

GC–MS Characterization and Neurostimulatory Potential of Seasonal Rhizome Essential Oils of *Hedychium spicatum*: A Comparative Study

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Abstract- Essential oils are extremely important plant products that are made up of a complex mixture of volatile substances that are naturally present in the plant. These oils have reportedly been shown to have a variety of biological effects, giving rise to the new product category known as aromaceuticals. The prevalence of mental health conditions including depression, anxiety, sleeplessness, and stress has grown along with increasing standards of life. As a result of their widespread usage in the treatment of certain ailments, essential oils have seen an increase in popularity. Therefore, the present study evaluated the central nervous system (CNS) stimulant activity of essential oils extracted from the rhizomes of *Hedychium spicatum* collected during winter and summer seasons. Essential oils were extracted by hydro-distillation and evaluated for physicochemical parameters including specific gravity, viscosity, refractive index, acid value and saponification value. Chemical profiling was carried out using Gas Chromatography–Mass Spectrometry (GC–MS). CNS activity was assessed in rodent models and

compared with the standard stimulant drug caffeine (100 mg/kg body weight). GC–MS analysis revealed the presence of several bioactive constituents. Evaluation of locomotor activity using an actophotometer demonstrated a marked increase in CNS activity. Essential oils obtained from winter season (HS-1) and summer rhizomes (HS-2) exhibited locomotor activity values of 92.14% and 93.33%, respectively, compared with 97% observed for caffeine. Acute toxicity studies indicated that both oils were safe up to the tested dose levels. The findings suggest that essential oils of wild *Hedychium spicatum* possess significant CNS stimulant activity and may serve as promising natural bioactive products for further pharmacological investigations.

Keywords: *Hedychium spicatum*, Essential oil, Physico-chemical properties, GC–MS, CNS stimulant activity, Aromaceuticals

Introduction

Essential oils are complex mixtures of volatile secondary metabolites produced by aromatic plants and are recognized for their characteristic fragrance and

diverse biological activities. These oils are rich sources of physiologically active compounds and have attracted considerable attention owing to their therapeutic applications. Previous studies have reported antimicrobial, antiviral, antioxidant, anti-inflammatory, antinociceptive and antitumor activities associated with various essential oils.

The growing prevalence of stress related disorders and increasing demand for natural health products have resulted in renewed interest in essential oils and aromaceuticals. Essential oils are increasingly incorporated into complementary healthcare systems due to their ability to influence physiological and psychological responses. The study of physicochemical characteristics provides valuable information regarding the quality, purity and commercial applicability of essential oils. Parameters such as colour, specific gravity, viscosity, refractive index, acid value and saponification value are widely employed as quality indicators and play an important role in determining the suitability of oils for pharmaceutical and industrial applications. The family Zingiberaceae is well known for its medicinally important species. *Hedychium spicatum* Sm. is a perennial rhizomatous herb belonging to the family Zingiberaceae (ginger family). It is commonly known as Kapurkachri (Indian trade name), Spiked Ginger lily and Van-haldi, (Chopra,). It is a leafy plant up to 1–2 m tall having strong aromatic horizontal rhizomes. Flowers are fragrant, off white with orange-red base, appearing in dense terminal spikes 15-25 cm at the top of stem and hence the name spiked ginger lily. Flowering

season is July to August. Leaves are about 30 cm or more in length, oblong, lanceolate, very variable in breadth and glabrous^[2-3]. Rhizomes are 15-20 cm long; 2.0-2.5 cm in diameter, externally yellowish-brown, but change to dark brown on storage⁴. (Honda) *H. spicatum* is found in the entire Himalayan region. It occurs throughout subtropical Himalayas in the Indian state of Assam, Arunachal Pradesh and Uttarakhand within an altitudinal range of 1800-3000 m a.s.l.^[5]. The plant forms clumps amongst moist rocks outside forest or on the borders of cultivated fields. The plant also found in Western and Central Himalayas at an altitude of 1000-2300 m a.s.l.^[6-7].

Traditionally, the rhizomes are used as stomachic, carminative, tonic and stimulant^[8]. The rhizomes are reported to be boiled and eaten with salt, roasted powder is used in asthma, and decoction of rhizome with sawdust is used in tuberculosis^[9]. Tuberous roots are reported to be edible^[10]. Fruit of the species is cooked and eaten with lentils in savory dishes^[11]. The rhizomes are white and starchy within and are employed in the preparation of Abir, a fragrant colored powder used during holi festival and in religious ceremonies. They are considered to have insect repelling properties and are used for preserving clothes. They may be employed as an auxiliary in dyeing to impart a pleasant smell to fabrics. They are used with henna to produce perfumed cloth known locally as Malagiri cloth^[12]. Rhizomes have bitter camphor-like taste and a strong aromatic odor, with a wide application in incense and fragrant preparations. They enter into the

preparation of cosmetic powders used for promoting hair growth. They are used in Bengal, after frying or mixing with other ingredients as chars or perfumed baits for fish. They are much used in veterinary medicine. The powdered rhizome in divided doses is used in conditions like bronchial asthma, cough, chest heaviness, sleeplessness, loss of appetite and pulmonary eosinophilia. The rhizomes are also used in dyspepsia, diarrhea, piles, liver complaints, ulcers, skin diseases and rheumatoid arthritis. The essential oil of the rhizome is used to check seed-borne diseases of crops and has mild tranquilizing activity. The rhizomes showed significant analgesic, anti-inflammatory, cytotoxic, antimalarial and pediculicidal activity. It also acts as antidote for snakebite. It is used in conditions like poor circulation due to thickening of blood vessels. It forms one of the ingredients of herbal vanishing cream^[13].

Material and Methods

Plant Material- *Hedychium spicatum* rhizomes were collected in winter and summer seasons through the year from Bataghat (on the way from Mussoorie to Dhanaulti), Uttarakhand, India, at an altitude, latitude and longitude ranging from 1900 m, 30.44984° N, 78.15848° E - 2000 m 30.45014° N, 78.15867° E. The identification and authentication of targeted specie was done by Systematic Botany Division, Forest Research Institute, Dehradun, Uttarakhand, India. Herbarium registration of *Hedychium spicatum* Sm. is 172345. Rhizomes were cut into small pieces and oven dried at 60–80°C for 48 hours. The dried materials were stored at room tempe-

ature until use.

Extraction of Essential Oil- Dried rhizomes collected during winter (300 g) and summer (600 g) seasons were subjected to hydro-distillation using a Clevenger-type apparatus for 6–8 hr. The volatile oil fractions obtained were collected, dried over anhydrous sodium sulfate and stored at 4–6°C until further analysis.

Physicochemical Evaluation of Essential Oils - Physicochemical characteristics provide a baseline assessment of essential oil quality and suitability. These characteristics of both oils HS-1 & HS-2 studied were colour, Specific gravity, Specific Viscosity, Refractive index, Acid number and Saponification number, ester value and glycerol percentage.

Acute Toxicity Study - This study examines the acute toxicity of five groups (n = 5) of male albino mice. Animals from all groups were fasted overnight before receiving a single dosage of samples (HS-1, HS-2) at doses of 50, 100, 150, 250, 500, 1000, 1500, and 2000 Mg/Kg body weight (P.O.) from the various groups. The behaviour of the animals was monitored for 72 hours following extract administration in a group of mice that received an identical volume of P.B.S. Animals were monitored for 14 days for any indication of toxicity and death. (Table-4)

GC-MS Analysis of the Extracted Essential Oils- Gas chromatography mass spectrometry (GC-MS) was used to analyse the extracted essential oils. The operational parameters listed below were standardised and applied: DB-5 ms capillary GC column split injection

mode (50:1), ultra-inert 5% Phenyl 95% dimethylarylene (30 m x 0.25 mm x 0.25 m), and helium as the carrier gas. When the analysis is complete, the injector temperature is increased to 240°C at a rate of 3°C per minute from the initial oven temperature of 50°C. Using a mass selective detector and electron impact ionisation (EID) at an energy of 70 eV, the eluted analytes were found.

Identification of Compounds: Chemical components were identified by comparing their mass spectra to those in the NIST and F&F libraries, those reported by Adams, and data from the MS literature, as well as by comparing their Kovats/Retention index with standard C7-C40 alkanes.

Neurostimulatory Potential (locomotor activity/CNS)- The actophotometer, which uses photoelectric cells and a counter to work, may be used to quickly assess the Neurostimulatory activity (locomotor activity/CNS)¹³ A count is made whenever a light beam strikes the photocell while it is on an animal. In an actophotometer, the animal can move in either a square or circular region. Rats and mice may both be utilised for testing this apparatus. Wistar rats of both sexes, weighing between 150 and 250 grams with a variation of ± 2.0 grams, were used. The rats were individually housed in elastic cages with wire tops prior to the start of the experiment. Before the study, all animals were fasted for at least 12 hours, with access only to water.

The rats were divided into groups of five animals each. Each rat was weighed individually and marked to distinguish one from another. The equipment used

for the study was checked to ensure proper functioning, and the photocell counter was set up to record the activity of each group for a duration of 10 minutes. At the end of the counting period, each group of rats was removed from the chamber. The drugs used in the study were administered orally according to the following schedule. After administration, the rats were retested for activity scores after 60 minutes, again for a duration of 10 minutes. The drug dose used was 100 mg/kg of body weight for the rats, and the reference drug used was caffeine at a dose of 100 mg/kg of body weight. Animals were divided into 5 groups and received different drugs as Control group receiving Tween 80, Standard groups receiving Caffeine and Test groups receiving HS-1&HS-2. **Table-4**

CNS motor activity was calculated as per the following formula: -

$$\% \text{ CNS activity} = \frac{\text{Initial no. of counts} - \text{Final no. of counts}}{\text{Initial no. of counts}} \times 100$$

Statistical Analysis- All data were expressed as mean SEM $\pm$ wherever applicable, the data were analysed statistically by student's t- test, using graph pad instant version 2.05a and one way ANOVA. The level of significance was $p < 0.05$ and n represents five per groups.

Results

Hydro-distillation yielded pale yellow coloured essential oils from both leaves and rhizomes. Physicochemical parameters are summarized in Table 1 Chemical composition is presented in Table-2, acute toxicity studies in Table-3, and neurostimulatory activity in Table-4.

Table-1 Physicochemical Characteristics of Essential Oils

Season	Yield (%)	Colour	Specific Gravity	Specific Viscosity	Refractive index	Saponification Value (mg/g)	Acid value (mg/g)	Ester value (mg/g)	Glycerol (%)
HS-1 Winter	1.16 ^a ± 0.012	Pale Yellow	.986	0.195	1.543	54.00 ^b ± 2.88	0.21 ^c ± 0.03	53.79 ^b ± 2.97	2.90 ^b ± 0.17
HS-2 Summer	1.64 ^c ± 0.018	Light pale yellow	.987	0.198	1.534	22.00 ^a ± 3.05	0.04 ^a ± 0.01	21.95 ^a ± 3.05	1.20 ^a ± 0.29
F-value	88.59					12.667	9.60	12.481	12.489
Sig. level (P-value)	0.000 (S)					0.002 (S)	0.005 (S)	0.002 (S)	0.002 (S)

Table-2 Chemical Composition of Essential Oils

Peak	RT	Chemical constituents		CKI	AKI	
		HS-1	HS-2			
1	6.187	1.97	0.84	α -Pinene	936	939
3	7.277		0.18	Sabinene	966	975
4	7.365	3.14	1.12	β -Pinene	969	975
5	7.812	0.11		β -Myrcene	986	990
7	9.022	14.83	21.81	1,8-Cineole	1026	1038
8	9.735	0.35	0.11	γ -Terpinene	1054	1059
9	10.102	0.11	0.16	cis-Sabinene hydrate	1067	1070
10	11.001	3.02	5.22	Linalool	1095	1096
11	11.359	0.07	0.11	Unidentified	1110	
12	12.97	0.38	0.44	Unidentified	1166	
13	13.135	0.81	0.83	Terpinene-4-ol	1174	1167
14	13.587	1.42	1.93	α -Terpineol	1186	1178
15	17.351	0.09	0.08	Unidentified	1325	
16	17.756	0.23	0.16	α -Cubebene	1340	1351
17	18.477	0.33	0.21	α -Copaene	1368	1376
18	18.813	0.23	0.17	β -Cubebene	1335	1326
19	18.864	0.17	0.19	β -Elemene	1383	1390
21	19.567	0.92	0.84	E- Carophyllene	1410	1417
22	19.841	0.11		β -copaene	1421	1432
23	20.158		0.12	Guaia-6,9-diene	1434	1444
24	20.251	0.12	0.14	Cadina-3,5-diene	1438	1446
25	20.325	0.24	0.29	cis-Murolene,3-5-diene	1441	1450
26	20.465	0.81	0.73	α -Humulene	1446	1454
27	20.558	0.92	0.76	Alloaromadendrene	1450	1458
28	20.637	0.26	0.21	cis-Murola-4(14),5-diene	1453	1466
29	20.912	0.13	0.08	trans-cadina-1(6),4-diene	1464	1476
30	20.991	0.63	0.42	γ -Murolene	1467	1479
31	21.112	0.35	0.24	Germacrene-D	1472	1481
32	21.299	0.13	0.1	Unidentified	1490	
33	21.364	0.41	0.27	Unidentified	1482	
34	21.499	1.24		4-Epicubebol	1488	1493
35	21.582	0.91	0.65	α -Murolene	1492	1500

36	21.731	0.31	0.6	BHT	1497	1514
37	21.927	2.41	1.78	γ -cadinene	1505	1513
38	21.983	1.35	1.02	Cubebol	1507	1515
39	22.085	4.63	3.8	δ -Cadinene	1512	1523
40	22.327		0.17	δ -Amorphene	1522	1512
41	22.378	0.47	0.18	cis-cadina-1,4-diene	1524	1533
42	22.476	0.35	0.3	α -Cadinene	1528	1538
43	22.514	0.23	0.23	α -Calacorene	1530	1545
44	22.849	7.05	10.07	α -Elemol	1544	1549
45	23.081	0.14	0.12	β -Calacorene	1554	1565
46	23.17	0.99	1.01	E-Nerolidol	1558	1563
47	23.44	4.7	4.65	Germacrene-D-4-ol	1547	1575
48	23.514	0.38	0.55	Caryophyllene oxide	1572	1583
49	23.71	0.7	0.68	Unidentified	1581	
50	23.905	0.24	0.23	Unidentified	1589	
51	24.026	0.22	0.22	Ledol	1594	1602
52	24.143	1.28	0.69	Unidentified	1599	1602
53	24.306	0.37	0.49	Unidentified	1606	
54	24.455	5.94	0.88	10-epi- γ -Eudesmol	1613	1622
55	24.594	0.48		Hinesol	1619	1640
56	24.706	3.91	4.17	γ -Eudesmol	1624	1618
58	25.037	8.06	8.27	τ -Muurolol	1638	
60	25.33	18.79	17.49	α -Cadinol	1651	1652
61	25.6	0.31		Unidentified	1664	
62	26.154		0.19	Unidentified	1689	
63	26.214	0.18		Unidentified	1691	
64	37.625	2.17	3.45	Unidentified	2284	
65	38.197		0.45	Unidentified	2418	

Table-3 Acute Toxicity Studies

Treatment	Dose (mg/kg PO)	No. of Animal	No. of Animal Dead	No. of Animal Survive	% dead animals
HS-1	50	5	0	5	0
	100	5	0	5	0
	150	5	0	5	0
	250	5	0	5	0
	500	5	0	5	0
	1000	5	0	5	0
	1500	5	0	5	0
	2000	5	0	5	0
HS-2	50	5	0	5	0
	100	5	0	5	0
	150	5	0	5	0
	250	5	0	5	0
	500	5	0	5	0
	1000	5	0	5	0
	1500	5	0	5	0
	2000	5	0	5	0

Table-4 Neuro-stimulatory Activity of Essential Oils

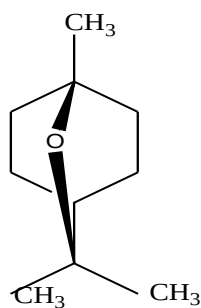
Treatment	Before	After	% Increase in Activity
Control	42.00 $\pm$ 0.21	42.33 $\pm$ 0.25	0.70
HS-1	42.00 $\pm$ 0.51	80.70 $\pm$ 1.16	92.14 $\pm$ 0.99
HS-2	42.66 $\pm$ 0.94	82.50 $\pm$ 1.66	93.33 $\pm$ 0.87
Caffeine (100 mg/kg)	42.33 $\pm$ 0.47	83.33 $\pm$ 0.78	97.00 $\pm$ 0.89

Discussion

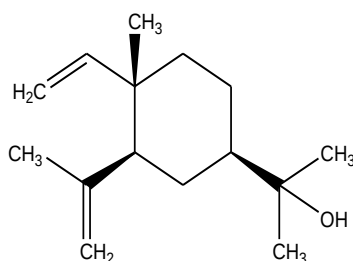
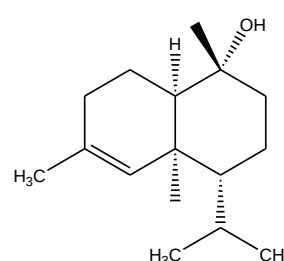
Physicochemical properties such as density, refractive index, viscosity, acid value, saponification value and chemical composition are important indicators of the quality and purity of essential oils. These parameters significantly influence stability, storage behaviour and commercial applicability.

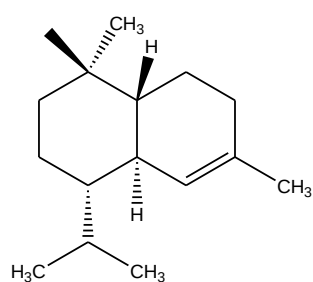
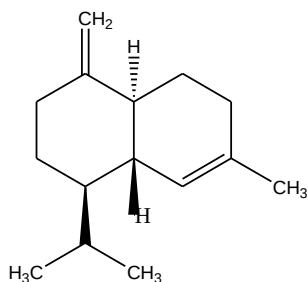
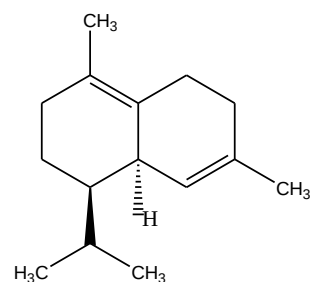
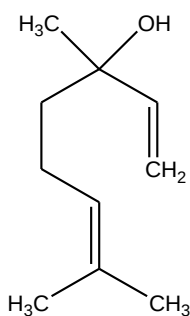
Density and refractive index are widely accepted markers of purity, while viscosity and acid value provide useful information regarding quality and shelf stability. Understanding these properties helps distinguish authentic essential oils from adulterated products. The essential oils compositions of *H. spicatum* in winter & summer seasons HS-1 and HS-2 were analyzed by GC-MS and total 65 peaks were identified as given in table 3.

In HS-1 55 compounds were present, out of which 43 compounds were identified which constitute 93.67% area and rest 6.33% remains unidentified. Out of the total identified compounds α -cadinol (18.79%), 1, 8-cineole (14.83%), τ -muurolol (8.06%) and α -elemol (7.05%) were major. In HS-2 season, 54 compounds were present, out of which 42 compounds were identified which constitute 92.82% area and rest 7.18% remain unidentified. The major chemical constituents were 1, 8-cineole (21.81%), α -cadinol (17.49%), α -elemol (10.07%) τ -muurolol (8.27%). Figure-1 Acute toxicity studies demonstrated a favourable safety profile, as neither oil produced mortality or observable toxic effects even at doses up to 2000 mg/kg body weight.

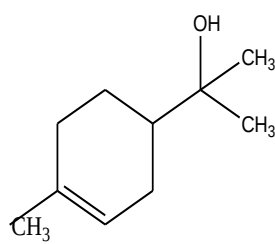
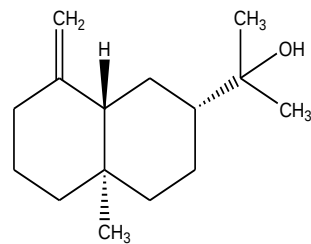
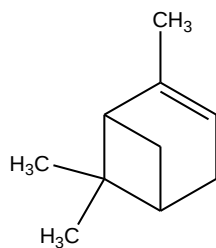
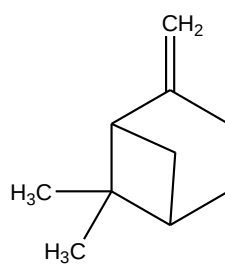
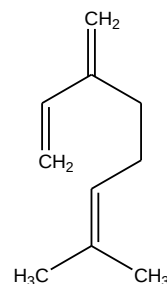
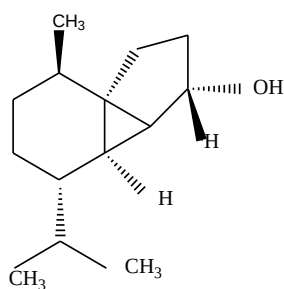


1, 8-Cineole

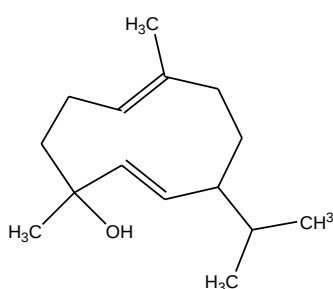
 α -Elemol τ -Muurolol

 α -Cadinol γ -Cadinine δ -Cadinene

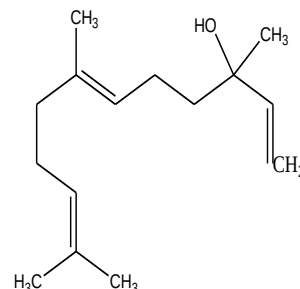
Linalool

 α -Terpineol γ -Eudesmol α -Pinene β -Pinene β -Myrcene

E-Cubebol



E-Nerolidol



Germacrene D-4-ol

Figure-1 Structures of major constituents identified in the rhizome essential oils of *Hedychium spicatum*.

Essential oils influence the nervous system primarily through olfactory path-ways. Upon inhalation, volatile molecules stimulate olfactory receptors, transmitting signals to the olfactory bulb, limbic system and hypothalamus. This interaction can modulate neuro-transmitter release and alter physio-logical and behavioural responses. The present study evaluated CNS activity using locomotor behaviour as an indicator. Increased locomotor activity is generally associated with enhanced alertness and CNS stimulation, whereas decreased activity reflects CNS depression. The results clearly indicate significant CNS stimulant activity for both essential oils. HS-1 and HS-2 produced locomotor activity increases of 92.14% and 93.33%, respectively, compared with 97% for caffeine. These findings suggest that the major phytochemical constituents present in the oils may contribute to their observed neuro-stimulatory effects.

Conclusion

The present study demonstrates that the rhizome essential oils of *Hedychium spicatum* exhibit significant seasonal variations in their physicochemical characteristics and chemical composition. GC–MS analysis revealed the presence of a diverse array of bioactive constituents, with 1,8-cineole, α -cadinol, α -elemol, and τ -muurolol identified as the major compounds in both winter and summer oils. Seasonal fluctuations influenced the relative abundance of these constituents, indicating that environmental conditions play an important role in determining oil quality and composition.

The physicochemical parameters obtained in the study were within acceptable limits, confirming the purity and quality of the extracted oils. Acute toxicity evaluation

further established their safety, as no mortality or adverse toxic effects were observed at doses up to 2000 mg/kg body weight. Assessment of central nervous system activity revealed that both seasonal oils significantly enhanced locomotor activity in experimental animals. The winter (HS-1) and summer (HS-2) oils produced increases in locomotor activity of 92.14% and 93.33%, respectively, which were comparable to the effect of the standard stimulant caffeine (97%). These findings suggest that the essential oils possess notable neurostimulatory properties, likely attributable to their rich content of oxygenated mono-terpenes and sesquiterpenes, particularly 1,8-cineole and related constituents.

Overall, the results validate the traditional importance of *Hedychium spicatum* and highlight its potential as a valuable natural source of bioactive essential oils for aromaceutical, pharmaceutical, and wellness applications. Further investigations focusing on the isolation of active constituents, elucidation of their mechanisms of action, and clinical evaluation are warranted to fully exploit the therapeutic potential of this Himalayan medicinal plant.

Conflict of Interest

The authors declare that there is no conflict of interest.

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