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Research Paper

Aroma profile of two *Juniperus* species from Alpine region in Uttarakhand

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ABSTRACT

The leaves (needles) of *Juniperus communis* var. *saxatilis* and *Juniperus wallichiana* were collected from three populations of North-West Himalaya. The essential oils were analyzed by GC and GC/MS in order to determine quantitative and qualitative variation in the oil composition. *J. communis* essential oils were characterized with α-pinene, as a first principal component in three investigated oils (31.8-49.5%). Limonene was the second major constituent (13.7-19.5%), whereas δ-3-carene afforded 14.7% and 9.7% in Oil 'A' and 'B', respectively and only 0.1% in the Gomukh oil of *J. communis*. In *J. wallichiana* oils, the major component was sabinene (32.5-51.0%) followed by terpinen-4-ol (7.3-14.0%). α-Pinene, the major compound of *J. communis* essential oils, constituted 6.2-12.6% in *J. wallichiana*. The amount of monoterpenes (76.2-81.4% in *J. communis*; 69.1-76.3% in *J. wallichiana*) dominated in each oil sample of both the species.

Keywords: *Juniperus communis; Juniperus wallichiana*; Essential oil; α-pinene; Sabinene.

INTRODUCTION

Juniperus L. (*Cupressaceae*), a genus of evergreen aromatic shrubs or trees is distributed in temperate and cold regions of the northern hemisphere (Anonymous, 2001). In alpine north-western Himalaya (Uttarakhand), four species of *Juniperus* viz. *J. communis*, *J. wallichiana*, *J. recurva* and *J. squamata* have been reported (Naithani, 1985). In Uttarakhand, *J. communis and J. wallichiana* commonly grow on dry rocky and sandy slopes and during survey we observed that these are used as incense by the local inhabitants. The essential oil is also widely used in the perfumery, cosmetics and pharmaceutical industries (Chatzopoulou and Katsiotis, 2006) Essential oils of *Juniperus* species reported antifungal activity (Cavaleiro, et al., 2006). It is well reported that great variability occurred in *Juniperus* species oil composition and detected α-pinene, sabinene, limonene, terpinen-4-ol, trans sabinyl acetate, β-thujone as the major components in *J. communis* and *J. wallichiana* from different parts of the globe (Chatzopoulou and Katsiotis, 1993; 2006; Adams and Chaoudhary, 1996; Pande and Mathela, 2000; Srivastava, et al., 2005; Butkiene, et al., 2006; Cavaleiro, et al., 2006; Gonny, et al., 2006; Kumar, et al., 2007; Chanotiya and Mathela , 2007; Adams, et al., 2010; Lohani et al., 2010).

In spite of above fact there is a lot of possibility to vary essential oils constituents both qualitative and quantitative in species among and within species collected even same place, therefore present study access the comparative assessments between two *Juniperus* species is being reported first time from same locations with the aim of establishing aroma profile of *Juniper from* Uttarakhand Himalaya.

MATERIALS AND METHODS

***Plant Material*:** *J. communis* and *J. wallichiana* were collected during September-October 2009 from three different Himalayan localities (Uttarakhand, India), viz., Sample A (Badrinath, altitude 3350m), Sample B (Vasudhara, altitude 3500m) and Sample C (Gomukh Glacier, altitude 4000m). The plant specimens were identified based on the herbarium records with Botanical Survey of India (BSI), northern circle, Dehradun. The voucher specimens have been deposited in the herbarium of BSI (BSD Acc. No. 112766-112771).

***Extraction of oil*:** Shade dried needles (leaves) of each plant population (300g) were separately hydro-distilled for 3 hours using a Clevenger apparatus (Clevenger, 1928). The oil contents [(v/w) (%)] were estimated on a dry weight basis. The oil samples obtained were dehydrated over anhydrous sodium sulphate and stored in sealed vials at 4°C before analysis

***Analyses of the essential oils*:** GC analyses were carried out by an Agilent Technology 6890 N gas chromatograph data handling system equipped with a split/split less injector and fitted with FID using N_2 as the carrier gas. The column was HP-5 capillary column (30m x 0.32mm, 0.25µm film thickness) and temperature program was used as follows: initial temperature of 60°C (hold: 2min) programmed at a rate of 3°C/min to a final temperature of 220°C (hold: 5min). Temperatures of the injector and FID were maintained at 210°C and 250°C, respectively.

GC/MS analyses were performed with a Perkin Elmer Clarus 500 gas chromatograph equipped with a split/split less injector (split ratio 50:1) data handling system. The column was Rtx®-5 capillary columns (60m x 0.32mm, 0.25µm film thickness). Helium (He) was the carrier gas at a flow rate 1.0ml/min. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the EI^+ mode. The mass spectra were generally recorded over 40-500 amu that revealed the total ion current (TIC) chromatograms. Temperature program was used as the same as described above for GC analyses. The temperatures of the injector, transfer line and ion source were maintained at 210°C, 210°C and 200°C, respectively.

The components of the oils were identified by comparison of their mass spectra with those of commercial libraries (NIST/ Pfleger /Wiley) or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in literature (Adam, 2009).

RESULTS

In *J. communis*, the oil yield was found maximum in Badrinath (2.2%) followed by 1.9% and 1.8% in Gomukh and Vasudhara oils, respectively. The present results revealed that monoterpene hydrocarbons dominated in all the samples (76.2-81.4%). Among these, α-pinene (31.8-49.5%) and limonene (13.7-19.5%) were the main components and clearly dominated in all the oils. It is interesting to note that δ-3-carene detected as the third main constituent in oil 'A' (14.7%) and 'B' (9.7%), but only 0.1% in the sample collected from Gomukh Glacier. Other important compounds were sabinene (0.8-6.7%), β-myrcene (2.4-5.6%), β-pinene (2.1-4.3%) and α-terpinyl

acetate (1.7-2.9%). The hydro-distillation of *J. wallichiana* needles produced light yellowish colour volatile oils with yields of 2.4% (oil B) and 2.1% (oil A), whereas oil 'C' afforded comparatively lesser amount 1.3%. Monoterpenoids (84.9-88.4%) comprised the most abundant class of compounds in the oils having monoterpene hydrocarbons (69.1-76.3%) as the predominant group followed by oxygenated monoterpenes (8.6-19.2%). Sabinene, the most abundant component in all the three *J. wallichiana* oils, constituted 32.5-51.0%. Terpinen-4-ol (7.3-14.0%) was detected as second major component, whereas *α*-pinene, the most abundant compound of *J. communis* oils, afforded 6.2-12.6%. *δ*-3-carene, which found in appreciable amount in two *J. communis* oils of the present investigation was absent in *J. wallichiana* oils. Other important compound limonene was detected in lesser amount in *J. wallichiana* oils (1.6-3.8%), though it was second major component in *J. communis* oils.

DISCUSSION

Showed qualitative similarity to the oils of present investigation another study conducted in *J. wallichiana* leaf oil from North-Western Himalaya also showed the presence of sabinene, terpinen-4-ol and α-pinene as the most abundant components, while quantitative variations occurred on the basis of comparison with present investigation (Kumar, et al., 2007). Pande and Mathela, 2000, detected that *J. communis* oil sample has sabinene (48.8%). Market sample is reported to contain sabinene (22.8%), β-pinene (10.7%) and γ-cadinene (10.6%) as the major components (Kumar, et al., 2007). These reports resemble with our tune in *J. wallichaina.* The leaf (needle) essential oils of *J. communis* var. *communis* reported α-pinene chemotype (Adams, 1998; 2004, Lawrence, 2006), agreement with our present investigation**.** These major compounds α-pinene were also recommended for pharmaceutical uses (Teuscher, 2006) and is widely used in perfumery industry due to the pleasant aroma Limonene used in the preparation of commercially available flea shampoos, mosquito, repellents and different agrochemicals (Dubey, et al., 2010). The bicyclic monoterpenes *cis*-sabinene hydrate and *cis*-sabinene hydrate acetate are considered to be responsible for the special flavor of marjoram (*Origanum majorana*) (Johannes, 2002). It is well reported that terpence content of distilled volatile oils have been known to differ enormously due to different geographical locations and between and among the species of the sample plants (Mathela, et al., 2008; Verma, et al., 2010). Variation of essential oil and its components associated with the different pheonological stage as well as seasons reported in Lamiaceae member *Oreganum vulagre* (Pino, et al., 2003). Variations in the chemical composition are possibly due to the variation of soil characteristics and the climatic conditions of the collection site.

CONCLUSION

J. communis dominated by monoterpene hydrocarbons in all the samples (76.2-81.4%) with α-pinene and limonene as the major components. *J. wallichiana* dominated by monoterpenoids (84.9-88.4%) with sabinene and terpinen-4-ol which found abundant for commercial explorations for industry.

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Table-1: Compounds identified in the essential oil of leaves of *J. communis* and *J. wallichiana.*

Components	$RI_{exp.}$	$RI_{lit.}$	% in Oil					
			J. communis			*J. wallichiana*		
			A	B	C	A	B	C
α-thujene	931	924	-	-	-	1.5	2.2	2.5
α-pinene	939	932	31.8	49.5	45.8	12.6	6.2	12.2
α-fenchene	945	945	0.9	0.3	0.1	-	-	-
camphene	953	946	0.3	0.2	0.8	-	-	0.2
sabinene	976	969	6.7	0.8	2.0	45.6	51.0	32.5
β-pinene	980	974	2.3	2.1	4.3	0.3	0.1	0.4
β-myrcene	991	988	3.1	2.4	5.6	4.0	3.2	4.7
δ-2-carene	1001	1001	0.2	0.1	0.5	0.2	-	0.3
δ-3-carene	1011	1008	14.7	9.7	0.1	-	-	-
α-terpinene	1018	1014	-	0.2	0.8	2.9	2.2	4.5
p-cymene	1026	1020	0.5	0.2	0.2	-	1.5	0.3
limonene	1031	1024	19.5	13.7	14.5	3.8	1.6	2.9
1,8-cineole	1033	1026	-	-	0.1	0.3	0.3	0.5
γ-terpinene	1062	1054	0.2	0.1	0.3	4.7	3.9	6.4
cis-sabinene hydrate	1068	1065	-	-	-	0.3	0.3	1.2
terpinolene	1088	1086	1.2	0.8	1.2	0.7	0.9	2.2
trans-sabinene hydrate	1097	1098	-	-	-	-	0.3	1.0
linalool	1098	1095	0.2	0.1	0.2	0.2	0.7	0.1
p-menth-2-en-1-ol	1121	1118	0.1	0.1	0.5	-	0.5	1.0
cis-verbenol	1141	1137	0.2	-	0.4	-	-	-
terpinen-4-ol	1177	1174	1.8	0.4	0.7	7.3	13.5	14.0
p-cymen-8-ol	1183	1179	0.2	0.3	0.1	-	-	-
α-terpineol	1189	1186	0.2	0.2	0.1	0.2	-	0.7
citronellol	1228	1223	0.2	0.2	0.2	-	-	-
myrtenyl acetate	1235	1224	0.2	-	0.4	-	-	-
methyl citronellate	1261	1257	0.4	0.1	1.2	-	-	0.2
bornyl acetate	1285	1284	0.7	0.5	1.2	-	-	0.4
α-terpinyl acetate	1350	1346	2.9	1.7	2.2	0.3	-	0.1
α-cubebene	1351	1345	-	-	-	0.1	0.2	-
β-cubebene	1390	1387	0.2	0.4	-	1.5	-	0.3
β-elemene	1391	1389	1.4	1.0	0.7	-	-	-
γ-elemene	1430	1434	-	-	1.0	-	-	-
trans-α-bergamotene	1431	1434	-	1.5	-	-	-	-
germacrene D	1483	1484	0.1	0.9	0.8	0.2	0.2	-
α-muurolene	1499	1500	0.5	0.3	0.4	0.6	0.1	0.2
α-bisabolene	1504	1505	0.2	0.3	-	-	-	-
β-bisabolene	1509	1506	0.4	0.9	0.2	-	-	-
γ-cadinene	1513	1513	0.7	1.5	0.8	0.8	-	-
δ-cadinene	1524	1522	2.5	1.7	1.8	3.4	0.9	1.0
hedycaryol	1530	1546	-	-	-	0.7	-	1.4
elemol	1549	1548	0.3	0.2	-	1.3	1.9	-
germacrene B	1556	1559	0.1	0.2	0.3	-	-	-
β-eudesmol	1649	1649	-	-	-	0.8	-	0.8
α-cadinol	1653	1652	-	-	2.4	1.3	1.0	1.0
α-bisabolol	1685	1685	-	1.5	0.6	-	-	-
Monoterpene hydrocarbons			81.4	80.1	76.2	76.3	72.8	69.1
Oxygenated monoterpenoids			7.1	3.6	7.3	8.6	15.6	19.2
Sesquiterpene hydrocarbons			6.1	8.7	6	6.6	1.4	1.5
Oxygenated sesquiterpenoids			0.3	1.7	3	4.1	2.9	3.2
Total identified			**94.9**	**94.1**	**92.5**	**95.6**	**92.7**	**93.0**

- A=Badrinath, B=Vasudhara, C=Gomukh Glacier

Table -2: Main constituents (>10%) of the leaf (needle) oils of *Juniperus* species from India.

Species	Source	Main components	References
J. communis	Cultivated in a garden (Nainital)	sabinene (48.8%)	7
	Dry leaves (needle) procured from market, Moradabad	sabinene (22.8%) β-pinene (10.7%) γ-cadinene (10.6%)	10
	Wildly growing in Shibok (Niti valley)	α-pinene (35.4%) Limonene (23.8%)	12
J. wallichiana	Wildly growing in Milam region	sabinene (46.7%)	11
J. indica *syn. J. wallichiana*	Wildly growing in Shibok (Niti valley)	sabinene (27.8%) Terpinen-4-ol (16.1%)	12
J. macropoda	Wildly growing in Hindolkhal	β-elemene (10.7%)	14
	Wildly growing in Mussorie	α-thujene (35.4%)	

