EFFECT OF SOIL PARAMETERS ON RHIZOME CHEMICAL PROFILES OF DIFFERENT ACCESSIONS OF *HEDYCHIUM SPICATUM* SW. GROWN IN UTTARAKHAND, INDIA

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ABSTRACT

Hedychium spicatum Sw. is utilized in treating several ailments due to its medicinally rich rhizome. Present study aims to analyze the effect of soil parameters on *H. spicatum* rhizome essential oils (HSREOs). Ten accessions of *H. spicatum* rhizomes were collected from various locations of Uttarakhand. HSREOs were extracted by hydro-distillation using a Clevenger-type apparatus and characterized by GC and GC-MS. Results revealed identification of 89.74-94.96% of HSREOs, composed of oxygenated monoterpenes (32.4-80.46%) followed by oxygenated sisquiterpene (2.08-38.59%), sisquiterpene hydrocarbon (0.26-19.84%) and monoterpene hydrocarbon (1.8-19.47%). Dominant oil chemicals were: 1,8-cineole, linalool, τ-cadinol, δ-cadinene, cubebol, γ-eudesmol, sabinene, germacra-4(15),5,10(14)-trien-1-α-ol, limonene and cis-γ-bisabolene. Pearson's analysis of soil effect on HSREOs indicated positive correlation of soil EC, Mn and K with oils' limonene; Mn and K with linalool and negative correlation of OC with limonene; K with 1,8-cineole and Zn with eudesmol. Thus, a quantitative effect on oils' composition was observed due to soil variation. To our knowledge, this is the first report on soil influence on rhizome essential oils *H. spicatum*.

Key words: *Hedychium spicatum* Sw., rhizomes, soil parameters, GC and GC-MS.

1. INTRODUCTION

Raw material dependence of traditional medicinal system as well as modern pharmaceutical industries on aromatic and medicinal plants (AMPs) is due to their pharmacological properties. This reliance can be judged by the fact that 85% of the traditional medicinal system and 70% of modern medication therapies use either extract or essential oils of these plants. Thus, studying various aspects of AMPs is always a matter of interest of scientific communities (Singh et al., 2008).

Hedychium spicatum Sw., a perennial rhizomatous herb from Zingiberaceae family, is a near endemic species of Himalayan region with wide distribution in South-east counties, Japan, China, Bhutan, India, Nepal, Pakistan and Madagascar. In India, all Himalayan states with sub-tropical and temperate habitats witness its extensive availability within an altitude range of 1000-2800 m above sea level, generally in moist soil (S. Rawat et al., 2018). It is commonly called as "Spiked Ginger lily", in Ayurvedic literature as "Shati", in Hindi as "Kapoor Kachari" and in vernacular language as "Van Haldi". The plant is approximately 2 m in height with broadly lanceolate leaves and fleshy, bitter tasted and camphor-like aromatic rhizomes (Rasool & Maqbool, 2019). The fragrant chemicals of rhizomes have applications in food, cosmetic & perfumery and pharmaceutical industries. Traditionally, the rhizomes were used to cure bronchitis pains, hiccough, vomiting, skin diseases, liver complaints and asthma etc. (S. Rawat et al., 2018). Several studies on secondary metabolites from various vegetative parts of *H. spicatum* investigated their applications as biological and pharmacological agents, *viz.* antioxidant, anti-inflammatory, antimicrobial, insecticidal, and antitumor etc. (A. Rawat et al., 2024; Rasool & Maqbool, 2019; S. Rawat et al., 2011).

Previous studies observed qualitative and quantitative variations in *H. spicatum* rhizomes essential oils (HSREOs) due several factors viz. altitude, different vegetative parts and plant harvest season etc.(Gangwar et al., 2024; A. Rawat et al., 2024; S. Rawat et al., 2020; S. Rawat et al., 2014;Verma & Padalia, 2010). To our knowledge, there is no systematic study exploring effect of soil characteristics on chemical profile of *H. spicatum* rhizomes. This research aims at collection, characterization and chemical profiling of rhizomes of *H. spicatum* from various locations and studying effect of soil parameters on quality and quantity of HSREOs. As the

overexploitation of wild population of this species for perfumery EO has put it in rare and vulnerable categories, this work would help in exploring environmental conditions utilized for developing conservational efforts of this species.

2. MATERIALS AND METHODS

2.1. Samples collection

Ten wild accessions of *H. spicatum* rhizomes were collected from various locations in October 2024 (Fig. 1). Plant was initially classified and identified by dichotomous key and the identification was further confirmed by online database Https://Powo.Science.Kew.Org/, 2024.

2.2. Essential oils extraction

Freshly collected rhizomes sample populations were cleaned with distilled water, weighed and then subjected to hydro-distillation for 5 h using Clevenger-type apparatus for volatile oils' extraction (Clevenger, 1928). The samples were hydro distilled twice. The percentage content of obtained essential oils was calculated on the basis of weight of fresh rhizomes.

$$\text{Oil yield } \% = \frac{\text{volume of extracted EO (ml)}}{\text{fresh weigh of plant sample (g)}} * 100\ \%$$

The oil samples were dried over anhydrous Na_2SO_4 and stored in sealed vials under refrigeration at 4^oC for further analysis.

2.3. Individual compound identification

Gas Chromatography (GC) A Nucon 5765 gas chromatograph equipped with dual flame ionization detector (FID), using two different stationary phases, Rtx-5 (30 m × 0.25 mm i.d., 0.25 μm film coating) and CP-Wax 52 CB (30 m × 0.32 i.d., 0.25 μm film thickness) fused silica columns were used for gas chromatographic (GC) analysis of volatile oils. Nitrogen and hydrogen were the carrier gases at 1.0 ml/min in nonpolar and polar columns, respectively. Temperature programming was from 70°C to 230°C at 4°C/min (CP-Wax 52 CB) and from 60°C to 210°C at 4°C/min (Rtx-5) with the initial hold time of 2 min. The injector and detector temperatures were 210°C and 220°C, respectively. The injection volume was 0.02 μl neat or 0.1 μl in hexane and split ratio was 1:30.

Gas Chromatography-Mass Spectrometry (GC-MS) For GC-MS analysis of oil samples, Agilent 5977B EI/CI MS instrument with model Agilent 8890/5977B Series II triple-axis detector with high energy dynode and long-life electron multiplier (EM) Auto sampler was used. The spectrometer was fitted with a DB-5MS (30 m X 0.25 mm X 0.25 μm) column. 1.0 μL of sample prepared in *n*-hexane was injected by splitless injection technique with injection temperature at 250°C. The oven temperature was programmed 50-300°C at 5°C/min using helium as carrier gas with a flow rate 30 ml/min. Mass spectra electron impact (EI) mode were taken at 70 eV with mass scan range of 30–550 amu.

Retention index (RI) Individual compounds were identified on the basis of their Retention Index (RI), determined experimentally with reference to homologous series of *n*-alkanes C9–C24 (Polyscience Corp., Niles, IL, USA) and confirmed by combining the MS Library search (NIST 20.L) with MS literature data (Adams, 2017).

2.4. Statistical analysis

Influences of soil on EOs' constituents were studied by Pearson's correlation analysis using IBM SPSS Statistics for Windows, Version 16.0 software (IBM Corp. 2007).

3. RESULTS AND DISCUSSION

3.1. Yield and color of HSEOs

The oil content of HSREOs found to be varying from 0.17% to 0.47% on basis of weight of fresh rhizomes and the appearance of oils was pale yellow to yellow and very light brown (Table 1). Highest yield of 0.47% was obtained from yellow color volatile oil extracted from HS 7 accession. Lowest yield was of 0.17% was recorded from HS 9 accession oil appeared light brown in color. Similar result was also observed in a previous study from Ramgarh (altitude 2100 m asl), Uttarakhand with yield of fresh *H. spicatum* rhizomes to be 0.18% (Gangwar et al., 2024). Other researches from similar geographical region observed higher yield on the basis of dry rhizomes weight (Bharadwaj et al., 2019; Raina & Negi, 2015). Thus, the observations in the present work related to yield and appearance of HSREOs were in good agreement with previous studies on this herb.

3.2. Composition analysis of HSREOs

GC and GC-MS analysis of HSREOs compositions revealed that 89.74-94.96% of the oils were identified with dominant compounds found to be: 1,8-cineole (8.96-55.41%), linalool (4.23-70.24%), τ-cadinol (0.52-17.94%), δ-cadinene (0.05-13.87%), cubebol (0.11-10.49%), γ-eudesmol (0.21-8.26%), sabinene (0.94-8.05%), germacra-4(15),5,10(14)-trien-1-α-ol (0.03-7.96%), limonene (0.03-5.68%) and cis-γ-bisabolene (0.07-5.24%), along with minor compounds (Table 2). Among the different accessions; HS 3, HS 4, HS 5, HS 6, HS 7, HS 8 and HS 10 essential oils had 1,8-cineole (26.49-55.41%) as most dominant chemical with low amount of linalool. Several previous studies conducted on the chemical composition of *H. spicatum* EO grown in the identical geographical region found 1,8-cineole to be most abundant compound of the volatile oil followed by other major compounds (Verma & Padalia, 2010; Raina & Negi, 2015; S. Rawat et al., 2020). However, HS 1 and HS 9 EOs constituted of a high percentage of linalool (51.18-70.24%) and a relatively lower amount of 1,8-cineole (8.96-17.54%). Earlier studies on HSREO composition from this region have not reported linalool chemotype of this species. In case of HS 2 accession, an almost comparable amount of linalool (34.52%) and 1,8-cineole (31.5%) were obtained. A. Rawat et al., (2024) observed a comparable amount of linalool and 1,8-cineole from Tehri and Pithoragarh accessions of Uttarakhand (HS-THE: linalool 29.9% & 1,8-cineole 16.8% and HS-PIT: linalool 10.1% & 1,8-cineole 5.8%).

Thus, based on chemical compositions analysis of HSREOs, ten accessions of *H. spicatum* could be grouped into three categories viz. first category with 1,8-cineole dominated accessions (HS 3, HS 4, HS 5, HS 6, HS 7, HS 8 and HS 10), second category with linalool dominated accessions (HS 1 and HS 9) and third category accession was HS 2 having almost comparable amount of linalool and 1,8-cineole. As mentioned earlier, previous research works on essential oil of *H. spicatum* from Uttarakhand Himalaya already reported 1,8-cineole dominated HSREO as well as linalool and 1,8-cineole dominated HSREO. However, linalool dominated HSREO was reported for the first time in the present research.

Table 2 also revealed that *H. spicatum* essential oils was dominated with oxygenated monoterpenes (OM) (32.41-80.46%) followed by oxygenated sisquiterpene (OS) (2.08-38.59%), sisquiterpene hydrocarbon (SH) (0.26-19.84%) and monoterpene hydrocarbon (MH) (1.8-

19.47%). Koundal et al., (2015) also found that in the essential oil of fresh *H. spicatum* rhizomes collected from Himanchal Pradesh, India, the largest contributor was OM (57.6–68.0%) followed by OS (9.9–25.3%). Similarly, chemical class composition of essential oils of H. spicatum rhizomes obtained by Raina & Negi, (2015) was rich in OM (18.3-75.7%), followed by OS (8.1-43.8%), SH (1.6-25.3%) and MH (0.9-10.0%). Present work further observed that monoterpene hydrocarbons constituted of α-pinene, sabinene, β-pinene, limonene, and γ-terpinene. Oxygenated monoterpenes were 1,8-cineole, cis-sabinene hydrate, linalool, trans-sabinene hydrate, terpinen-4-ol and α-terpineol. Sisquiterpene hydrocarbons were isolongifelene, β-caryophyllene, β-acoradiene, γ-muurolene, germacrene D, β-guaiene and cis-γ-bisabolene. Oxygenated sisquiterpenes were cubebol, δ-cadinene, elemol, ledol, γ-eudesmol, τ-cadinol, α-eudesmol, α-cadinol, 14-hydroxy-(Z)-caryophyllene, germacra-4(15),5,10(14)-trien-1-α-ol and 8-cedren-13-ol.

Furthermore, by comparing the chemical composition of EOs of *H. spicatum* rhizome samples, present work observed a quantitative variation in their chemical composition. However, no qualitative change was observed in oils' composition. Previous studies on volatile oil composition of various aromatic plants also investigated quantitative variations in oil constituent, supporting the results of present study (Padalia et al., 2014; Loya et al., 2023). There are several genetic or/and biotic and abiotic environmental factors such as altitude, soil nutrient level, physical nature of soil, rainfall, temperature, exposure to sunlight etc. responsible for variations in EO yield and phytochemicals quantity of plants' secondary metabolites as found in various previous studies (Fellah et al., 2018; Negi et al., 2020; Moghaddam et al., 2024). Thus, analysis of effect of these factors might lead to identify an ideal condition of growing these aromatic medicinal herbs with desirable constituents and high yield of their essential oils.

3.3. Soil physicochemical parameters

Various soil physicochemical characteristics were analyzed, where soil samples were collected from ten different sampling sites (Table 3). The values of calculated soil parameters were represented with standard deviation value. The soil properties were found to be varying across the experimental accessions.

In the case of soil texture; soil sand content varied from 67% to 75%, the lowest being for HS 7 and HS 8 (67%) and the highest being for HS 1 (75%). The silt content of the soil fluctuated from 18% to 23%, with HS 1 soil composed of the minimum silt content. Moreover, comparatively low clay content of 5-10% across all sites was observed with a minimum amount for HS 10 soil. Thus, soil texture across all sampling sites was predominantly sandy.

In the case of soil pH, EC, WHC, and OC; the variation of soil pH from the lowest value of 2.67 (for HS 8 associated soil) to the highest value of 7.2 (HS 2 associated soil) indicated that the nature of the soil across all sampling sites was highly acidic to almost neutral. Water holding capacity (WHC) fluctuated from 25.41% (HS 1) to 89.77% (HS 2), showing a striking variation across all selected sites. Small variations in soil electrical conductivity (EC) from 0.025-0.059 dSm^{-1} with lowest value for HS 7 & HS 8 and highest value for HS 1 indicated non-saline soil conditions along all sampling sites. Soil organic carbon (OC) content ranged from 2.66% to 5.24%, with HS 1 observing the lowest soil OC, whereas HS 10 recorded the highest soil OC value.

In the case of micronutrients; manganese (Mn) varied from 1.22 to 2.36 mg kg^{-1}, with the lowest Mn amount in the HS 9 soil sample and the highest concentration in the HS 1 soil sample. Iron (Fe) content in soil ranged from 2.12 to 4.83 mg kg^{-1}**,** with a minimum value at HS 9 and maximum values at HS 1. Copper (Cu) proportion in soil varied from 0.09 (for the HS 10 soil sample) to 0.22 mg kg^{-1} (for the HS 2 sample). Zinc (Zn) concentrations ranged from 0.38 to 0.53 mg kg^{-1}**,** with the minimum Zn amount in HS 3 soil and the maximum Zn amount in HS 10 soil. Overall, low levels of changes were observed for all micronutrients across all sampling sites.

In the case of available macronutrients; available nitrogen (N) content varied from 105.01 to 200.7 kg ha^{-1} with the lowest value for HS 9 soil and highest for HS 10 soil, indicating considerable fluctuation in soil N across the selected sites. Available phosphorus (P) ranged from 12.02 to 15.44 kg ha^{-1}, with the lowest value for HS 9 soil and maximum value for HS 6 soil. Available potassium (K) varied from 108.56 to 120.25 kg ha^{-1} with HS 6 soil marked for its lowest value and HS 1 for its highest value.

3.4. Effect of soil properties on HSREOs

Pearson's correlation analysis was used to study the effect of soil on HSREOs (Table 4). Among the major components; limonene correlated positively to soil EC ($r = 0.756$, $\rho < 0.05$) that means soil with high EC was responsible for greater concentration of limonene in the EO of *H. spicatum* grown in that soil. Similarly, limonene was also correlated positively to soil Mn ($r = 0.720$, $\rho < 0.05$) and K ($r = 0.733$, $\rho < 0.05$) implied that soil with higher content of Mn and K favored the enhancement of limonene content in the EO of *H. spicatum* grown in that soil. However a negative correlation of limonene with soil OC ($r = -0.636$, $\rho < 0.05$) indicated that lower organic carbon in soil favored higher amount of limonene in the oil of *H. spicatum* grown in that soil. Previously also, effects of soil pH, its organic carbon content, macronutrients level such as N, P and K were also studied on the essential oil composition of medicinal herb (Efendi et al., 2021).

Similar to limonene, linalool amount was also correlated significant positively to soil Mn ($r= 0.695$, $\rho < 0.05$) and K ($r = 0.651$, $\rho < 0.05$) indicted that soil with higher content of Mn and K favored the increment of limonene content in the EO of *H. spicatum* grown in that soil. In a previous research work, a significant positive correlation was also found between linalool content in the volatile oil of kaffir lime and leaf K content (Efendi et al., 2021).

1,8-cineole percent was correlated significant negatively with soil K ($r = -0.696$, $\rho < 0.05$) implied that soil with lower potassium amount favored greater 1,8-cineole fraction in the EO of *H. spicatum* grown in that soil. Previously also, influence of soil potassium on essential oil composition mainly 1,8-cineole of aromatic medicinal plants such as sweet basil (*Ocimum basilicum* L.) (Nurzyńska-wierdak & Borowski, 2011) and *Lavandula angustifolia* (Mill.) (Chrysargyris et al., 2017) was reported.

γ-Eudesmol content was correlated negatively by Zn ($r = -0.728$, $\rho < 0.05$), thus lower Zn containing soil found good for accumulation of higher percentage of γ-eudesmol in the EO of *H. spicatum* grown in that soil. A previous research on *Ocimum basilicum* L. or sweet basil by Ghorbanpour et al., (2016) also investigated that soil Zn content influenced yield, chemical compositions of plant essential oil.

Thus, correlation study between soil and HSREOs revealed that soil was one of the crucial environmental factors influencing quantitative composition, mainly limonene, linalool, 1,8-cineole and γ-eudesmol content, of HSREOs composition. Therefore, by maneuvering soil physico-chemical conditions a *H. spicatum* could be grown with desired amount of above chemicals in its essential oil. To our knowledge, this is the first report of its kind for HSREOs.

4. CONCLUSIONS

This study presents a detail account of compositional variation among the essential oils of wild accessions of *H. spicatum* collected from different locations of Uttarakhand Himalaya. Research observed 1,8-cineole, linalool, τ-cadinol, δ-cadinene, cubebol, γ-eudesmol, sabinene, germacra-4(15),5,10(14)-trien-1-α-ol, limonene and cis-γ-bisabolene as the dominant compounds along with the minor compounds present in the oils. The research also concluded soil to be one of the EO influencing environmental factors and analyzed that limonene positively correlated with soil EC, Mn and K and negatively with soil OC; 1,8-cineole influenced negatively by soil K; linalool related positively to soil Mn and K and eudesmol varied negatively by Zn. Thus, soil influenced HSREOs' composition quantitatively. The growing conditions of chemically diverse wild *H. spicatum* could be utilized for growing a desirable cultivar of this species for pharmaceutical or other industry.

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Table 1: Collection sites, EO yield and EO color of *H. spicatum* rhizome populations

S. No.	Sample	Collection Site (District)	EO % yield	EO color
1	HS 1	Bhimtal (Nainital)	0.20	Very light brown
2	HS 2	Chanffi (Nainital)	0.21	Pale yellow
3	HS 10	Maneshwar (Champawat)	0.36	Very light brown
4	HS 3	Matial (Nainital)	0.20	Yellow
5	HS 9	Khalkariya (Champawat)	0.17	Light brown
6	HS 7	Manar (Champawat)	0.47	Yellow
7	HS 8	Khark Karki (Champawat)	0.30	Very light brown
8	HS 5	Paharpani (Nainital)	0.44	Pale yellow
9	HS 6	Narshingh hill (Champawat)	0.44	Pale yellow
10	HS 4	Dhanachuli (Nainital)	0.31	Pale yellow

Table 2: Composition analysis of HSREOs

S. No.	Component	*RT (min)	*RI[a]	*RI[b]	% of identified compounds									
					HS 1	HS 2	HS 10	HS 3	HS 9	HS 7	HS 8	HS 5	HS 6	HS 4
1	α-Pinene	5.133	941	939	2.83	0.72	0.71	1.05	3.88	0.73	1.63	1.37	1.34	0.49
2	Sabinene	6.013	978	975	2.5	1.7	0.94	3.38	8.05	2.49	1.39	1.55	3.37	1.51
3	β-Pinene	6.238	982	979	1.06	0.63	0.05	0.13	4.43	0.23	0.04	0.21	0.18	0.13
4	Limonene	6.852	1034	1029	5.68	0.54	0.03	1.26	2.97	-	0.04	-	-	-
5	**1,8-cineole**	7.453	1038	1031	**8.96**	**31.5**	**42.34**	**26.49**	**17.54**	**30.62**	**44.5**	**36.82**	**55.41**	**44.89**
6	γ-Terpinene	7.888	1063	1059	0.09	0.09	0.07	0.15	0.14	-	0.1	-	0.09	0.06
7	cis-Sabinene hydrate	8.728	1074	1070	0.37	0.19	0.13	0.03	0.31	0.11	0.11	0.12	0.19	0.13
8	**Linalool**	9.043	1101	1096	**70.24**	**34.52**	**4.39**	**4.23**	**51.18**	**4.95**	**5.57**	**8.43**	**10.11**	**9.33**
9	trans-Sabinene hydrate	11.012	1104	1098	0.59	0.6	0.18	0.03	0.59	0.37	0.61	0.61	0.34	0.37
10	Terpinen-4-ol	11.33	1180	1177	0.12	0.88	0.55	0.77	0.2	0.6	0.95	0.55	0.93	0.88
11	α-Terpineol	11.75	1192	1188	0.18	1.22	1.61	0.86	0.16	1.19	1.72	1.42	1.85	1.9
12	isolongifelene	18.718	1396	1390	0.02	0.33	1.1	0.81	0.11	1.01	0.36	0.6	0.33	0.63
13	β-Caryophyllene	19.652	1418	1408	0.02	0.7	1.84	0.77	-	2.79	0.99	1.39	0.92	0.91
14	β-Acoradiene	19.96	1473	1470	-	0.33	0.33	0.7	-	0.33	0.21	0.31	0.21	0.29
15	γ-Muurolene	20.543	1479	1479	0.02	0.07	0.42	0.3	0.15	0.34	0.15	0.37	0.16	0.35
16	Germacrene D	20.97	1481	1481	0.02	0.72	1.02	1.94	0.05	1.11	0.65	0.79	0.19	0.39
17	β-Guaiene	21.603	1495	1493	0.06	2.12	0.35	1.09	0.06	1.17	0.12	1	1.16	1.15
18	cis-γ-Bisabolene	21.762	1517	1515	0.07	1.92	1.64	5.24	0.11	1.87	1.48	1.51	1.2	1.7
19	Cubebol	22.578	1517	1515	0.28	0.55	7.01	0.95	0.11	10.49	2.65	4.15	1.88	5.42
20	δ-Cadinene	23.355	1525	1523	0.05	1.77	8.31	1.92	0.06	6.93	1.35	13.87	2.59	7.61
21	Elemol	24.662	1555	1549	0.07	0.94	3.76	1.2	0.14	4.75	3.19	3.01	0.22	0.27
22	Ledol	24.922	1608	1602	0.23	0.56	1.78	1.2	0.19	3.12	1.45	0.49	0.45	1.4
23	γ-Eudesmol	25.233	1637	1632	0.21	2.94	1.55	8.26	0.23	3.09	3.22	2.86	1.91	3.21
24	τ-Cadinol	25.693	1645	1640	0.52	7.9	10.9	17.94	1.01	15.39	10.89	9.08	5.46	9.67
25	α-Eudesmol	27.922	1653	1653	0.23	-	0.38	0.13	0.29	-	0.45	1.35	0.15	0.08
26	α-Cadinol	28.453	1655	1654	-	-	0.03	0.1	-	-	4	-	2.08	-
27	14-hydroxy-(Z)-Caryophyllene	29.357	1670	1667	0.31	-	-	0.66	0.61	-	0.1	0.1	0.08	0.11

28	Germacra-4(15),5,10(14)-trien-1-α-ol	32.012	1687	1686	0.03	-	0.67	7.96	0.04	-	0.36	-	0.22	-
29	8-Cedren-13-ol	33.427	1690	1689	0.2	-	0.06	0.19	0.03	-	3.35	2.38	1.61	-
Total identified %					**94.96**	**93.44**	**92.15**	**89.74**	**92.64**	**93.68**	**91.63**	**94.34**	**94.63**	**92.88**
Monoterpene Hydrocarbon (MH)					12.16	3.68	1.8	5.97	19.47	3.45	3.2	3.13	4.98	2.19
Oxygenated Monoterpene (OM)					80.46	68.91	49.2	32.41	69.98	37.84	53.46	47.95	68.83	57.5
Sesquiterpene Hydrocarbon (SH)					0.26	7.96	15.01	12.77	0.54	15.55	5.31	19.84	6.76	13.03
Oxygenated Sesquiterpene (OS)					2.08	12.89	26.14	38.59	2.65	36.84	29.66	23.42	14.06	20.16

*RT: Retention time; RI[a]: Experimental retention indices (calculated); RI[b]: Retention indices according to literature (Adams, 2017); Compounds in s. no. 1,2,3,4, and 6 were MH; Compounds in s. no. 5,7,8,9,10 and 11 were OM; Compounds in s. no. 12 to 18 were SH; Compounds in s. no. 19 to 29 were OS

Table 3: Physico-chemical parameters of soil samples

Soil parameters / Locations		HS 1	HS 2	HS 10	HS 3	HS 9	HS 7	HS 8	HS 5	HS 6	HS 4
Soil texture	Sand	75±2.00	69±1.00	74±2.00	70±1.15	68±0.00	67±1.00	67±2.00	71±1.00	73±2.00	69±1.15
	Silt	18±2.00	21±1.00	21±0.00	22±2.00	23±0.00	23±2.00	23±1.00	20±1.00	21±1.00	21±2.00
	Clay	7±1.00	10±2.00	5±2.00	8±0.00	9±1.00	10±1.00	10±2.00	7±1.00	6±1.00	8±0.00
pH		6.56±0.71	7.2±0.52	6.09±0.59	6.71±0.56	5.29±1.05	5.98±0.58	2.67±0.59	5.09±0.65	5.69±0.50	5.86±0.59
*WHC		25.41±0.62	89.77±0.61	28.88±0.68	33.15±0.41	27.88±0.67	27.29±0.34	27.01±0.34	32.11±0.33	44.45±0.65	35.76±0.92
*EC		0.059±0.03	0.028±0.01	0.028±0.02	0.036±0.05	0.026±0.02	0.025±0.01	0.025±0.01	0.038±0.01	0.026±0.01	0.033±0.01
*OC		2.66±0.47	4.76±0.45	5.24±0.34	4.57±0.35	3.97±0.62	3.66±0.55	3.43±0.26	4.19±0.13	4.26±0.29	4.77±0.40
Mn		2.36±0.16	1.74±0.44	1.48±0.41	1.42±0.06	1.22±0.25	1.26±0.11	1.27±0.03	1.39±0.04	1.38±0.05	1.44±0.05
Fe		4.83±0.79	3.22±0.1	4.12±0.12	3.66±0.26	2.12±0.56	2.89±0.42	2.58±0.22	3.23±0.09	3.54±0.17	3.69±0.19
Cu		0.13±0.03	0.22±0.10	0.09±0.04	0.14±0.03	0.13±0.02	0.15±0.02	0.15±0.03	0.12±0.02	0.19±0.04	0.15±0.03
Zn		0.52±0.10	0.49±0.13	0.53±0.02	0.38±0.07	0.48±0.05	0.44±0.03	0.45±0.04	0.4±0.07	0.47±.03	0.39±0.06
Available N		112.89±1.141	175.61±1.169	200.7±1.822	150.52±1.11	105.01±1.026	108.36±0.924	110.32±0.764	155.89±0.763	124.18±0.875	153.32±0.789
Available P		12.43±0.99	15.22±1.26	13.47±0.73	12.53±0.33	12.02±0.81	12.08±0.87	12.11±0.91	12.54±0.47	15.44±0.84	13.01±1.06
Available K		120.25±1.54	113.47±0.43	115.22±0.84	112.04±0.89	113.45±0.42	113.38±0.56	113.88±0.43	111.54±0.42	108.56±1.37	112.67±0.71

*WHC = water holding capacity (%); EC = electrical conductivity (dS/m); OC = organic carbon (%)

Table 4: Pearson's correlation coefficient (r) between HSREOs and soil parameters

	Sabinene	Limonene	1,8-Cineole	Linalool	cis-γ-Bisabolene	Cubebol	δ-Cadinene	γ-Eudesmol	τ-Cadinol	Germacra-4(15),5,10(14)-trien-1-α-ol	Sand	Silt	Clay	pH	WHC	EC	OC	Mn	Fe	Cu	Zn	N	P	K
Sabinene	1	0.433	-0.448	0.46	-0.219	-0.425	-0.485	-0.22	-0.43	0.092	-0.209	0.349	0.171	0.024	-0.169	-0.13	-0.184	-0.234	-0.484	-0.02	0.065	-0.523	-0.212	-0.112
Limonene		1	-.851**	.908**	-0.38	-0.543	-0.555	-0.388	-.635*	0.014	0.386	-0.486	-0.055	0.26	-0.219	.756*	-.636*	.720*	0.357	-0.192	0.414	-0.4	-0.301	.733*
1,8-Cineole			1	-.775**	0.142	0.345	0.389	0.157	0.324	-0.149	-0.059	0.237	-0.24	-0.358	0.137	-0.598	0.508	-0.527	-0.119	0.221	-0.225	0.304	0.488	-.696*
Linalool				1	-0.584	-0.592	-0.556	-0.589	-.815**	-0.273	0.278	-0.459	0.078	0.285	0.091	0.586	-0.535	.695*	0.19	0.058	0.541	-0.335	-0.072	.651*
cis-γ Bisabolene					1	0.063	0.08	.975**	.849**	.886**	-0.188	0.271	0.078	0.249	0.12	-0.14	0.444	-0.255	0.06	0.071	-0.603	0.353	0.013	-0.355
Cubebol						1	.659*	0.031	0.534	-0.228	-0.186	0.257	-0.032	-0.066	-0.307	-0.329	0.197	-0.373	-0.023	-0.312	-0.14	0.151	-0.214	-0.085

δ-Cadinene							1	0.072	0.351	-0.191	0.064	-0.137	-0.332	-0.043	-0.164	-0.075	0.389	-0.288	0.072	-0.389	-0.364	0.465	-0.087	-0.267
γ-Eudesmol								1	.840**	.850**	-0.308	0.302	0.201	0.115	0.091	-0.127	0.329	-0.289	-0.028	0.128	-.728*	0.219	-0.064	-0.388
τ-Cadinol									1	0.588	-0.372	0.45	0.183	0.004	-0.047	-0.351	0.391	-0.455	-0.082	-0.055	-0.578	0.299	-0.146	-0.336
Germacra-4(15),5,10(14)-trien-1-α-ol										1	-0.007	0.168	-0.044	0.255	-0.094	0.095	0.224	-0.094	0.139	-0.105	-0.46	0.152	-0.144	-0.161
Sand											1	-.813**	-.878**	0.391	-0.096	0.603	0.002	.638*	.840**	-0.29	0.481	0.334	0.331	0.315
Silt												1	0.563	-0.416	-0.082	-.843**	0.194	-.849**	-.831**	0.096	-0.276	-0.251	-0.232	-0.457
Clay													1	-0.218	0.271	-0.308	-0.285	-0.222	-.653*	0.47	-0.251	-0.436	-0.27	0.007
pH														1	0.444	0.323	0.315	0.48	0.522	0.199	0.145	0.416	0.397	0.147
WHC															1	-0.215	0.399	0.172	-0.049	.807**	0.131	0.418	.768**	-0.225

EC																1	-0.501	.852**	.729*	-0.267	0.105	-0.075	-0.229	.662*
OC																	1	-0.397	-0.017	0.039	-0.166	.824**	0.47	-0.501
Mn																		1	.770**	0.051	0.467	0.068	0.139	.741*
Fe																			1	-0.203	0.255	0.377	0.215	0.47
Cu																				1	-0.049	-0.106	.672*	-0.376
Zn																					1	0.066	0.277	0.54
N																						1	0.437	-0.074
P																							1	-0.405
K																								1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).



Fig. 1: Full blossom stage of *H. spicatum* plant collected from Matial (Nainital) at an altitude of 1668 m.