monoterpene metabolic pathways in *H. flavescens* in agreement with its morphological and chemical dissimilarity with others. Out of the twenty-five constituents identified from *H. flavescens*, sixteen are common to *H. coronarium* and thus, the Sr. on chemical data between the two is 50% (Table 3). Chemical relation of *H. flavescens* with *H. spicatum* var. *acuminatum* is 28% because, constituents found common to them are fifteen out of the total forty-one constituents identified from the latter and with *H. venustum*, it is only 24% (Table 3) as sixteen constituents are common out of the total fifty-seven. Lower percentage of β-pinene and higher percentage of 1,8-cineole in *H. spicatum* var. *acuminatum* and *H. venustum* (Fig. 1) support their close similarity in morphological and chemical inter-relationships. The monoterpenes, viz., myrcene, limonene, p-cymene, camphene and γ-terpinene, were identified from all the four taxa studied here (Fig. 2). These monoterpenes were reported as common constituents in all *Hedychium* species previously studied (Medeiros *et al*., 2003). The relative percentages of these monoterpenes are highest in *H. flavescens* (Fig. 2).

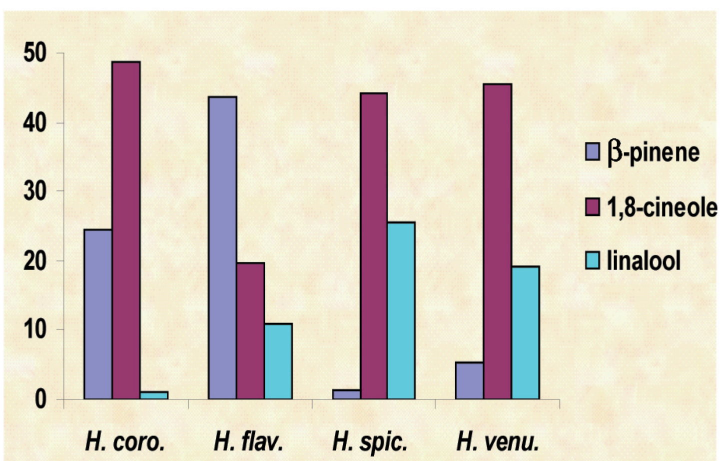

Figure 1. Percentage of major monoterpenes found in four species of *Hedychium* in South India.

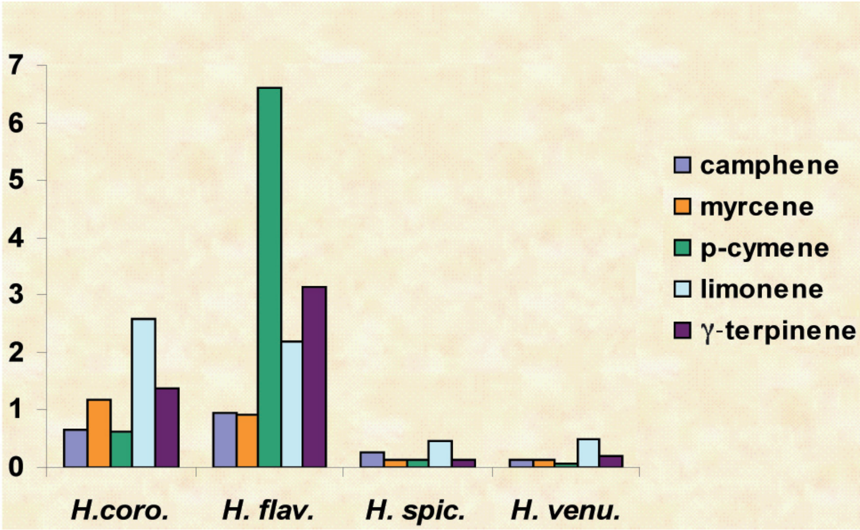

Figure 2. Percentage of minor monoterpenes common to four *Hedychium* species in South India.

Sesquiterpenes, with farnesyl pyrophosphate (FPP) as its precursor, are more evolved in the biosynthetic pathway than monoterpenes, with geranyl pyrophosphate (GPP), as its precursor. The sesquiterpene distribution is more dominant in *H. venustum* compared to others. The number and percentage of sesquiterpenes and their derivatives in *H. venustum* were 38 and 24.0%, respectively. The number and percentages of sesquiterpenes in *H. spicatum* var. *acuminatum*, *H. coronarium* and *H. flavescens* were 23 (22.1%), 4 (3.1%) and 6 (1.3%) respectively (Fig. 3). The role of essential oil constitunets as markers in chemotaxonomic studies is described by Hegnauer (1982) and very well proved by many botanists as well as chemists. Among these four south Indian taxa of *Hedychium*, *H. venustum* closely resembles *H. spicatum* var. *acuminatum* morphologically as well as in mono- and sesquiterpene distribution indicating their phylogenetic affinity. Thirty-nine constituents are common in the essential oils of these two species and resulted in 66% chemical similarity between them (Table 3). The current data also suggest that transformation of the farnesyl pyrophosphate to various sesquiterpenes by terpenoid synthases is most advanced in *H. venustum*, followed by *H. spicatum* var. *acuminatum*. The major monoterpene component, β-pinene, and relatively very low numbers of sesquiterpenes in the oil suggest the sharp difference in mono- and sesquiterpene pathways in *H. flavescens* and also indicate its primitiveness in phylogeny.

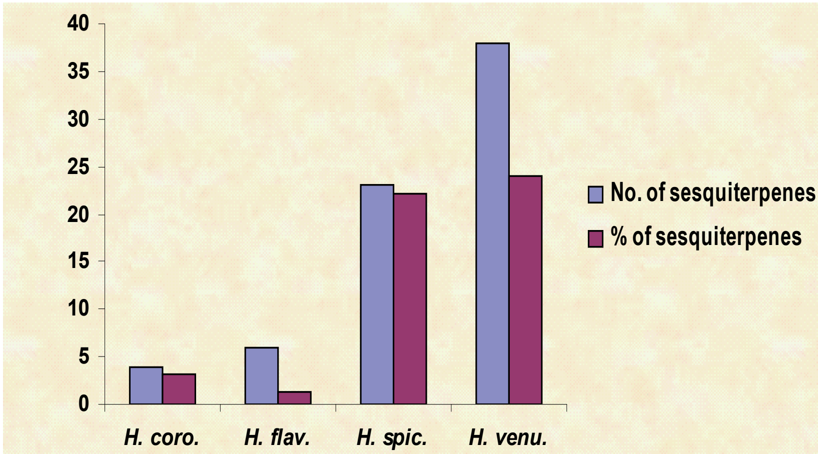

Figure 3. Number and percentage of sesquiterpenes found in four species of *Hedychium* in South India.

Conclusion

The major constituents of the rhizome oil of all the four south Indian *Hedychium* species are monoterpenes, viz., 1,8- cineole, β-pinene and linalool (70-75%). The inter-relationship of the four taxa based on chemical data is well in tune with that of morphological data. *H. venustum* is morphologically closer to *H. spicatum* var. *acuminatum* and they also show 66% chemical similarity to each other. *H. flavescens* is distinct from others morphologically as well as chemically. The species-specific chemical profiles of essential oils from these four *Hedychium* taxa are chemotaxonomically significant.

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