COMPARATIVE ANALYSIS OF ESSENTIAL OIL CONTENTS OF *JUNIPERUS EXCELSA* M. BEIB. FROM BALOCHISTAN

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Abstract

Cones/berries of *Juniperus excelsa* from three provenances in Balochistan, Pakistan were collected and essential oil was extracted by solvent method. Oil contents were analyzed on Gas Chromatography Mass Spectrometry (GCMS). Identification and quantification was made by using Wiley and NIST library and HP Chemstation software. Quantitatively the mean berry oil yield was 5.8%, 6.5% and 4.5% for Ziarat, Zarghoon Ghar and Harboi District Kalat respectively. Ziarat being the richest in number of compounds while Harboi in compound diversity. Quantitative and qualitative differences were found among three locations. Ziarat was found to be rich in α-Pinene, Cedrol, Camphene, Copaene, Phyllocladene, Ferruginol, Podocarp-7-en-3-one, and Pimara-8(14), 15-dien. Zarghoon Ghar was found to be rich in α-Pinene, Limonene, Germacrene D, widdrol, Phyllocladene, Ferruginol, Androst-4-ene-3,6-dione and Harboi was represented by α-Pinene, β-Myrcene, Limonene, Cedrene, Clovene, Cadinene, Patchoulene, Cedrol, Spathulenol, Abietatriene, Norkaur, Pimaric acid and Neoabietic acid. In all 99 compounds are reported for the first time from the oil of cones of *Juniperus excelsa* in Balochistan.

Introduction

The genus *Juniperus* belongs to the family *Cupressaceae* consisting of 55 species, all of which occur throughout the northern hemisphere of the world (Farjon 1998) except *Juniperus procera* which is the only species of the genus that grows naturally in the southern hemisphere (Adams 1993). In Balochistan *J. excelsa* has natural stands distributed between 20° 9'N and 30° 37'N and between 67° 1'E, as well as in some isolated dry valleys from 1200m to 3000m above sea level (Rafi 1965). There are three spatially segregated pockets of Juniper forests, namely Zarghoon, Ziarat and Harboi (in districts Quetta, Ziarat and Kalat respectively). Balochistan has approximately 141,000 hectors of *Juniperus excelsa* forests, out of which about 86,000 hectors of these are found in Ziarat and Loralai districts. *J. excelsa* trees typically grow as pure stands, and form characteristically open and multistoried forests between elevations 2000 to 3000 meters (Sheikh 1985). Conifers are known as a renewable source of essential oil. Oil contents are believed to be genetically determined and little influenced by the environment. Besides having economic value oil contents play an important role in plant defense system against fungus and insect attacks (Koukos *et al* 2001, 2002). In addition to genetic makeup the oil composition of Junipers has also been reported to change due to geographic variation, age of plant, season of harvesting, and inter-specific differences (Medini, 2010; Almaarri 2010; Adams *et al.*, 1993 ;Alam, 2001). The wood of juniper is used for fuel, beams and for pencil making. The wood and berries are also used as incense. Cone (berry) is traditionally used as a medicine by the local people (WWF 1998). Juniper berry oil is known to be an antiseptic, analgesic, and sedative and has been reported to cure tuberculosis, jaundice, and eczema among so many other ailments (Adams 1999; Ucar 2002; Marina *et al*. 2004; Unlu *et al*. 2008; Derwich *et al*. 2010; Orav *et al*. 2010). Variation in essential oil contents of conifer species due to multiple factors has been reported by many investigators but previously no work has been conducted to investigate the cone oil contents of *Juniperus excelsa* in Balochistan. The purpose of this study was to investigate the composition of berry oil contents of *Juniperus excelsa* and compare the quantitative and qualitative differences among the three different natural stands of Juniper forests.

Materials and Methods

Plant material and extraction: cone/berries were collected from the Juniper forests of Ziarat valley (2700m elev.), Zarghoon Ghar (2500m elev.) and Harboi (2350m elev.) District Kalat. Samples were collected from healthy mature trees having bluish-black ripe cones in fall October 2006. Ten trees were randomly selected in each location and berries were collected from all sides of the crown. Seeds were manually separated from the pulp. The oil was isolated from crushed dried juniper berries (100 g) by SDE with n-hexane (Fluka >99.0%) as solvent (0.5 mL), using a Marcusson type micro-apparatus (Bicchi *et al.*, 1990). The SDE process was carried out for 2 h. The oil amount (%) was determined using n-tetradecane (Reachim >99.9%) as the internal standard (2 μL). The reproducibility of three parallel SDE procedures with a single juniper sample showed the variation coefficients were below 20%.

***Chemical analysis*: GC and GC-MS Analysis:** For GC and GC-MS, SPB-5 and DB-5 MS columns (30 m length, 0.32 mm ID and 0.22 μm df) were used respectively. Both the GC and GC-MS analysis were performed with the identical gradient thermal ramping and temperatures as follows: the column was kept at 50°C for two min, and then ramped to 260°C at a rate of 5°C. In case of GC, injector and detector were kept at 260°C. For GC-MS injector was kept at 260°C, transfer line kept at 280°C and EIMS was operated at 70 eV, at 250°C. Helium (He) was used as carrier with a flow of 1.8 ml/min. 5μl of filtered sample was injected at a split ratio of 1:40.

GC-FID was performed on Shimadzu (Japan) GC-17A coupled with Class GC-10 software while GC-MS was performed on an Agilent (USA) GC 6890 coupled with Jeol (Japan), 600H MS. For the spectral library search, NIST 2008 was used. The quantization was performed using area normalization method on Class GC-10 software.

Table 1. The composition of essential oil *Juniperus excelsa* cone extracts as percentage of total peak area

S.No	Compound	Ziarat	Zarghoon Ghar	Harboi
1	α-Pinene	15.92	1.70	15.92
2	β-Pinene	1.48	n. d.	n. d.
3	Camphene	2.96	n. d.	n. d.
4	Limonene	0.60	1.32	1.46
5	Terpinene α	n. d.	n. d.	0.81
6	β-Myrcene	n. d.	n. d.	1.09
7	n-Butyl butyrate	0.12	n. d.	0.12
8	Calarene	n. d.	0.07	n. d.
9	Bornyl acetate	1.00	n. d.	0.84
10	Cedrene	0.92	n. d.	1.73
11	Himachala-2,4-diene	0.46	0.17	n. d.
12	α- Cedrene	2.11	n. d.	n. d.
13	Copaene	0.06	n. d.	n. d.
14	Cubenol	n. d.	0.15	n. d.
15	Guaiene	n. d.	0.17	n. d.
16	Elemene	n. d.	0.28	n. d.
17	Germacrene D	n. d.	1.24	n. d.
18	Thujopsene-(12)	n. d.	0.92	n. d.
19	Guaia-1(5),7(11)-diene	n. d.	0.32	n. d.
20	Cyprene	n. d.	0.45	n. d.
21	Cyclosativene	n. d.	0.11	n. d.
22	Clovene	n. d.	n. d.	0.47
23	Cadinene	n. d.	n. d.	1.16
24	Patchoulene	n. d.	n. d.	1.76
25	3-Hexyloxyacetophenone	1.56	n. d.	n. d.
26	Cycloisolongifolene	0.38	0.11	n. d.
27	Nonane	1.51	n. d.	n. d.
28	Cedrol	8.50	n. d.	8.63
29	Lanceol,cis	0.54	0.20	n. d.
30	2-(3,4-methylenedioxyphenyl)-1-methylhexahydropyremidine	n. d.	0.12	n. d.
31	7R,8R-8-hydroxy-4-isopropylidene-7-methylbicyclo(5,3,1)undec-1-ene	n. d.	0.18	n. d.
32	Spathulenol	n. d.	n. d.	0.41
33	2,2,7,9-tetramethyl-3oxatricyclo dodecane	0.79	n. d.	n. d.
34	Widdrol	n. d.	1.94	0.57
35	Selina-6-en-4-ol	n. d.	0.44	0.66
36	8βH-cedran-8-ol	1.00	n. d.	n. d.
37	n-Hexadecanoic acid	0.79	0.20	1.35
38	1H-cyclopenta[a] phenanthrene	n. d.	n. d.	0.17
39	Abietatriene	n. d.	0.80	2.21
40	2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentaene	1.83	n. d.	0.85

S.No	Compound	Ziarat	Zarghoon Ghar	Harboi
41	Octahydrophenanthrene	n. d.	0.44	n. d.
42	Phyllocladene,(-)-	2.57	3.97	10.73
43	Kaur-16-ene,(8β,13β)-	0.50	n. d.	0.50
44	Cupressene	0.27	2.60	n. d.
45	Indan,2-butyl-5-hexyl-	1.70	n. d.	n. d.
46	Norkaur-15-ene,13,methyl-,(8β,13β)-	1.00	1.21	2.94
47	6-Methyl-benzo[c] phenanthrene	0.93	n. d.	n. d.
48	Unidentified Diterpene (MW = 275)	n. d.	n. d.	0.39
49	4-epiabietal,dehydro	n. d.	0.67	n. d.
50	Preg-17(20)-ene	0.49	0.18	n. d.
51	Ferruginol	1.79	1.68	1.46
52	Unidentified (MW = 286)	3.72	14.28	n. d.
53	Androstadien-17β-ol-3-one	0.25	0.78	n. d.
54	Unidentified (MW = 286)	0.31	n. d.	n. d.
55	Podocarp-7en-3-one-13β-methyl-13-vinyl-	2.03	3.44	3.51
56	Totarol	n. d.	0.61	0.60
57	Unidentified (MW = 286)	n. d.	4.17	n. d.
58	Androst-4-ene-3,6-dione	8.18	8.81	n. d.
59	Unidentified (MW = 286)	n. d.	1.61	n. d.
60	Unidentified (MW = 286)	n. d.	0.16	n. d.
61	Unidentified (MW = 286)	n. d.	n. d.	0.84
62	Unidentified (MW = 286)	n. d.	n. d.	0.61
63	Verticiol	0.44	n. d.	n. d.
64	Unidentified (MW = 288)	0.73	n. d.	n. d.
65	Chrysene	n. d.	n. d.	0.55
66	9(1H)-Phenanthrenone	0.63	1.35	0.23
67	Tretinoin	0.09	n. d.	n. d.
68	Abieta-8,11,13-trien-	n. d.	0.61	0.50
69	Unidentified (MW = 302)	0.55	n. d.	n. d.
70	Unidentified (MW = 302)	4.58	n. d.	n. d.
71	Pimara-8(14),15-dien	4.31	3.92	5.85
72	Unidentified (MW = 302)	0.90	n. d.	n. d.
73	Unidentified (MW = 302)	0.31	n. d.	n. d.
74	Unidentified (MW = 302)	n. d.	4.15	n. d.
75	Unidentified (MW = 302)	n. d.	1.50	n. d.
76	Unidentified (MW = 302)	n. d.	1.34	n. d.
77	Unidentified (MW = 302)	n. d.	8.42	n. d.
78	Unidentified (MW = 302)	n. d.	4.15	n. d.
79	Pimaric acid	n. d.	n. d.	1.22
80	Neoabietic acid	n. d.	n. d.	2.77
81	Unidentified Diterpene (MW = 316)	1.95	n. d.	n. d.
82	Unidentified Diterpene (MW = 316)	1.79	n. d.	n. d.
83	Unidentified Diterpene (MW = 316)	1.79	n. d.	n. d.
84	Unidentified Diterpene (MW = 329)	n. d.	n. d.	0.60
85	Unidentified Diterpene (MW = 329)	0.33	n. d.	n. d.
86	Unidentified Diterpene (MW = 330)	1.08	n. d.	n. d.
87	Unidentified Diterpene (MW = 330)	0.65	n. d.	n. d.
88	Unidentified Diterpene (MW = 330)	0.09	n. d.	n. d.
89	Unidentified Diterpene (MW = 330)	n. d.	1.60	n. d.

S.No	Compound	Ziarat	Zarghoon Ghar	Harboi
90	Unidentified Diterpene (MW = 330)	n. d.	0.61	0.50
91	Unidentified Diterpene (MW = 330)	n. d.	2.12	n. d.
92	Unidentified Diterpene (MW = 330)	n. d.	n. d.	1.49
93	Unidentified Diterpene (MW = 330)	n. d.	n. d.	0.06
94	Unidentified Diterpene (MW = 393)	0.42	n. d.	n. d.
95	Unidentified Diterpene (MW = 406)	1.83	n. d.	n. d.
96	Unidentified Diterpene (MW = 406)	n. d.	1.55	n. d.
97	Unidentified Diterpene (MW = 406)	n. d.	n. d.	2.37
98	Cholest-7en-3-ol-15-one,14-methyl-	n. d.	0.10	n. d.
99	10-Methyldotriacontane	n. d.	0.35	0.46

MW= Molecular Weight; n. d. = Not Detected

Results and Discussion

Ninety Nine Compounds with their relative mean percentage at three different locations are listed in table 1. The oil contents of *Juniperus excelsa* are consisting of monoterpenes, sesquiterpenes, diterpenes and oxygenated hydrocarbons. The monoterpene content of oil was 20.96, 3.02 and 19.28% at Ziarat, Zarghoon Ghar and Harboi, respectively. Among monoterpenes α-Pinene was 15.92 % at Ziarat and Harboi while Zarghoon Ghar was 1.70 %. Sesquiterpenes were 15.11% at Ziarat, 6.75 % at Zarghoon Ghar and 14.82% at Harboi. Among sesquiterpenes Cedrol was most abundant at Ziarat and Harboi but it was not detected at Zarghoon Ghar. Diterpene content of oil was 20.35% Ziarat, 25.70% Zarghoon Ghar and 20.68% Harboi. Oxygenated hydrocarbons include Bornyl acetate, dodecane, 3- Hexyloxyacetophenone, n-Hexadecanoic acid, Phenanthrene, Verticiol, Pimara, Tretinoin, Guaiene, Widdrol, Selina-6-en-4-ol.

Table 1 shows 12 compounds unique to Ziarat are β-Pinene, Camphene, Alpha Cedrene, Copaene, 3-Hexyloxyacetophenone, 2,2,7,9-tetramethyl-3oxatricyclo dodecane, 8βH-cedran-8-ol, Nonane, Tretinoin, Verticiol, 6-Methyl-benzo[c] Phenanthrene, Indan,2-butyl-5-hexyl-, while 14 compounds unique to Zarghoon Ghar include Calarene, Cubenol, Guaiene, Elemene, Germacrene D, Thujopsene-(12), Guaia-1(5),7(11)-diene, Cyprene, Cyclosativene, 2-(3,4-methylenedioxyphenyl)-1-methylhexahydropyremidine, 7R,8R-8-hydroxy-4-isopropylidene-7-methylbicyclo(5,3,1)undec-1-ene, Octahydrophenanthrene, 4-epiabietal dehydro, Cholest-7en-3-ol-15-one,14-methyl-, and 10 compounds unique to Harboi are Terpinene α, β-Myrcene, Clovene, Cadinene, Patchoulene, Spathulenol, Chrysene, Pimaric acid, Neoabietic acid 1H-cyclopenta(a) phenanthrene.

There are 9 compounds which were found common to all the three locations, these include α-Pinene, Limonene, n-Hexadecanoic acid, Phyllocladene, Norkaur-15-ene,13,methyl-, (8β,13β), Ferruginol, Podocarp-7en-3-one-13β-methyl-13-vinyl-, 9(1H)-Phenanthrenone, Pimara-8(14),15-dien.

Conclusion

1. Quantitative and qualitative differences in the cone oil of *Juniperus excelsa* found at three different locations in Balochistan Pakistan may be considered primarily due to geographic variation.
2. Although Ziarat and Zarghoon Ghar areas of Juniper forests are located adjacent to each other, there is more similarity of compounds between Zarghoon Ghar and Harboi which are distantly located from each other almost 100 km apart, therefore, it may be concluded that Juniper forests of Harboi are the extensions of Zarghoon Ghar or vice versa.
3. Compound diversity among the three locations and the compounds unique to each location call for further investigation and determination of phyllogenetic relationship of the forests on the genomic level.
4. Given the economic and medicinal significance of essential oils of *Juniperus excelsa*, in future the forest potential in this regard may be exploited.

References

Adams, R.P. (1999). Investigation of *Juniperus* species of the United States for new sources of Cedar wood oil. *Economic Botany*. 41: 48-54.

Adams, R.P. (2001). Geographical variation in leaf essential oils and RAPDs of *Juniperus polycarpos* K. Koch in central Asia. *Biochemical Systemics and Ecology* 29: 609-619.

Adams, R.P., Demeke, T. and Abulfatih, H.A. **(**1993). RAPD DNA fingerprints and terpenoids: clues to past migrations of *Juniperus* in Arabia and east Africa *Theor. Appl. Genet* 87: 22-26.

Almaarri, K., Alamir, L., Yasmin, J. and De-Yu Xie. (2010). Volatile compounds from leaf extracts of *Juniperus excelsa* growing in Syria via gas chromatography mass spectrometry. *The Royal society of Chemistry Anal. Methods* 2: 673-677.

Bicchi, C., Amato, D., Nano, A. and Frattini, C. (1990). Improved method for the analysis of small amounts of essential oils by microdistillation followed by capillary gas chromatography. *Journal of Chromatography* 279: 409-416.

Derwich, E., Benziane, Z., Taouil, R., Senhaji, O. and Touzani, M. (2010). A Comparative study of the chemical composition of the leaves volatile oil of *Juniperus phoenicea* and *Juniperus oxycedrus* Middle-East. *Journal of scientific Research* 5(5): 416-424.

Farjon, A. (1998). world checklist and bibliography of conifers. Royal Botanic Gardens, Kew chemistry of natural compounds 44(1): 2008.

Koukos, P.K., Papadopoulou, K.I. and Papagiannopoulos, A.D. (2002). Variation in the chemical composition of the berry oil of *Juniperus* oxycedrus L grown in north and west Greece. *Holz als Roh-und werkstoff* 60: 152-153.

Koukos, P.K., Papadopoulou, K.I. and Papagiannopoulos, A.D. (2001). Essential oils of the twigs of some Conifers grown in Greece. *Holz als Roh-und werkstoff* 58: 437-438.

Marina, D., Sokovic, R.M. and Grubisic, D. (2004). Chemical composition and antifungal activity of the essential oil from *Juniperus excelsa* berries. *Pharmaceutical Biology* 42: 328-331

Medini, H., Elaissi, A., Khouja, M.L., Chraief, I., Farhat, F., Hammami, M., Chemli, R. and Skhiri, H.F. (2010). Leaf essential oil of *Juniperus oxycedrus* L. (Cupressaceae) harvested in northern Tunisia: composition and intra-specific variability. *Chem Biodivers* 7(5):1254-66

Orav, A., Kailas, T. and Müürisepp, M. (2010). Chemical investigation of the essential oil from berries and needles of common juniper (*Juniperus communis* L.) growing wild in Estonia. *Natural Product Research* 24: 1789-1799.

Rafi, M. (1965). Vegetation types of Balochistan Province. Pakistan Govt, Printing Press, Lahore, Pakistan.

Samoylenko, V., Dunbar, D.C., Gafur, M.A., Khan, S.I., Ross, S.A., Mossa, J.S., El-Feraly, F.S., Tekwani, BL., Bosselaers, J. and Muhammad, I. (2008). Antiparasitic, nematicidal and antifouling constituents from *Juniperus* berries. *Phytother Res*. 22(12): 1570-6.

Sheikh, I.S. (1985). Afforestation in Juniper forests of Balochistan. Pakistan Forest Institute, Peshawar, Pakistan.

Ucar, G. and Balaban, M. (2002). The composition of volatile extractives from the wood of *Juniperus excela, Juniperus foetidissima and Juniperus oxycedrus. Holz als Roh-und werkstoff* 60: 356-362

Unlu, M., Vardar-Unlu, G., Vural, N., Donmez, E. and Cakmak, O. (2008). Composition and antimicrobial activity of *Juniperus excelsa* essential oil. *Chemistry of Natural Compounds* 44: 100-101.

WWF. (1998). Assessment of biodiversity resource of Zarghoon Juniper ecosystem. A report by WWF Pakistan. pp 28.