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Research Article

Essential oil composition of rhizomes of *Valeriana wallichii* DC. grown in temperate zone, Uttarakhand, India

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Abstract

Valeriana wallichii DC. (Valerianaceae) is an important medicinal and aromatic plant species (MAPs) used in traditional, Ayurvedic, Unani and modern system of medicines. Valerian rhizomes used in herbal tea formulations for sleep aid due to sedative properties. Volatile oil used in perfumery, flavour, pharmaceutical and fragrance industries and insect repellent formulations. Over utilization threatened its status in natural habitat and sustainable use of species is recommended. Considering increased demand, experimental cultivation initiated at Bharsar, Uttarakhand (1900 m asl). Samples were collected from experimental cultivation trials (for yield) during the month of March 2020. Essential oil was extracted from freshly harvested samples using a Clevenger type apparatus and analyzed by GC/FID and GC/MS. Essential oil content was 0.46% on fresh weight basis. Patchouli alcohol (39.78%) was the major constituent of the essential oil and other important constituents were α-guaiene (9.29%), α-bulnesene (6.96%), seychellene (5.1%), α-patchoulene (3.29%), isovaleric acid (2.41%), α-santalene (1.90%), camphene 1.82%), *etc.* Variations in the composition of the essential oil may be attributed to factors related to the environment including temperature, relative humidity and photoperiod at cultivation sites along with genetic makeup of the species. This study opens new areas for cultivation, pharmacological and biological activities for the studied germplasm.

1. Introduction

The genus *Valeriana* (Family-Valerianaceae) is distributed throughout the world and consist of approximately 250 species worldwide [1]. Twelve species of this genus are found distributed in moist temperate and cool regions of India [2]. Plants are erect pubescent herb, having horizontal thick rhizomes with descending fibrous roots. The plant is well known for the drug '*Valerian*', which consists of the subterranean parts of species including the rhizomes and roots. The genus is well known for medicinal and aromatic uses in traditional system of medicines (TSM). Rhizomes and roots of the plants used for the treatments of cardiac debility, ulcers, convulsion, jaundice, dry cough, asthma, skin diseases, leprosy, seminal weakness, general debility and for sleep enhancement [3]. Valerian are used as a

mild sedative and sleep-promoting agent [4]. It has also been used in the treatments of many diseases in Ayurvedic and Unani system of medicines. Valerian is often used as a mild alternative or a possible substitute for stronger synthetic sedatives, such as the benzodiazepines, in the treatment of states of nervous excitation and anxiety-induced sleep disturbances [4]. It is also used to treat epilepsy, gum sores, headaches, nausea, sluggish liver, urinary tract disorders, vaginal yeast infections, throat inflammations and as an emmenagogue, antiperspirant, antidote to poisons, diuretic, anodyne and decoction for cold [5]. A number of clinical investigations have demonstrated the effectiveness of Valerian as a sleep aid and minor sedative [4-5]. On account of sedative properties; valerian rhizomes are being used in herbal tea formulations for sleep aid in different parts of the world. Volatile oil isolated from the rhizomes and roots are used in perfumery and insect repellent formulations. The oil of the species is also used in flavour, pharmaceutical and fragrance industries and about 30 products are commercially available [2]. Rhizomes and roots of plant were prescribed for hysteria, hypochondriasis and nervous unrest [6].

Valeriana wallichii DC. (syn. *V. jatamansi* Jones) commonly known as Indian Valerian (Sugandhbala, Muskbala, Tagar), is a perennial herb, distributed in temperate Himalaya up to an altitude of 3000 m asl. The species used as an ingredient of herbal medicine in Indian systems of medicine (ISM) and also used as a substitute of European *V. officinalis*. The roots of the plant yield essential oil which gives a musky, woody, sweet and balsamic odor. *V. wallichii* is reported to be rich in two major groups of constituents; the valepotriates and sesquiterpenoids; which are responsible for the pharmacological activities of the species. Essential oil of this species contains terpenoids like α-and β-patchoulene, sesquifenchene, valeranone, maaliol, xanthorrhizzol, patchouli alcohol [7-10], along with existence of chemotypes [11] (for detail see Table 2). On the other hand, qualitative and quantitative variations have also been reported in the essential oil composition of *V. wallichii* [7-13].

The rhizomes and roots are highly aromatic and extracted Valerian oil is in great demand and annual consumption was 123 Metric Tons which is sourced from wild [14]. On account of commercial harvesting and other biotic pressures prevailing in Himalayan region this species had been enlisted under threatened category and prioritized for cultivation by National Medicinal Plant Board (NMPB), India. Considering multiple uses and increasing demand, efforts are being made to develop a cultivation protocol for *V. wallichii*. Germplasm collected from different natural populations and is being evaluated in Bharsar. One of the germplasm collected from Tigaddu (2000 m asl) near Bharsar showed variations in the essential oil composition and has not been published so far. Therefore, the detail of essential oil from that germplasm is presented here. However, further studies on growth performance and oil evaluation in other germplasm is under investigation.

2. Materials and methods

2.1. Plant material and essential oil extraction

Samples for present study were collected during the age of fifth year in the month of March 2020 from experimental cultivation trials (for yield) established in Bharsar (1900 m asl), Pauri Garhwal, Uttarakhand, India. In general, the soil of cultivation site is deep clay-loam and has profile ranging from 1 to 2 m. The soil is slightly acidic having pH 5.5, EC 0.21 dSm^{-1}, organic carbon 0.9% with Nitrogen 290 kg/ha, P_2O_5 23 kg/ha and K_2O 380 kg/ha. Generally, soil is rich in potassium, medium in phosphorous and nitrogen contents, with the exception of some cultivated fields. The climate is represented with mild summer; higher precipitation and severe cold prolonged winter season. Generally, days of Bharsar are fairly warm followed by cool nights. The area also receives heavy precipitation during monsoon and occasional snow fall during winter season [15].

Voucher specimen was authenticated in Botanical Survey of India, Dehradun and deposited for future reference (BSD 0614796). Rhizomes of uprooted plants were separated and washed thoroughly with tap water to remove the soil particles and chopped in 2 cm size. The fresh and chopped rhizomes (250g) were used in triplicate for the essential oil extraction using hydro-distillation method in a Clevenger-type apparatus for 4 h. After decanting, oil samples were dried with anhydrous Na_2SO_4 and stored at 4°C prior to analysis.

2.2. GC/FID and GC/MS analysis

Gas Chromatography/ flame ionization detector (GC/FID) analysis was carried out using a Perkin Elmer Clarus 500 GC equipped with a 30m×0.32mm Elite-5MS capillary column (0.32 μm film thickness). Oil sample (1 μL) was diluted with diethyl ether (200 μL) and then injected (0.5 μL) in the 'split' mode (1:30) with a column temperature program of 40°C for 5 min, then increased to 280°C at 4°C/min and finally held for 10 min. Injector and detector temperature were set at 250°C and 300°C, respectively, and the carrier gas was He at 1.0 mL/min (head pressure of 12.0 psi).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis was carried out using a Perkin Elmer Clarus 500 GC equipped with a Clarus 500 mass spectrometer using the same capillary column and chromatographic condition as for the GC/FID analysis. Mass spectra were acquired over 40-500 *amu* range at 1 scan/sec with ionizing electron energy of 70 eV, and ion source at 200°C. The transfer line was set at 300°C, while the carrier gas was, He at 1.0 ml/min. The identification of the oil components was performed by the determination of their retention indices (RI), by comparison with authentic reference compounds as well as with published mass spectra [16] and by peak-matching library search [17]. Retention Index (RI) were calculated according to Adams [16] using a *n*-alkane series (C_6-C_{32}) under the same GC conditions as for the samples. Retention indices were used to convert retention times into system-independent constants. Therefore, the obtained retention indices are independent of the GC conditions and are a characteristic feature of each compound. Tables of retention indices were used to identify peaks by comparing measured retention indices with the tabulated values. The relative amount (%) of individual components of the essential oil was expressed as percent peak area relative to total peak area from the GC/FID analysis of the whole essential oil, assuming an equal response factor for all the detected compounds.

3. Results and discussion

Hydro-distillation of the rhizomes yields 0.46±0.06% yellow essential oil (fresh weight basis). Essential oil content in the present study slightly varied as compared to earlier studies [10,13], and not reported so far. Thirty-three compounds constituting 94.4% of the total essential oil were detected using GC-FID and GC-MS analysis. Two unknown sesquiterpene compounds ($C_{15}H_{24}$ #16 and $C_{15}H_{24}O$ #30, Table 1) were tentatively identified by comparison of their MS spectra with those of known compounds and reported as unidentified sesquiterpenes. Composition of the essential oil is presented in Table 1, along with the Retention Index (RI) of each constituent. A chromatographic profile of the essential oil is also presented in Fig. 1.

Table 1. Essential oil composition from the rhizomes of *V. wallichii* grown at Bharsar

S.N.	Compounds	RI	RI lit[c]	Percentage
1	Isovaleric acid	836	827	2.41
2	α-Pinene[a]	928	932	1.29
3	α-Fenchene[a]	942	945	1.12
4	Camphene[a]	944	946	1.82
5	3-Methyl valeric acid	956	939	1.49
6	β-Pinene[a]	972	974	0.24
7	p-Cymene[a]	1021	1020	0.27
8	Limonene[a]	1025	1024	0.37
9	Borneol[a]	1167	1165	0.13
10	Thymol methyl ether[a]	1222	1232	0.19
11	Carvacrol methyl ether[a]	1237	1241	0.09
12	Bornyl acetate[a]	1280	1374	0.78
13	β-Patchoulene[b]	1378	1379	1.60
14	β-Elemene[b]	1385	1389	0.85
15	α-Santalene[b]	1414	1416	1.90
#16	Unidentified sesquiterpene $C_{15}H_{24}$[b]	1430	-	3.72
17	α-Guaiene[b]	1440	1437	9.29
18	Seychellene[b]	1449	1444	5.01
19	α-Patchoulene[b]	1453	1454	3.29
20	γ-Gurjunene[b]	1477	1475	0.23
21	(z)-β-Guaiene[b]	1487	1492	0.27
22	Valencene[b]	1489	1496	1.34
23	α-Selinene[b]	1492	1498	1.16
24	α-Bulnesene[b]	1501	1509	6.96
25	7-Epi-α-selinene[b]	1511	1520	1.18
26	Kessene[b]	1521	1529	1.64
27	Maaliol[b]	1561	1566	1.63
28	Viridiflorol	1585	1592	0.85
29	Guaiol[b]	1595	1600	1.38
#30	Unidentified sesquiterpene $C_{15}H_{24}O$[b]	1650	-	1.35
31	Patchouli alcohol[b]	1661	1656	39.78
32	Bulnesol[b]	1673	1670	0.71
33	Xanthorrhizol[b]	1759	1751	0.06

[a] monoterpenes; [b] sesquiterpenes; [c] from Adams, 2007[16]; RI: Retention index.

The present study revealed that major constituent of

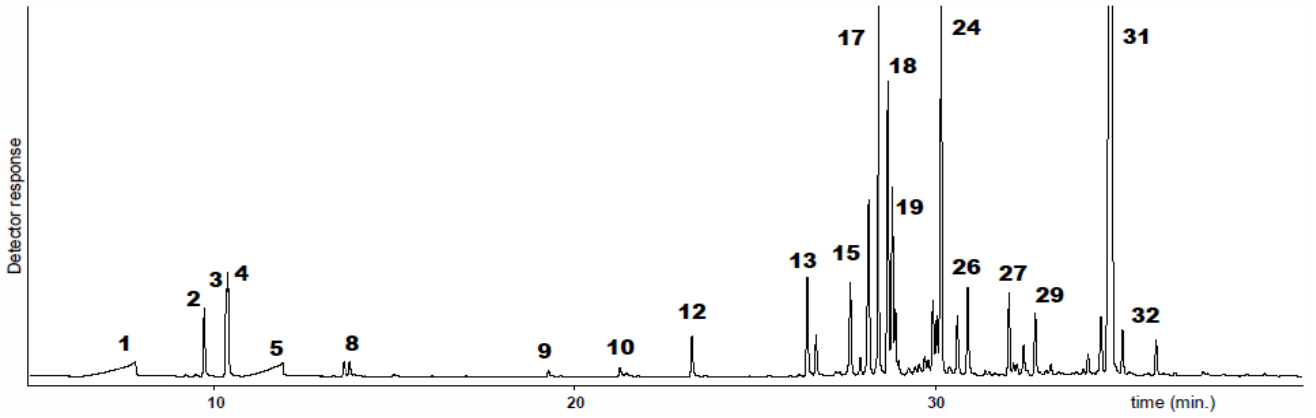

Figure 1. Chromatographic profile of the essential oil of *V. wallichii* grown at Bharsar, Uttarakhand (See Table 1 for compound identification).

Table 2. Variations in the important constituents of the essential oils of *V. wallichii* from the different studies

Compounds	Present study	Composition of the essential oil in previous studies					
		Ref. 7	Ref. 8	Ref. 9	Ref. 10	Ref. 12	Ref. 13
Isovaleric acid	2.41	1.05	0.5	-	-	-	1.4
α-Pinene	1.29	-	-	-		0.59	1.9
α-Guaiene	9.29	-	4.3	0.7-2.3	3.1	3.86	2.7
Seychellene	5.01	3.37	2.8	4.1-27.4	5.4	4.64	4.8
α-Patchoulene	3.29	0.35	1.7	0.8-6.6		2.2	2.4
α-Bulnesene	6.96	-	7.6	0.7-6.3	-	-	-
Patchouli alcohol	39.78	60.7	48.5	0.4-63.7	51.3	44.3	36-39

the essential oil was patchouli alcohol (39.78%). The other important constituents were isovaleric acid (2.41%), α-pinene (1.29%), camphene (1.82%), 3-methyl valeric acid (1.49%), α-santalene (1.90%), α-guaiene (9.39%), seychellene (5.01%), α-patchoulene (3.29%), α-bulnesene (6.96%), malliol (1.63%). Two sesquiterpenes ($C_{15}H_{24}$ and $C_{15}H_{24}O$) could not be identified and further study is continuing for proper identification. The study reveals that the essential oil of *V. wallichii* analyzed here is a good source of patchouli alcohol. This compound had earlier been reported from same species [7-10, 12-13] and also, from *Nardostachys jatamansi* [18], belonging to the same genus. Patchouli alcohol has been reported to have antibacterial, antiplaque and fungicidal activities [19]. Isovaleric acid, 3-methyl valeric acid, α-pinene, camphene, α-santalene, α-guaiene, seychellene, α-patchoulene, α- bulnesene, malliol, guaiol, etc. were also present in appreciable amounts. The various compounds identified in the present study had also been reported in one or other studies from this species [10-13, 20], however, their percentage varied in different studies. These compounds are very useful for pharmaceutical as well as flavour and fragrance industry.

A list of the important compounds of the essential oil from *V. wallichii* collected from different parts of the Himalayan region at the same phenophase as in present study, is presented in Table 2. Quantitative and qualitative composition of the essential oil varied in different studies as compared to present study (Table 2). Comparison revealed that the cultivated strain of *V. wallichii* in present study showed a remarkably high content of isovaleric acid, α-guaiene and seychellene (Table 2). The present study revealed quantitative and qualitative variations in the composition of the essential oil of *V. wallichii*. Such variations may be attributed to factors related to the soil and environment including temperature, relative humidity and photoperiod along with altitude of experimental site. The quantitative composition of the essential oils of many aromatic plants has been shown to be greatly influenced by the genotype. Based on these findings, it is likely that nutrient level,

temperature regime, relative humidity, irradiance and photoperiod may play a specific role in the composition of the oil, in addition to the well-established genetic diversity. Quantitative and qualitative variations in some of the constituents of the essential oil may be helpful to understand physiological pathway of these constituents in *V. wallichii* [21-22].

4. Conclusions

Study revealed that major constituent of the essential oil was patchouli alcohol (39.78%) in studied germplasm of *V. wallichii*. Two sesquiterpenes compounds $C_{15}H_{24}$ and $C_{15}H_{24}O$ could not be identified and further study is continuing for proper identification. Characterization of the essential oil of this germplasm opens new areas for research on medicinal, aromatic and industrial applications, along with large-scale cultivation to fulfil international market demand. Further studies may be extended on identification of unknown compounds, pharmacological and biological activities of the constituents of the essential oil.

Authors' contributions

Rajendra Singh Chauhan, Dinesh Tewari and Bhagwati Prasad Nautiyal designed experiment, collected germplasm, evaluated and prepared MS, Virendra Singh Rana and Aldo Tava analyzed essential oil and corrected MS as per need.

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Conflicts of interest

The authors have declared that no conflict of interest exists.

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