Crop-Ecology and Post-harvest distillation method: The key regulators for yield and composition of essential oil of *Valeriana jatamansi*

Babit Kumar Thakur

Council of Scientific and Industrial Research-Institute of Himalayan Bioresource Technology (CSIR-IHBT)

Shivani -

Council of Scientific and Industrial Research-Institute of Himalayan Bioresource Technology (CSIR-IHBT)

Mitali Mahajan

Council of Scientific and Industrial Research-Institute of Himalayan Bioresource Technology (CSIR-IHBT)

Probir Kumar Pal (✉ palpk@ihbt.res.in)

Council of Scientific and Industrial Research-Institute of Himalayan Bioresource Technology (CSIR-IHBT)

Research Article

Keywords: Crop-ecology, essential oil, patchouli alcohol, seychellene, α -Santalene, β -elemene

Posted Date: August 3rd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-744915/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Valeriana jatamansi is an important temperate herb, which is used for pharmaceutical and essential oil industries. In India, this species is now at the verge of extinction due to the over-exploitation of its rhizomes from its natural habitat. It is hypothesized that the variations in bioactive compounds in its essential oil are very high among the wild populations as well as cultivated sources. Thus, the study was conducted to evaluate the chemical profiling of essential oil of four wild populations (Rupena, Kugti, Garola, Khani) and two cultivated sources (CSIR-IHBT, Saloonie), which were distilled at three consecutive days. The variation in oil concentration in root/rhizome was found significant ($P \leq 0.05$), and the maximum value (0.35%) was registered with the population collected from Kugti and Khani. In essential oil, irrespective of population and distillation day, patchouli alcohol was the major compound, which ranged from 19 to 63.1%. The maximum value (63.1%) was recorded with the essential oil obtained from Garola's population and distilled on the first day. The percentage of seychellene was abruptly increased with subsequent days of extraction in all the populations. The multivariate analysis revealed that the essential oil profiles of Rupena, Kugti, Garola, and CSIR-IHBT populations were found similar during the first day of distillation. However, during the second day Rupena, Kugti, Khani, and CSIR-IHBT come under the same ellipse of 0.95% coefficient. The results suggest that the population of Kugti is superior in terms of oil concentration (0.35%) with a higher proportion of patchouli alcohol (63% on the first day). Thus, repeated distillation is recommended for higher recovery of essential oil. Moreover, repeated distillation can be used to attain *V. jatamansi* essential oil with differential and perhaps targeted definite chemical profile.

Introduction

Valeriana jatamansi Jones (Syn. *V. wallichii* DC.), commonly known as Indian valerian, belongs to the family of Valerianaceae; However, recently it has been categorized under Caprifoliaceae^{1,2}. It is a small perennial medicinal herb, and naturally distributed in the temperate Himalaya between an altitude of 1000 and 3300 m. Asl^{3,4}. In India and Nepal, *V. jatamansi* is widely used as a substitute for *V. officinalis*, which is mostly used in Europe⁵. *V. jatamansi* is widely used in the Indian Ayurvedic and Unani systems of medicine since ancient times⁶. Now it becomes the most acceptable sedative over modern medicines^{6,7,8}. *V. jatamansi* possesses a sedative property due to the presence of bioactive compounds like γ -valerpotriates in roots/rhizome^{9,10}, which is a non-glycosidic iridoid esters¹¹. It is also used in Chinese traditional systems of medicine for its tranquilizing hypnotic and antiviral activities¹².

Importantly, the essential oil of *V. jatamansi* is now widely used in flavor and fragrance industries, and about 30 pharmaceutical and essential oil-based products are commercially available¹³. Moreover, *V. jatamansi* has also been reported as a psychopharmacological agent^{10,14,15}, and highly effective against leprosy¹⁶, epilepsy and hysteria¹⁷, nerve diseases¹⁸, scorpion bite¹⁹, cholera²⁰, anxiolytic properties²¹ and curing lewy body dementia²². However, the present study has been emphasized only on essential oil content and composition of different populations of *V. jatamansi* from the western Himalayan region in India.

In Himachal Pradesh, a Himalayan state of India, this plant is naturally grown on a moist slope of hills in the district of Chamba. However, a wide diversity of *V. jatamansi* exists in nature in this region²³. Now in India, this species is at the edge of extinction due to the over-exploitation of its roots/rhizome from its natural habitat to meet the burgeoning industrial demand. The industrial demand for this plant is increasing day by day for phytopharmaceutical as well as essential oil-based products. Since the major portion of the *V. jatamansi* is contributed through wild collections, there is a major concern about a consistent supply of quality raw material. Moreover, the collectors do not follow the standard protocol for harvesting to maintain the quality of essential oil. Essential oil of *V. jatamansi* is a complex mixture of volatile molecules including monoterpenes and sesquiterpenes. More than 290 compounds have been identified in the essential oil of *V. jatamansi* so far⁸. The major compounds of *V. jatamansi* oil are patchouli alcohol, β -patchoulene, α -patchoulene, seychellene, α -selinene, δ -guaiene etc. The relative proportion of major compounds determines the quality of the essential oil. The content of essential oil in roots/rhizome is varied largely with a range from 0.05-2.00%²⁴⁻²⁹.

The variations in oil concentrations in roots/rhizome as well as the percentage share of different compounds are largely influenced by seasonal variations³⁰, populations²⁸, plant parts^{4,27,31}, geographical regions^{32,33}, type of materials²⁹ and genetic makeup^{4,33,34}. The variations in secondary metabolites of *V. jatamansi* due to habited and altitude differences have also been reported³⁵. The differences between wild and cultivated source of *V. jatamansi* in terms of essential oil content and antioxidant activities have also been noticed³⁶. The variations in chemical compositions of essential oil of *Rosa damascena* have been noticed due changes of ambient temperature and humidity during the time of flowering³⁷. Since *V. jatamansi* is widely found in nature in the Chamba region, there is a possibility of large variations in oil content and compositions among the populations. However, the information about the variety, chemotype, or population that occurs in this region is limited. Farmers collect/harvest roots/rhizomes from wild without having the information about chemical profiles. Farmers also use wild populations as planting material for cultivation in the under-utilized land or in orchards as an intercrop.

Another important issue with *V. jatamansi* is distillation methods, which largely influence the oil recovery and quality of essential oil. Generally, farmers distil their harvested roots/rhizome in a commercial hydro-distillation unit after partial drying. Both dry and fresh roots/rhizome are used for oil extraction, and the variations in oil concentration between fresh and dry materials have been reported^{29,36,38}. Among the distillation conditions, time/duration is a key factor for the composition of essential oil like pine (*Pinus ponderosa* Dougl. ex Laws) and Sweet Sagewort (*Artemisia annua* L.)^{39,40}, Japanese cornmint (*Mentha canadensis* L.)⁴¹, lemongrass (*Cymbopogon flexuosus* Steud.), and palmarosa (*Cymbopogon martini* Roxb.)⁴². Moreover, the effect of distillation time on recovery of essential oil of *V. jatamansi* has been reported^{29,43,44}. However, information regarding the effects of repetitive distillation on oil recovery and the quality of the oil is missing. It has been reported that essential oil fractions with specific chemical profiles can be managed through altering the time of hydro-distillation⁴⁵. In case of rose essential oil, higher methyl eugenol concentration is noticed with long-term fermented flowers⁴⁶.

We hypothesized that repetitive distillation would be effective to increase the oil recovery and modification of the composition of the oil. It is a fact that the pH level of water influences the hydrolytic reactions during hydro-distillation. Seeing the above issues, there is a pressing need to understand the best population and effective distillation method for higher oil yield and desire quality. This experiment, therefore, conducted to (i) investigate the profile of essential oil of different populations of *V. jatamansi*, and (ii) to understand the effects of repetitive distillation on recovery and quality of essential.

Results

Essential oil content (%)

Analyzed data revealed that essential oil of *V. jatamansi* was obtained up to the third day of distillation; however, in the case of Rupana and Garola, oil was not obtained (Fig. 1a-e) on the third day. Irrespective of sources of roots/rhizome, the recovery rates (%) were higher on the first day of distillation, and thereafter the trends were declined. The total recovery (addition of 2/3 days' samples) of essential oil (%) was found significant ($P \leq 0.05$) variations among the natural populations and cultivated source (Fig. 1f). The populations collected from Kugti and Khani registered significant ($P \leq 0.05$) higher oil content (0.35%) compared with other populations, a cultivated source at CSIR-IHBT, and oil obtained from the traditional extraction method. The lowest oil content (0.17%) was recorded with the population of Garola.

Composition of essential oil

GC and GC-MS analysis data revealed that a maximum of 18 volatile compounds was identified from the essential oil obtained from hydro-distillation of *V. jatamansi* roots/rhizome of different populations and cultivated sources (Fig. 2). These compounds contributed up to 81.8% of the total volume (Fig. 2) in this study. The highest number (16) of volatile compounds were identified with the cultivated source from CSIR-IHBT during third-day distillation (Fig. 2e) whereas the lowest number (9) of compounds were identified with the population collected from Garola on Day-one distillation (Fig. 2c). However, the maximum (81.8%) and minimum (62.1%) sharing by the identified compounds in a total volume of essential oil was observed with the day-one distillation of the Kugti population and day-two distillation of Garola population, respectively (Fig. 2b, c). In all populations, the numbers of identified compounds were higher in the essential oil extracted on the second day compared to the first day even these values were higher than the Saloonie sample (traditional practice).

The chemical profiles of the volatile oils from different natural and cultivated sources of *V. jatamansi* have been presented in Table 2. The data on compositions of essential oils revealed a great variation among the different sources of material and distillation process. Irrespective of the source of materials and distillation day, the major compounds detected in the essential oil were patchouli alcohol, δ -guaiene, selinene < 7-epi- α ->, α -selinene, α -patchoulene, seychellene, and β -patchoulene, which have been illustrated in the representative chromatograms of essential oil in Fig. 3. Patchouli alcohol was found the most abundant compound in all the essential oil obtained from different populations and distilled on different days (Table 2). Patchouli alcohol ranged from 19 to 63.1%. The maximum value (63.1%) was recorded with the essential oil obtained from Garola's population and distilled the first day whereas the lowest value (19%) was recorded with the population collected from Khani and distilled third day (Table 2). The essential oil of *V. jatamansi* extracted through the traditional method (Saloonie sample) registered maximum percentage of β -patchoulene (10.2%), α -guaiene (5.5%), α -humulene (5.0%), and δ -guaiene (7.3%) compared with the rest of the samples in this experiment. Humulene epoxide II was found only in three populations (Rupena, Garola, and Khani) during second-day distillation, and the maximum value (2.5%) was recorded with Garola's population. Seychellene concentration was found maximum (13.3%) during the third day of distillation of the population collected from Kugti. Similarly, β -caryophyllene concentration was found maximum (3.8%) with Khani and CSIR-IHBT populations during the third day of distillation (Table 2).

Table 2
The dynamic of composition of essential oil in different populations of *V. jatamansi* under repetitive distillation method

Compound	Rupena		Kugti		Garola			Khani		CSIR-IHBT			Salooni	
	Day-1	Day-2	Day-1	Day-2	Day-3	Day-1	Day-2	Day-1	Day-2	Day-3	Day-1	Day-2	Day-3	Day-1
β-Patchoulene	4.5 ± 0.33	3.8 ± 0.27	1.9 ± 0.09	-	-	1.9 ± 0.11	-	2.0 ± 0.07	2.5 ± 0.12	-	1.7 ± 0.01	2.0 ± 0.08	-	10.2 ± 0.05
β-Elemene	-	1.2 ± 0.00	0.4 ± 0.01	0.7 ± 0.03	1.5 ± 0.03	-	1.3 ± 0.16	-	0.9 ± 0.02	1.7 ± 0.06	-	0.7 ± 0.02	2.9 ± 0.14	0.8 ± 0.03
Santalene < alpha>	-	1.1 ± 0.04	-	1.2 ± 0.01	1.2 ± 0.02	-	0.8 ± 0.20	-	1.1 ± 0.02	1.5 ± 0.05	-	0.7 ± 0.02	1.7 ± 0.04	-
β-Gurjunene	-	-	-	0.9 ± 0.03	-	-	-	0.9 ± 0.02	2.8 ± 0.05	-	-	-	1.5 ± 0.03	-
α-Guaiene	1.7 ± 0.02	2.2 ± 0.14	1.8 ± 0.08	0.7 ± 0.02	1.1 ± 0.03	1.9 ± 0.05	1.2 ± 0.16	1.7 ± 0.02	2.9 ± 0.15	4.1 ± 0.24	1.9 ± 0.18	1.1 ± 0.03	2.3 ± 0.13	5.5 ± 0.29
Trans(Beta.)-Caryophyllene	0.9 ± 0.01	3.0 ± 0.01	1.1 ± 0.01	2.3 ± 0.04	3.1 ± 0.08	-	1.6 ± 0.02	0.9 ± 0.02	2.5 ± 0.03	3.8 ± 0.22	1.3 ± 0.02	1.8 ± 0.04	3.8 ± 0.06	1.8 ± 0.03
Seychellene	3.7 ± 0.05	11.5 ± 0.10	4.9 ± 0.04	10.4 ± 0.39	13.3 ± 0.40	5.7 ± 0.01	10.6 ± 0.04	3.8 ± 0.03	8.4 ± 0.10	12.4 ± 0.47	3.4 ± 0.04	4.8 ± 0.03	9.2 ± 0.14	9.2 ± 0.11
α-Humulene	1.0 ± 0.02	-	0.8 ± 0.01	1.5 ± 0.15	-	2.3 ± 0.03	0.8 ± 0.05	1.7 ± 0.03	3.4 ± 0.34	1.2 ± 0.04	1.5 ± 0.02	1.3 ± 0.04	3.3 ± 0.46	5.0 ± 0.06
α-Patchoulene	1.9 ± 0.06	4.8 ± 0.12	2.1 ± 0.02	1.3 ± 0.09	4.2 ± 0.14	1.6 ± 0.09	2.6 ± 0.24	1.6 ± 0.06	4.9 ± 0.18	4.0 ± 0.76	1.3 ± 0.05	1.8 ± 0.02	3.8 ± 0.16	3.2 ± 0.00
β-Selinene	-	0.9 ± 0.03	-	0.6 ± 0.13	0.8 ± 0.04	-	1.9 ± 0.01	-	-	1.7 ± 0.11	-	-	2.2 ± 0.04	-
α-Selinene	-	3.2 ± 0.33	0.5 ± 0.09	0.8 ± 0.03	2.3 ± 0.20	-	2.3 ± 0.11	-	2.4 ± 0.04	2.3 ± 0.10	-	1.1 ± 0.00	1.7 ± 0.24	-
δ-Guaiene	3.5 ± 0.26	2.7 ± 0.55	2.6 ± 0.12	3.14 ± 0.41	4.7 ± 0.90	2.6 ± 0.06	3.2 ± 0.37	3.4 ± 0.09	6.0 ± 0.64	6.3 ± 1.86	2.9 ± 0.06	5.8 ± 0.20	3.9 ± 0.84	7.3 ± 0.23
Selinene < 7-epi-alpha->	0.5 ± 0.10	3.1 ± 0.12	1.1 ± 0.02	1.0 ± 0.14	3.5 ± 0.06	1.4 ± 0.04	3.0 ± 0.01	-	1.5 ± 0.01	3.3 ± 0.10	-	1.9 ± 0.03	2.6 ± 0.07	2.2 ± 0.04
Kessane	-	-	0.4 ± 0.00	0.9 ± 0.06	-	-	-	1.1 ± 0.00	1.9 ± 0.07	1.2 ± 0.17	0.9 ± 0.02	0.4 ± 0.00	1.0 ± 0.03	-
Spathulenol	-	-	-	-	-	-	0.7 ± 0.02	-	-	-	-	-	2.8 ± 0.06	1.6 ± 0.08
Epiglobulol/ Globulol	1.8 ± 0.07	1.3 ± 0.03	1.2 ± 0.01	0.9 ± 0.06	1.1 ± 0.07	1.1 ± 0.09	-	-	-	-	1.9 ± 0.02	1.5 ± 0.04	0.7 ± 0.05	1.3 ± 0.01
Humulene epoxide II	-	0.7 ± 0.02	-	-	-	-	2.5 ± 0.04	-	0.6 ± 0.04	-	-	-	-	-

Compound	Rupena		Kugti		Garola		Khani		CSIR-IHBT		Salooni			
Patchouli alcohol	61.7 ± 0.49	33.6 ± 1.78	63.0 ± 0.91	44.1 ± 1.82	27.9 ± 0.77	63.1 ± 0.65	28.8 ± 1.85	55.8 ± 0.47	31.2 ± 1.05	19.±0.46	60.2 ± 0.63	53.8 ± 0.56	33.5 ± 0.68	30.7 ± 0.49

The dynamic of important compounds in the essential oil of *V. jatamansi* populations has been influenced by the distillation days (Fig. 4). The results indicated that maximum percentages of patchouli alcohol, irrespective of the source of materials, were registered with the oils obtained during the first day of distillation (Fig. 4a). The patchouli alcohol concentrations in the oils obtained during the first day of distillation, irrespective of populations, were found substantially higher than the oil obtained from the traditional distillation method (Saloonie). However, the concentrations of patchouli alcohol were sharply declined during subsequent days of extraction (Fig. 4a). In contrast, the percentage of seychellene was abruptly increased with subsequent days of extraction in all the populations (Fig. 4b). The concentration of δ -guaiene in the essential oil of *V. jatamansi* was increased with second and third-day samples of Kugti, Garola, Khani, and CSIR-IHBT populations (Fig. 4c). However, the trend of α -patchoulene concentration was not found consistent across the populations (Fig. 4d).

Principal component analysis (PCA)

The multivariate analysis in terms of PCA was performed to explore the relationship among the volatile compounds in the essential oil of *V. jatamansi* populations and to understand how these populations were different from each other. The compounds of the essential oil were treated as dependent variables. The separate PCAs were constructed for all three days of distillation revealed that the first two components, PC1, and PC2, jointly explained 79.86, 63.06, and 86.03% of the total variations for first, second, and third-day distilled samples, respectively (Fig. 5a-f). The eigenvalues of these two PCs are 9.53 and 2.45, respectively. The relationships among the compounds of essential oil the magnitude of their contributions in the space of PC1 and PC2 have been presented in Fig. 5a, c, and e for the first, second and third day, respectively. For first day sample, PC1 has positive coefficients with β -patchoulene, β -elemene, α -guaiene, seychellene, α -humulene, α -patchoulene, δ -guaiene, selinene < 7-epi- α ->, and spathulenol; the loading values of these compounds were quite high (> 0.83). Hence, it is clear that these eight compounds are highly correlated with each other (Fig. 5a). However, PC1 has a negative coefficient with patchouli alcohol with a loading value of - 0.94. The PCA bi-plot of the first-day sample indicated that there were three distinct clusterings among the populations and cultivated sources. The populations from Kugti, Garola, Rupena, cultivated source of CSIR-IHBT were placed in the negative coordinate of both PCs, and all four samples came under the same ellipse of 0.95% coefficient (Fig. 5b). The population of Khani and Saloonie created independent groups.

During the second day of distillation, β -patchoulene, α -guaiene, α -humulene, δ -guaiene were situated in the positive coordinate of PC1 with loading value of 0.81, 0.56, 0.81, and 0.87, respectively (Fig. 5c). The compound like β -elemene, santalene, β -selinene, α -selinene, and humulene epoxide II, were also separated by the PC1 and placed in the negative coordinate with loading value of 0.78, 0.75, 0.78, 0.87, and 0.71, respectively. On the third day of distillation, the compounds were separated by both PCs, but there was no distinct cluster among the compounds (Fig. 5e). The PCA bi-plots of second and third days of distillation also indicated three distinct groups among the natural populations and cultivated sources (Fig. 5d, f). For the second-day sample, Kugti, Khani, and CSIR-IHBT fall under the same group, whereas CSIR-IHBT is separated from Kugti and Khani for the third-day sample. The oil from Saloonie did not form any group with the rest of the populations in this study.

Physico-chemical properties of soil of naturally distributed locations of *Valeriana jatamansi*

The physico-chemical properties of the soil of different sample collecting locations have been presented in Table 2. The soil texture of the different locations ranged from sandy loam to silty clay. Sandy loam soil was found in Rupena and Saloonie, whereas loamy soil was observed at Khani and Kugti. The soil of Palampur was heavy (silty clay). The significant ($P \leq 0.05$) differences in terms of chemical properties (pH, EC, OC, available N, P, and K) of the soil were found among the locations. The highest pH (8.85) of soil was registered at Kugti that was significantly ($P \leq 0.05$) different from the rest of the locations. The lowest pH value (5.59) was registered with the soil collected from Palampur. The EC values of soils from different locations were also found significant ($P \leq 0.05$) difference, and the highest and lowest values were recorded in Garola and Palampur, respectively. Soil OC ranged from 0.71% at Garola to 3.34% at Saloonie (Table 2). The bulk densities of soil collected from Kugti, Khani, Garola, and Palampur were found almost similar value (~ 1.48 g cc⁻¹), but this value was significantly ($P \leq 0.05$) higher than the values recorded from Rupena (1.25 g cc⁻¹) and Saloonie (1.34 g cc⁻¹). Significant ($P \leq 0.05$) variations in available N, P, and K content in soil were found among the locations. Maximum N and K contents were registered in the soil of Rupena, whereas maximum P was recorded with soil from Saloonie (Table 3). Thus, it is evidenced from the result that the *V. jatamansi* is grown in a wide range of soil types with different nutritional statuses. Analysis of variance outcomes for determining the effect of location

on oil concentration in different populations of *V. jatamansi* and on soil properties have been presented in Table 4. The values of mean squares with corresponding level of significant are presented.

Table 3
Physico-chemical properties of soil of different locations of sample collection

Location	Soil Texture	pH	EC _(25 °c) (dS m ⁻¹)	Organic Carbon (%)	Bulk Density (g cc ⁻¹)	Available N (kg ha ⁻¹)	Available P (kg ha ⁻¹)	Available K (kg ha ⁻¹)
Rupena (Chamba)	Sandy Loam	7.61	0.847	3.22	1.25	284.53	18.54	1231.83
Kugti (Chamba)	Loam	8.85	0.484	1.23	1.48	80.22	12.45	642.54
Garola (Chamba)	Silty Clay Loam	7.81	1.074	0.71	1.47	61.12	33.75	1044.37
Khani (Chamba)	Loam	6.67	0.411	2.05	1.48	139.29	14.76	185.38
CSIR-IHBT, Palampur	Silty Clay	5.59	0.057	1.35	1.48	77.47	13.90	295.98
Saloonie (Chamba)	Sandy Loam	6.40	0.210	3.34	1.34	190.89	37.62	218.68
SEm (±)		0.12	0.00	0.04	0.03	7.25	1.45	19.35
CD (<i>P</i> = 0.05)		0.38	0.01	0.12	0.09	23.15	4.63	61.76

Table 4

Analysis of variance for determining the effect of location on oil concentration in different populations of *V. jatamansi* and on soil properties (values of mean squares are presented).

Source of variation	df	Parameters tested							
		Oil %	pH	EC _(25 °C) (dS m ⁻¹)	Organic Carbon (%)	Bulk Density (g cc ⁻¹)	Available N (kg ha ⁻¹)	Available P (kg ha ⁻¹)	Available K (kg ha ⁻¹)
Location	5	0.016**	4.07**	0.442**	3.592**	0.027**	22,306.07**	361.875**	604,904.63**
Error	12	0.00	0.042	0.00	0.003	0.002	157.491	6.529	960.476
df - degree of freedom; EC- Electrical conductivity of soil.									
** <i>P</i> < 0.01.									

Discussion

In this study, significant variations were found in the essential oil concentrations, the main attribute of *V. jatamansi*, among the naturally distributed populations in Chamba region and cultivated sources. The highest oil concentration (0.35%) was registered with the population collected from Kugti and Khani. These differences among the populations were probably due to variations in micro-climatic conditions, soil characteristics, and genetic make-up. The altitude of these two locations is higher. Although the physicochemical properties of the soils were found differences among the locations, no definite correlation was found between soil characteristics and essential oil concentration. Despite the higher altitude, the lowest concentration of oil (0.17%) was registered with the population from Garola. This location is characterized by higher EC (1.07 dS m⁻¹) and relatively low OC (0.71%) and available N (61.12 kg ha⁻¹) that might be another cause for the low concentration of essential oil in roots/rhizome. Crop-ecological factors such as light, ambient temperature and soil nutrients availability control the biosynthesis of essential oil⁴⁷. In the mountainous region, variation in micro-climate is common, and this variation influences the qualitative and quantitative parameters of medicinal and aromatic plants^{48,49,50}. The variations in essential oil concentrations in the wild populations and cultivated populations of *V. jatamansi* have also been reported earlier^{31,36,44}. Despite proper agronomic practices, the oil

concentration in the cultivated population (CSIR-IHBT) was lower than the populations from Kugti and Khani. This result was probably due to altitudinal variations and chemotype differences. The populations difference in terms of oil content has been reported from Himachal Pradesh, and the major chemical constituents and essential oil content are negatively correlated with altitude³³. The low percentage of patchouli alcohol with the population from Khani during first cycle of distillation was noticed probably due to the edaphic factor, particularly soil available K content. Patchouli alcohol is synthesized in various stages through cis- farsenylpyrophosphate⁵¹. The variations in essential oil composition of *V. jatamansi* due to growing conditions and location have also been reported^{44,52}. Thus, the population having higher amount of patchouli alcohol could be used for mass multiplication for commercial cultivation. It is also suggested that variation in oil quality can be reduced by collecting the raw material from the cultivated sources.

Despite 6 h distillation on the first day, some quantities of essential oil of *V. jatamansi* were obtained up to the third day of distillation for the populations collected from Kugti and Khani and cultivated sources (CSIR-IHBT). The populations of Rupena and Garola also produced oil on the second day. These results were because some compounds of essential oil of *V. jatamansi* like β -elemene, santalene, and seychellene were not removed from the hypodermal layer in the root cortex region. The outcome is noteworthy, and the result can be used to maximize the recovery of essential oil of *V. jatamansi*. The effects of duration of distillation time on recovery of essential oil have been reported in many aromatic plants^{39,42,45,53–58}.

In this study maximum of 18 volatile compounds were identified, which contributed up to 81.8% of the total volume (Fig. 2), and the maximum number (16) of volatile compounds were identified with the cultivated source from CSIR-IHBT during third-day distillation (Fig. 2e). This result could be due to the fact that the plants at CSIR-IHBT were grown with proper agronomic practices, which ultimately facilitated better conditions for the synthesis of a large number of compounds compared with other naturally distributed populations. The variations in genetic makeup among the populations may be another reason for changing the chemical profile of essential oil. On the other hand, the minimum number of volatile compounds were identified in the oil obtained at first-day distillation from the Garola population (Fig. 2c). The variations in the number of compounds in the essential oil of *V. jatamansi* have been reported in the literature^{4,25,30,59}. Thus, it is established from the present study that, irrespective of populations, the number of compounds has been increased either at first-day or on the second day of distillation (Fig. 2a-f).

The variations in compositions of essential oils were found noticeable due to populations and distillation methods (Table 2). The differences in crop-ecology may be another cause of variations in compositions of essential oil among the populations. In this study, variations in oil characteristics and altitudinal were observed (Table 1, 2). The variations in essential oil composition from the different parts of India have been reported in *V. jatamansi*^{31,36,43,52}. Patchouli alcohol, the most abundant compound in all the populations, was found noticeable differences, and the maximum concentrations were recorded on the first day of distillation, irrespective of populations (Table 2). The essential oil with different in fractions was probably due to boiling point, degree of solubility, molecular weight, and polarity of the compounds. The extraction of polar compounds during hydro-distillation is easy over that of terpene hydrocarbons⁶⁰.

Table 1

Geophysical positioning of different locations of sample collection

Location	Altitude (m)	Latitude	Longitude
Rupena (Chamba)	1810	32°27'68" N	76°04'54" E
Kugti (Chamba)	2140	32°34'35" N	72°27'82" E
Garola (Chamba)	2003	32°25'11" N	72°28'32" E
Khani (Chamba)	2014	32°27'39" N	76°29'1" E
CSIR-IHBT, Palampur	1354	32°06'61" N	76°33'77" E
Saloonie (Chamba)	2032	32°26'43" N	76°32'10" E

The concentration of patchouli alcohol in Saloonie (distilled in traditional method) was substantially lower compared with the rest of the populations distilled on the first day. This result could be due to the fact that patchouli alcohol was lost during the drying and storage or post-harvest practices. Low concentrations of patchouli alcohol were also found in the second and third days' oil for all populations. This result could be due to the fact that hydrolytic reactions occurred in the remaining hydrolat, which leads to the breakage of the functional group in patchouli alcohol.

However, the higher concentrations of β -patchoulene, α -guaiene, α -humulene, and δ -guaiene were found in the Saloonie sample probable due to the generation of more heat during the traditional distillation method. Disproportionation, as well as the cyclization process, occurs in

monoterpenes at elevated temperatures⁶¹. Moreover, at high temperatures compounds are formed through termination reactions⁶². Moreover, the higher concentration of humulene epoxide II (2.5% on the second day) makes difference to Garola from other populations.

Interestingly, the concentrations of seychellene were abruptly increased with subsequent days of extraction for all the populations. The use of seychellene compound as a non-selective candidate for inhibitor cyclooxygenase on pre-osteoblast cells has been reported⁶³. The α -santalene, generally used in cosmetic, perfumery, and aromatherapy industries⁶⁴, was found only in second and third days. The α -santalene is the precursor of α -santalol, which is the main component of sandalwood oil from eastern India⁶⁵. Similarly, β -elemene was found in the second and third days' oil. β -elemene is a novel anticancer agent and also reported for antitumor activity⁶⁶. The improvement and/or generation of the new compound during the second and third days was probably due to oxidation, thermal degradation, chemical degradation, eventually a chemical conversion. This finding confirmed that the distillation method can be used to obtain the higher quantity of essential oil of *V. jatamansi* with variance chemical profiles. For example, if a high-patchouli alcohol oil is desirable, 6 h distillation is required for a single day. Similarly, if high concentrations of seychellene, α -santalene, or β -elemene are desirable, roots/rhizome of *V. jatamansi* need to be distilled for second and/or third times. The effects of cyclic/repeated distillation on the dynamics of compounds in the essential oil of *V. jatamansi* are explained in the form of a schematic diagram (Fig. 6).

The results of this study have been proven the hypothesis that crop-ecology, source of materials, and distillation method determine the yield and composition of the essential oil of *V. jatamansi*. The results of the present study also demonstrate that the species *V. jatamansi* has wide adaptability, and it can be commercially grown with an altitudinal range of 1354–2140 m. It can also be grown in sandy loam to silty clay soil with a wide pH range of 5.59–8.85 and different nutritional levels.

Conclusions

The results of the present study elucidate that yield and composition of essential oil of *V. jatamansi* is largely governed by the crop-ecology and distillation methods. Our results also confirm that different chemo-types exist in natural populations in Chamba (H.P., India). The total recovery of essential oil (%) was found significant ($P \leq 0.05$) variations among the source of materials, and the maximum value (0.35%) was recorded with the populations from Kugti and Khani. All the populations are characterized by the rich in patchouli alcohol. This study also confirmed that the repetitive distillation method can be used to obtain a higher quantity of essential oil of *V. jatamansi* with variance chemical profiles. The concentrations of some compounds like seychellene, α -santalene, or β -elemene can be increased through repetitive distillation. It can also be concluded that the species *V. jatamansi* has wide adaptability, and it can be commercially cultivated with an altitudinal range of 1354–2140 m. It can also be grown in sandy loam to silty clay soil with a wide pH range of 5.59–8.85 and different nutritional levels. However, to discriminate the effects of population and crop-ecology and to identify the elite material, all the collected and/or characterized populations should be evaluated under the same conditions.

Materials And Methods

Study material

The fresh rhizomes with fibrous roots samples of four populations of *V. jatamansi* were collected from four different locations of Chamba district of Himachal Pradesh, India, which fall under the western Himalayan region. One sample was collected from a cultivated source from an Institutional experimental farm (Council of Scientific and Industrial Research-Institute of Himalayan Bioresource Technology, Palampur, India). The cultivated plant material was harvested at the age of two years. However, all the samples were collected during September. Since the wild populations were not distributed evenly, the samples were collected from about 20–30 m² area in each location. The samples were collected in three replicates from the 20–30 m² area. The population density was not uniform for all the locations. The plant collection method complies with local and national. The wild populations have been identified by Dr. Sanjay K. Uniyal (CSIR-IHBT, Palampur). The voucher specimen of this material has also been deposited in our Institutional herbarium (a recognized and publicly available herbarium). The voucher specimen number has also been received (Accession # 11701).

Harvested rhizomes and fibrous roots were washed with running tap water, and excess water was removed with the help of blotting paper. After recording the fresh weight of roots/rhizome, samples were placed in air-tight polyethylene bags and brought to the laboratory for extraction of essential oil. The same procedures were followed for the cultivated sample from the Institutional farm. Besides, one oil sample was collected in three replicate from the farmer, which was extracted through a commercial hydro-distillation unit after partial drying of the roots/rhizome in the Saloonie region (Chamba). This is the common practice, which is being followed by the growers. This sample is also considering as a cultivated source. The collected oil sample was placed in an ice bag for further analysis. Geophysical positioning of the sampling locations was recorded with the help of the Garmin-eTrex 30x GPS, and the details have been presented in Table 1.

Soil collection and analysis

The soil samples were collected from all locations, where fresh roots/rhizome were collected. The soil samples were collected up to a depth of 15 cm after removing the litter from the surface. The soil samples were also collected in three replicates from the plant sampling area. Then these soil samples were brought to the lab for further analysis. The collected soil samples were dried under shade and then grind with pestle and mortar passing through the sieve of spacing 2 mm. Prepared samples were used for the analysis of pH, electrical conductivity (EC), soil organic carbon (SOC), available nitrogen (N), available phosphorus (P), and available potassium (K). For determination of pH and EC value of the collected sample, the soil water suspension in the ratio of 1:2 was prepared as per standard method described by Jackson⁶⁷. Then the pH and EC of soil water suspension (1:2) were determined with the help of pH meter (model Eutech Instruments pH 510) and EC meter (model Century), respectively. The soil OC was analyzed by the wet oxidation method⁶⁸. Available N, P, and K were estimated as per standard methods reported in the literature^{69,70}.

Essential oil extraction

The fresh samples were chopped into small pieces and placed in 5L flasks. The samples size for all population was 400 g, and the samples included only roots and rhizomes. The size of the rhizome was upto 15 cm. The ratio between water and fresh roots/rhizome was 2:1 (v/w) during distillation in laboratory conditions. The oil was extracted through the hydro-distillation process in a Clevenger-type apparatus for 6 hours. After the collection of oil, the Clevenger apparatus was left as it is without removing the water and roots/rhizome for re-distillation. The next day, the respective Clevenger apparatus were run again without changing the water for 6 hours, and the oil recovery of recorded and collected separately. The same procedure was repeated in 3rd day. The amount of oil extracted was recorded daily, and the percentages of oil recovery (v/w) for each cycle were calculated based on the plant material used at first day. The total oil yield and oil percentage were calculated by adding all three days' recovery. Then the extracted oil was dehydrated over anhydrous sodium sulfate (Merck), and stored in sealed glass vials at 4 °C for further studies.

GC–MS analysis

The GC-MS analysis of essential oil of *V. jatamansi* was done by a QP2010 (Shimadzu Corp., Tokyo, Japan) GC–MS system, which was fitted out with an AOC 5000 Auto-injector and a ZB-5 (SGE International, Ringwood, VIC, Australia) silica capillary column with a dimension of 30 m × 0.25 mm i.d., and 0.25 µm film thickness. The temperature of the oven was programmed in such a way that started from 70°C (for 4 min) to 220°C with a slope of 4°C min⁻¹ and detention time of 5 min. The fixed temperatures for injector and interface were 240°C and 250°C, respectively. The flow rate of helium, a carrier gas, was 1.05 mL min⁻¹, and the voltage for ionization was 70 eV.

GC analysis

GC analyses of essential oil of *V. jatamansi* were carried out by a Shimadzu GC-2010 gas chromatograph (Shimadzu, Tokyo, Japan) that was attached with a flame ionization detector (FID) and a ZB-5 MS capillary column (30 m 9 0.25 mm, fused silica, and film thickness 0.25 µm). The temperature of the oven was programmed at 70°C for 3 min and gradually increased up to 220°C with a slope of 4°C min⁻¹ and detention time of 5 min. The temperatures for injector and interface were 220°C and 250°C, respectively. Then the individual compounds were quantified based on the peak-area percentage of the chromatogram.

Identification of components

The different compounds of essential oil of *V. jatamansi* were identified through using retention indices (RI) that were computed with reference to homologous series of n-alkanes (C8-C24). The compounds were confirmed through a comparison between RI and mass spectra with the National Institute of Standards and Technology mass spectral (NIST-MS) database⁷¹. The GC/ GC-MS analyses were conducted for all three replications and oil obtained at all rounds of extraction. The total percentage shares of compounds were computed by addition of the individual percentage of all identified compounds.

Experimental design and statistical analysis

The data obtained from the four populations from wild and one cultivated samples were subjected to analysis of variance (ANOVA). Differences among the populations in terms of oil concentration were assessed with the Fishers least significant differences (LSD) test values only when the *F-test* in ANOVA was found significant ($P = 0.05$). In case of soil parameters, Fishers LSD *post-hoc* test was applied to compare the means. Principal component analysis (PCA) was performed to categorize the populations based on chemical compositions of essential oil. The PCA was conducted separately for all three types of oil samples, which were obtained from three different distillation days. However, a sample received from traditional practice was included in all the PCA for comparative studies. All the ANOVA and PCA were performed with the help of statistical software (Statistica 7 software; Stat. Soft Inc., Tulsa, Oklahoma, USA). The data on compounds of

essential oil of this study were presented as mean $\pm$ standard error (SE) since many compounds were not detected during different distillation cycles.

Declarations

Acknowledgement

The authors are grateful to the Director of CSIR-IHBT, for providing the necessary facilities. The authors are also thankful to Shiv Kumar for technical support. The authors acknowledge the CSIR, Government of India, for financial support. The CSIR-IHBT communication number of this paper is 4785.

References

1. The Angiosperm Phylogeny (APG). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants, APG III. <https://doi.org/10.1111/j.1095-8339.2009.00996.x>. (2009).
2. Reveal, J. L. & Chase, M. W. : Bibliographical information and synonymy of Magnoliidae. *Phytotaxa*. **19**(1),71. [doi:10.11646/phytotaxa.19.1.4](https://doi.org/10.11646/phytotaxa.19.1.4) (2011).
3. Rather, A. M. et al. Bioactive compounds & medicinal properties of *Valeriana jatamansi* Jones-a review. *J. Life Sci.* **9**(2),952–955(2012).
4. Jugran, A. K., Bhatt, I. D., Rawal, R. S., Nandi, S. K. & Pande, V. Patterns of morphological and genetic diversity of *Valeriana jatamansi* Jones in different habitats and altitudinal range of West Himalaya, India. *Flora*. **208**,13–21(2013).
5. Mathela, C. S., Chanotiya, C. S., Sammal, S. S., Pant, A. K. & Pandey, S. Compositional diversity of terpenoids in the himalayan valeriana genera. *Chem. Biodivers.* **2**(9),1174–1182. [doi:10.1002/cbdv.200590087](https://doi.org/10.1002/cbdv.200590087) (2005).
6. Dhiman, B., Sharma, P. & Pal, P. K. Biology, chemical diversity, agronomy, conservation and industrial importance of *Valeriana jatamansi*: a natural sedative. *JARMAP*.100243. [doi:10.1016/j.jarmp.2020.100243](https://doi.org/10.1016/j.jarmp.2020.100243) (2020).
7. Rivera, J. O., Loya, A. M. & Ceballos, R. Use of herbal medicines and implications for conventional drug therapy medical sciences. *Alter. Integr. Med.* **2**(6),130. [doi:10.4172/2327-5162.1000130](https://doi.org/10.4172/2327-5162.1000130) (2013).
8. Jugran, A. K., Rawat, S., Bhatt, I. D. & Rawal, R. S. *Valeriana jatamansi*: An herbaceous plant with multiple medicinal uses. *Phytother. Res.* **33**,482–503. <https://doi.org/10.1002/ptr.6245>(2019).
9. Houghton, P. J. *Valerian the genus Valeriana*. HarwoodAcademicPublishers, pp. 155–178 (1997).
10. Mishra, L. C. *Scientific Basis for Ayurvedic Therapies*. CRC Press, New York, USA (2004).
11. Baby, R., Cabezas, M., Castro, E. & Filip, R. & Walsöe de Reca, N. E. Quality control of medicinal plants with an electronic nose. *Sens. Actuators B Chem.* **106**(1),24–28. [doi:10.1016/j.snb.2004.05.049](https://doi.org/10.1016/j.snb.2004.05.049) (2005).
12. Ming, D. S., Yu, D. Q., Yang, Y. Y. & He, C. H. The structures of three novel sesquiterpenoids from *Valeriana jatamansi* Jones. *Tetrahedron Lett.* **38**,5205–5208. [doi: 10.1016/S0040-4039\(97\)01112-X](https://doi.org/10.1016/S0040-4039(97)01112-X) (1997).
13. Prakash, V. : *A monograph on medicinally important family*. ScientificPublishers,India, pp. 1–2 (1999).
14. Violon, C., van Cauwenbergh, N. & Vercruysse, A. Valepotriate content in different in vitro cultures of *Valerianaceae* and characterization of *Valeriana officinalis* L. callus during a growth period. *Pharm. Weekbl.* **5**(5),205–209. [doi:10.1007/bf02332944](https://doi.org/10.1007/bf02332944) (1983).
15. Ernst, E. Scientific basis for ayurvedic therapies. *Focus Altern. Complement Ther.* **9**,243(2010).
16. Kaur, R. et al. In vitro propagation of *Valeriana jatamansi*. *Plant Cell, Tissue Organ Cult.* **59**,227–229. [doi: 10.1023/A:1006425230046](https://doi.org/10.1023/A:1006425230046) (1999).
17. Wagner, H., Jurcic, K. & Schaette, R. Comparative studies on the sedative action of *Valeriana* extracts, valepotriates and their degradation products. *Planta Medica.* **39**(08),358–365. [doi:10.1055/s-2008-1074930](https://doi.org/10.1055/s-2008-1074930) (1980).
18. Diapher, A. & Hindwarch, I. A double blind placebo controlled investigation of the effects of two doses of valerian preparation on the sleep, cognitive and psychomotor function of sleep disturbed order adults. *Phytother. Res.* **18**(10),831–836(2004).
19. Chopra, R., Nayar, S. & Chopra, I. 1956. *Glossary of Indian medicinal Plants, 1st Ed., New Delhi, India*: CSIRPublication; **251**, 14 (1956).
20. Pande, A. & Shukla, Y. N. Naphthoic acid derivative from *Valeriana wallichii*. *Phytochemistry.* **32**(5),1350–1351. [doi:10.1016/s0031-9422\(00\)95120-3](https://doi.org/10.1016/s0031-9422(00)95120-3) (1993).
21. You, J. S. et al. Evaluation of anxiolytic activity of compound *Valeriana jatamansi* Jones in mice. *BMC Complement Altern. Med.* **12**(1). [doi:10.1186/1472-6882-12-223](https://doi.org/10.1186/1472-6882-12-223) (2012).

22. Bagchi, P. & Hopper, W. Virtual screening of compounds with Valerianajatomansi with α -synuclein. International Conference on bioscience, biochemistry and bioinformatics. IPCBEE, Singapore. 5(3), 1–4 (2011).
23. Purohit, S. S. & Vyas, S. P. "Medicinal plant cultivation-A scientific approach Agrobios." Jodhpur, India, pp. 228 (2005).
24. Bos, R., Woerdenbag, H., van Putten, F., Hendriks, H. & Scheffer, J. Seasonal variation of the essential oil, valerenic acid and derivatives, and valepotriates in Valeriana officinalis roots and rhizomes, and the selection of plants suitable for phytomedicines. Planta. Medica. **64**(02), 143–147. doi:10.1055/s-2006-957392 (1998).
25. Sundaresan, V. et al. Impact of geographic range on genetic and chemical diversity of Indian valerian (Valeriana jatamansi) from North-Western Himalaya. Biochem. Genet. **50**(9–10), 797–808. DOI 10.1007/s10528-012-9521-5 (2012).
26. Liu, X. C., Zhou, L. & Liu, Z. L. Identification of insecticidal constituents from the essential oil of Valeriana jatamansi Jones against Liposcelis bostrychophila Badonnel. J. Chem. ID 853912. http://dx.doi.org/10.1155/2013/853912(2013).
27. Singh, S. K., Katoch, R. & Kapila, R. K. Chemotypic variation for essential oils in Valeriana jatamansi Jones populations from Himachal Pradesh. J. Essent. Oil Res. **25**(2), 154–159. doi: 10.1080/10412905.2013.767757 (2013).
28. Verma, R. S., Padalia, R. C. & Chauhan, A. Chemical differentiation of rhizome and root essential oils of Indian Valerian (Valeriana jatamansi Jones). J. Essent. Oil-Bear. Plants. **16**(6), 835–840. DOI: 10.1080/0972060X.2013.862082 (2013).
29. Raina, A. P. & Negi, K. S. Essential oil composition of Valeriana jatamansi Jones from Himalayan regions of India. In. J. Pharm. Sci. **77**(2), 218 (2015).
30. Singh, R. D. et al. Seasonal variation of bioactive components in Valeriana jatamansi from Himachal Pradesh, India. Ind. Crops Prod. **32**(3), 292–296. doi:10.1016/j.indcrop.2010.05.006 (2010).
31. Singh, S. K., Katoch, R. & Kapila, R. K. Chemotypic variation for essential oils in Valeriana jatamansi Jones populations from Himachal Pradesh. J. Essent. Oil Res. **25**(2), 154–159. doi: 10.1080/10412905.2013.767757 (2013).
32. Sati, S., Chanotiya, C. S. & Mathela, C. S. Comparative investigations on the leaf and root oils of Valeriana wallichii DC from Northwestern Himalaya. J. Essent. Oil Res. **17**(4), 408–409. doi:10.1080/10412905.2005.9698945 (2005).
33. Singh, S. K., Katoch, R. & Kapila, R. K. Genetic and biochemical diversity among Valeriana jatamansi populations from Himachal Pradesh. Sci. World J. 1–10. doi:10.1155/2015/863913 (2015).
34. Rajkumar, S., Singh, S. K., Nag, A. & Ahuja, P. S. Genetic structure of Indian valerian (Valeriana jatamansi) populations in Western Himalaya revealed by AFLP. Biochem. Genet. **49**(9–10), 674–681. doi:10.1007/s10528-011-9448-2 (2011).
35. Jugran, A. K. et al. Impact of altitudes, and habitats on valerenic acid, total phenolics, flavonoids, tannins and antioxidant activity of Valeriana jatamansi. Appl. Biochem. Biotechnol. **179**(6), 911–926 (2016).
36. Bhatt, I. D. et al. Characterization of essential oil composition, phenolic content, and antioxidant properties in wild and planted individuals of Valeriana jatamansi Jones. Sci. Hortic. **136**, 61–68. doi:10.1016/j.scienta.2011.12.032 (2012).
37. Mahajan, M. & Pal, P. K. Flower yield and chemical composition of essential oil from Rosa damascena under foliar application of Ca(NO₃)₂ and seasonal variation. Acta Physiol. Plant. **42**, 23 (2020).
38. Thusoo, S. et al. Antioxidant activity of essential oil and extracts of Valeriana jatamansi roots. BioMed. Res. Int. http://dx.doi.org/10.1155/2014/614187 (2014).
39. Zheljazkov, V. D., Astatkie, T., Jeliaskova, E. A., Tatman, A. O. & Schlegel, V. Distillation time alters essential oil yield, composition and antioxidant activity of female Juniperus scopulorum trees. J. Essent. Oil Res. **25**(1), 62–69 (2013a).
40. Zheljazkov, V. D., Astatkie, T., Horgan, T., Schlegel, V. & Schlegel, X. Distillation Time Effect on essential oil yield, composition, and antioxidant capacity of Sweet Sagewort (Artemisia annua L.) Oil. Hort. Sci. **48**(10), 1288–1292 (2013).
41. Valtcho, D., Zheljazkov, V. D. & Astatkie, T. Effect of distillation time on Mentha canadensis essential oil yield and composition. Hort. Sci. **47**(5), 643–647. DOI: https://doi.org/10.21273/HORTSCI.47.5.643 (2012).
42. Cannon, J. B., Cantrell, C. L., Astatkie, T. & Zheljazkov, V. D. Modification of yields and composition of essential oils by distillation time. Ind. Crops Prod. **41**, 214–220 (2013).
43. Verma, R. S. et al. Chemical diversity in the essential oil of Indian Valerian (Valeriana jatamansi Jones). Chem. Biodivers. **8**(10), 1921–1929. doi:10.1002/cbdv.201100059 (2011).
44. Rawat, S. et al. Essential oil composition and antioxidant activity in Valeriana jatamansi Jones: influence of seasons and growing sources. J. Essent. Oil Res. **29**(1), 101–107. DOI: 10.1080/10412905.2016.1189856 (2017).
45. Harsal, A. E. et al. Influence of extraction time on the yield, chemical composition, and antibacterial activity of the essential oil from Origanum elongatum (E. & M.) harvested at Northern Morocco. J. Essent. Oil-Bear. Plants. **21**(6), 1460–1474. DOI: 10.1080/0972060X.2019.1572545 (2018).

46. Baydar, H., Schulz, H., Krüger, H., Erbas, S. & Kineci, S. Influences of fermentation time, hydro-distillation time and fractions on essential oil composition of Damask Rose (*Rosa damascena* Mill.). *J. Essent. Oil-Bear. Plants*. **11**(3)224–232(2008).
47. Lawrence, B. M. Essential oil production. A discussion of influencing factors. in Parliament TH, Croteau R (eds) *Biogenesis of aroma. American Chemical Society, New York*, pp 363–369(1986).
48. Telci, I., Sahbaz, N., Yilmaz, G. & Tugay, M. E. Agronomical and chemical characterization of Spearmint (*Mentha spicata* L.) originating in Turkey. *Econ. Bot.* **58**(4),721–728. doi:10.1663/0013-0001(2004)058[0721:aaccos]2.0.co;2 (2004).
49. Telci, I., Bayram, E., Yilmaz, G. & Avci, B. Variability in essential oil composition of Turkish basil (*Ocimum basilicum* L.). *Biochem. Syst. Ecol.* **34**(6),489–497. doi:10.1016/j.bse.2006.01.009 (2006).
50. Rapposelli, E. et al. Relationship between soil and essential oil profiles in *Salvia desoleana* populations: preliminary results. *Nat. Prod. Commun.* **10**,1615–1618(2015).
51. Yamada, K., Kyotani, Y., Manabe, S. & Suzuki, M. Total synthesis of (±)-patchouli alcohol and (±)-seychellene via a common homoisotwistane intermediate. *Tetrahedron.* **35**,293–298(1979).
52. Das, J., Mao, A. A. & Handique, P. J. Terpenoid compositions and antioxidant activities of two Indian valerian oils from the Khasi hills of north-east India. *Nat. Prod. Commun.* **6**,129–32(2011).
53. Miguel, G. et al. *Salvia officinalis* L. essential oils: effect of hydrodistillation time on the chemical composition, antioxidant and antimicrobial activities. *Nat. Prod. Res.* **25**,526–541(2011).
54. Zheljazkov, V. D., Astatkie, T. & Hristov, A. N. Lavender and hyssop productivity, oil content, and bioactivity as a function of harvest time and drying. *Ind. Crop. Prod.* **36**(1),222–228(2012).
55. Zheljazkov, V. D., Cantrell, C. L., Astatkie, T. & Jeliazkova, E. Distillation time effect on lavender essential oil yield and composition. *J. Oleo Sci.* **62**(4),195–199(2013b).
56. Zheljazkov, V. D. et al. Essential oil yield and composition of garden sage as a function of different steam distillation times. *Hort. Sci.* **49**(6),785–790(2014).
57. Liu, X., Yang, D., Liu, J. & Ren, N. Analysis of essential oils from *Voacanga africana* seeds at different hydro distillation extraction stages: chemical composition, antioxidant activity and antimicrobial activity. *Nat. Prod. Res.* **29**,1950–1953(2015).
58. Ratri, P. J., Ayurini, M., Khumaini, K. & Rohbiya, A. Clove oil extraction by steam distillation and utilization of clove buds waste as potential candidate for eco-friendly packaging. *JBAT.* **9**(1),47–54. DOI: <https://doi.org/10.15294/jbat.v9i1.24935> (2020).
59. Jugran, A. K., Rawat, S., Bhatt, I. D. & Rawal, R. S. Essential oil composition, phenolics and antioxidant activities of *Valeriana jatamansi* at different phenological stages. *Plant Biosystems - An International Journal Dealing with all Aspects of Plant Biology.* DOI: 10.1080/11263504.2020.1810803 (2020).
60. Moghrani, H. & Maachi, R. Valorization of *Myrtus communis* essential oil obtained by steam driving distillation. *Asian J. Sci. Res.* **1**,518–524(2008).
61. Dieckmann, R. H. & Palamand, S. R. Autoxidation of some constituents of hops. I. The monoterpene hydrocarbon, myrcene. *J. Agric. Food Chem.* **22**,498–503(1974).
62. Velasco, J. & Dobarganes, C. Oxidative stability of virgin olive oil. *Eur. J. Lipid Sci. Technol.* **104**,661–76(2002).
63. Raharjo, J. S., Chanif, M., Nurdiana, N., Takheshi, K. & Fatchiyah, F. In vitro and in silico: selectivities of seychellene compound as candidate cyclooxygenase isoenzyme inhibitor on pre-osteoblast cells. *Curr. Enzyme Inhib.* **13**(1),2–10(2017).
64. Dwivedi, C. et al. Chemopreventive effects of alpha-santalol on skin tumor development in CD-1 and SENCAR mice. *Cancer Epidemiol. Biomarkers Prev.* **12**,151–156(2003).
65. Baldovini, N., Delasalle, C. & Joulain, D. Phytochemistry of the heartwood from fragrant *Santalum* species: a review. *Flavour Fragr. J.* **26**,7–26(2010).
66. Chen, W. et al. Beta-elemene inhibits melanoma growth and metastasis via suppressing vascular endothelial growth factor-mediated angiogenesis. *Cancer Chemother. Pharmacol.* **67**,799–808(2011).
67. Jackson, M. L. *Soil Chemical Analysis*. Prentice-Hall, Englewood Cliffs, New Jersey, USA (1967).
68. Walkley, A. & Black, I. A. An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **37**,29–37(1934).
69. Subbiah, B. V. & Asija, G. L. A rapid procedure for the determination of available nitrogen in soils. *Curr. Sci.* **25**,259–60(1956).
70. Mehlich, A. Mehlich-3 soil test extractant: a modification of Mehlich-2 extractant. *Commun. Soil Sci. Plant Anal.* **15**(12),1409–16(1984).
71. Stein, S. E. *Mass Spectral Database Software, Version 3.02*. Gaithersburg, MD: National Institute of Standards and Technology (2005).

Figures

Figure 1
Concentration of essential oil in the different populations of *V. jatamansi* under repetitive distillation method

Figure 2

Number of volatile compounds identified in the essential oil of *V. jatamansi* under repetitive distillation method

Figure 3

Representative GC/MS chromatograms of *V. jatamansi* oil sample

Figure 4

The dynamic of patchouli alcohol (a) seychellene (b) δ -guaiene (c) and α -patchoulene (d) under repetitive distillation method for the different populations

Figure 6

Schematic diagram showing the effects of cyclic/repeated distillation on the dynamics of patchouli alcohol, α-santalene, and seychellene in the essential oil of *V. jatamansi*. The concentrations of patchouli alcohol were sharply declined during subsequent days of extraction. In contrast, the percentages of seychellene and α-santalene were abruptly increased with subsequent days of extraction in all the populations.