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Original Article

Variation in the essential oil of *Origanum vulgare* (L) growing at different geographical locales of Kashmir Himalayas, India

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ABSTRACT

The agro climatic conditions of Kashmir valley provide an ideal habitat for the growth of variety of aromatic and medicinal plant species. The present study was conducted to analyse the essential oils obtained from *Origanum vulgare* (L.) family Lamiaceae and the variability in oil composition of the same species growing at different geographical locales viz. Srinagar, Pulwama and Tangmarg regions of Kashmir. The essential oil was obtained by hydro distillation of the aerial parts and analysed by Gas Chromatography coupled with Mass Spectrometry (GC-MS) for their chemical composition. Overall (34) thirty four different constituents were isolated and identified in its oil and the major compounds found are Gamma terpinene, Paracymene, Carvacrol, Cymene, Sabinene and Cis- sabinene hydrate acetate. Moreover, the percentage composition of the main components of its essential oil showed marked differences as a result of changing geographical regions.

Key words: *Origanum vulgare,* phytochemistry, GC-MS, essential oil.

INTRODUCTION

Origanum vulgare (L.) is one of the important perennial ethno medicinal plant having Ayurvedic importance and trade values as well. It is locally known as Jungali Tulsi or Oregano or Himalayan marjoram and belongs to the family Lamiaceae, tribe Mentheae. This is the only species of genus *Origanum* which is found in India. It is found in temperate Himalayas from Kashmir to Sikkim at an altitude of 1500-3600m. It is particularly grown in Shimla Hills, Gilgit, Nilgris and in the Kashmir valley. Genus Origanum comprises of 42 species and 18 hybrids widely distributed in Eurasia and North Africa (Ietswaart,1980 ; Duman *et al*., 1988, Kokkini,1997). It is native to the mountainous parts of Mediterranean region of Europe and Asia.

Oregano is the commercial name of those *Origanum* species that are rich in the phenolic monoterpenoids, mainly carvacrol and occasionally thymol (D'antuono *et al*., 2000). A number of chemically related compounds i.e. *p*-cymene, γ-terpinene, carvacrol methyl ethers, thymol methyl ethers, carvacrol acetates and thymol acetates, *p*-cymen-8-ol, thymoquinone, and thymo hydroquinone are found in the oil. The other chemical compounds, quantitatively less significant, are acyclic monoterpenoids such as geraniol, geranyl acetate, linalool, linalyl acetate and β-myrecene. Besides some sesquiterpenoids such as β- caryophyllene, β-bisabolene, β-bourbonene, germacrene-D, bicyclogermacrene, α-humulene, α- muurolene, γ-muurolene, γ-cadinene, allo-aromadendrene, α-cubebene, α-copaene, α-cadinol, β-caryophyllene oxide and germacrene-D-4-ol are also present. In some *Origanum* plants sabinyl compounds such as *cis-* and/or *trans*-sabinene hydrate, α-thujene, sabinene, *cis-* and *trans*-3 sabinene hydrate acetates, *cis-* and *trans*-sabinol and sabina ketone can also be found (Skoula and Harborne 2002).

There have been previous investigations on the chemical composition and content of the essential oil of *O. vulgare* from the Turkey (Sezik *et al*., 1993), India (Pande *et al*., 2000)), Bulgaria (Kula *et al*., 2007), Brazil (Cleff *et al*., 2010), Kumaon Himalayas (Verma *et al*., 2010). However, there is no report on the comparative study of the essential oil of *O. vulgare* growing at different geographical locales of the Kashmir Himalaya. Thus this study is aimed at the chemical analysis and the comparative assessment of essential oils of *O. vulgare* grown at different geographical locales of Kashmir Himalayas.

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MATERIALS AND METHODS

Essential oil isolation: The plant material of *Origanum vulgare* (L.) collected from three different geographical locales of Kashmir Himalaya (viz. Sanantnagar- Srinagar, Bonnera-Pulwama and Yaarikhah- Tangmarg) were identified at KASH herbarium, University of Kashmir, under voucher specimen Nos. 1822, 1823 and 1900 respectively. The aerial parts like stem, branches and leaves weighing 100 grams each, were separately shade dried, crushed and subjected to hydro distillation using a Clevenger apparatus until oil distillation ceased after 3 hours, according to the protocol described in the European Pharmacopeia (Council of Europe, 1997). The volume of essential oil was determined from a calibrated trap. The essential oils in the distillate were dried over anhydrous sodium sulphate (Na_2SO_4) so as to remove water if any, after extraction. The essential oils were separately stored in sealed vials at 4^0C until analysed. The oil yield was calculated on fresh weight basis percentage of volume by weight.

Analysis of Essential Oils: Analysis of Essential Oil was done using Gas Chromatography coupled with mass spectrometry (GC/MS) to know the composition of oil and quantity of each compound present.

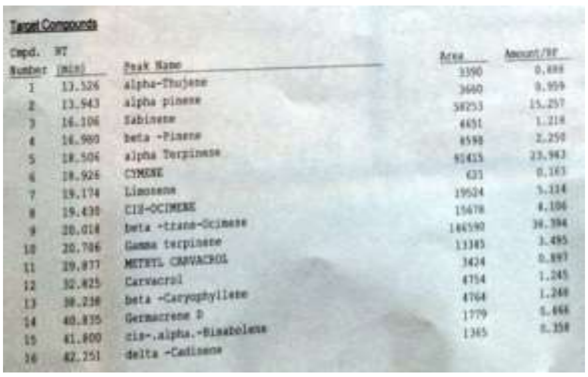
Gas Chromatography-Mass Spectrometry (GC/MS) analysis: GC-MS analysis were carried on a Varian Gas Chromatograph series 3800 fitted with a VF-MS fused silica capillary column (60m x 0.25mm, film thickness 0.25 µm) coupled with a 4000 series mass detector under the following conditions: injection volume 0.5µl with split ratio 1: 60, Helium as a carrier gas 1.0 ml / min constant flow mode , injector temperature 230 ^{0}C, oven temperature 60 to 280^0C at 3^0C / min. Mass spectra: electron impact (EI^+) mode 70 ev and ion source temperature 250 ^{O} C. Mass spectra were recorded over 50 - 500 amu range.

Identification of essential oil constituents was done on the basis of retention Index (RI), determined with respect to homologous series of n- alkanes (C_5-C_{28}, Polyscience Corp., Niles IL) under the same experimental conditions, co injection with standards (Sigma Aldrich and standard isolates), MS Library search by comparing with the MS literature data (Jennings and Shibamoto, 1980; Adams, 2007).The relative percentages of individual components were calculated based on GC peak area (FID response) without using correction factors.

RESULTS

Chemical composition of the essential oil: Chemical composition of essential oil of *Origanum vulgare* (L.) growing at different geographical locales of Kashmir Himalaya, the percentage yield of the compounds is presented in Table1. In total 16-34 different compounds were identified in its essential oil. In the present study sixteen compounds were identified in the essential oil of *Origanum vulgare* (L.) growing at (Sanantnagar) Srinagar, while as twenty five each were found in the essential oil plant species growing at Pulwama and Tangmarg locales of Kashmir valley. The essential oil composition of the three plant populations appeared quite different and allows us to identify three different chemotypes. The study revealed marked difference not only in the oil composition but also in the quantity of compounds present in the oil of plant species growing at different locales.

The most abundant components in the essential oil of *Origanum vulgare* (L.) growing at Srinagar, which is at an altitude of 1585mts absl are γ-terpinene (38.394%), followed by cymene (23.943%), Sabinene (15.257%), cis- oscimene (5.114%), β trans ocimene (4.106%), methyl carvacrol (3.495%), alpha terpinene (2.250%) (fig 1).



Target Compounds

Cmpd. Number	RT (min)	Peak Name	Area	Amount/RF
1	13.526	alpha-Thujene	3390	0.888
2	13.943	alpha pinene	3660	0.959
3	16.106	Sabinene	58253	15.257
4	16.980	beta -Pinene	4451	1.218
5	18.506	alpha Terpinene	8598	2.250
6	18.926	CYMENE	91435	23.943
7	19.174	Limonene	631	0.163
8	19.430	CIS-OCIMENE	19524	5.114
9	20.018	beta -trans-Ocimene	15678	4.106
10	20.786	Gamma terpinene	146590	38.394
11	29.877	METHYL CARVACROL	13385	3.495
12	32.825	Carvacrol	3424	0.897
13	38.238	beta -Caryophyllene	4754	1.245
14	40.835	Germacrene D	4764	1.248
15	41.800	cis-,alpha.-Bisabolene	1779	0.466
16	42.251	delta -Cadinene	1365	0.358

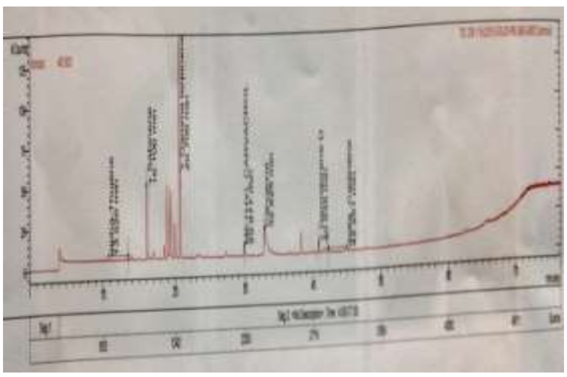


FIG.1: GC/MS analysis of oil sample obtained from specimen growing at Srinagar.

The phytochemical analysis of the essential oil of *Origanum vulgare* (L.) growing at Pulwama (Bonera) locale of Kashmir Himalaya which is at an altitude of about 1830mts absl revealed that the major components of its essential oil are γ-terpinene (25.725%), followed by p-cymene (21.123%), sabinene (18.083%), carvacrol (14.782%), β trans ocimene (3.430%) and β caryophyllene (2.953%) (Fig.2). Precursors of phenolic compounds like γ -terpinene and p-cymene (the two monoterepene hydrocarbons) were much higher in Pulwama area. In case of the phenolic compounds, the metabolic pathway is through the auto oxidative conversion of γ -terpinene to p-cymene followed by hydroxylation of p-cymene to thymol carvacrol as is quite markedly found in our observations. The same is confirmed by Poulose *et al*., (1978).

FIG 2: GC/MS analysis of oil sample obtained from specimen growing at Bonnera Pulwama.

In the examination of the essential oils of *Origanum vulgare* (L.) growing at (Yaarikhah) Tangmarg (2180mts absl, highest among the three), the major component of its essential oil was found to be p-cymene (33.290%), followed by carvacrol (27.198%), cis-sabinene hydrate acetate (8.737%), γ -terpinene (8.429%), sabinene (6.527%) and 4- terpineol (2.442%) (fig 3). Cis-sabinene hydrate acetate is a compound of intense spicy "marjoramy" aroma and was found only in *Origanum vulgare* growing at Tangmarg (high altitude) area. So, selection of this geographic altitude for isolation of Cis-sabinene hydrate acetate is a remarkable observation of our analysis.

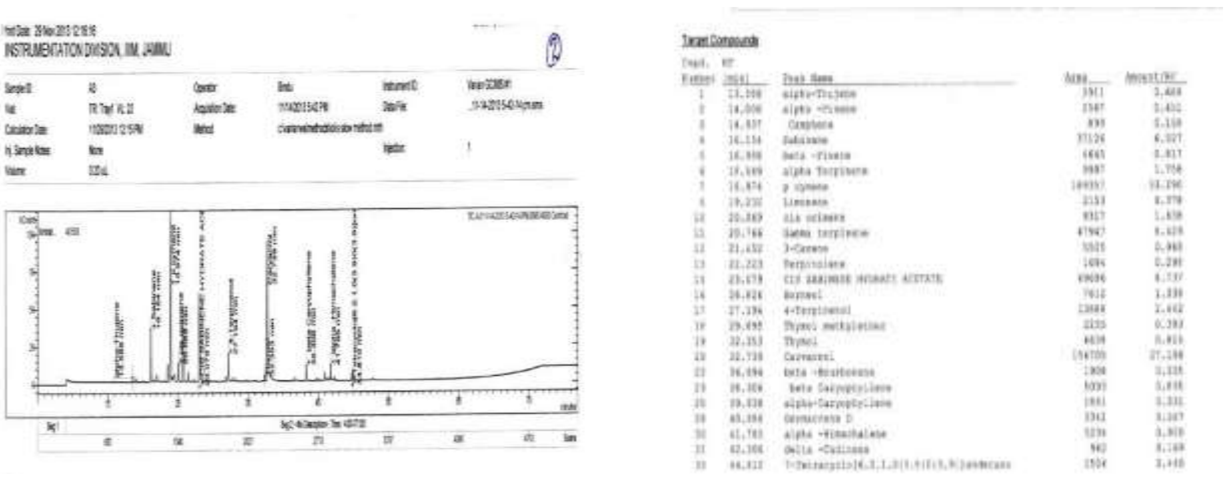


FIG 3: GC/MS analysis of oil sample, obtained from specimen growing at Yaarikhah Tangmarg.

DISCUSSION

The total oil contents of plant and %age contribution of major oil constituents like Carvacrol, Sabinene, γ-terpinene and p-cymene varied markedly in specimens growing at different localities. The observed increase in carvacrol and p- cymene with corresponding decrease in γ -terpinene content indicates a biosynthetic correlation between the two compounds. Reports suggest that at high altitudes carvacrol biosynthetic pathway is favoured and becomes more efficient than the thymol biosynthetic pathway as is remarkably found in our analysis as well. The present study indicated that the oil of *Origanum vulgare* from the Kashmir region is mainly a carvacrol- rich chemotype which has good importance because of its high biological as well as its antioxidant activity.

In the examination of the essential oil content, geographical variations have an impact on it because its oil mainly contains mono terpenes and sesquiterpene which easily change by environmental conditions and geographic origins. A number of studies have shown that variation in chemical features may occur within a single Origanum species. Furthermore, it has been found that the pattern of variation of a single species follows its geographical distribution or depends on the season of plant collection as well as the conditions used for drying and storage (Kokkini *et al*., 1994, 1996, 1997; Dorman *et al*., 2000).

From the present study it is quite evident that the *Origanum vulgare* (L.) growing under cultivated conditions at (Srinagar) is a new chemo type whose chemical profile is neither matching with the plant specimens growing wild at different geographical locales (Pulwama and Tangmarg) of Kashmir valley nor with the *Origanum vulgare* (L.) growing elsewhere in the world. Cymene was found only in the sample growing at Srinagar (lower altitude) and is totally absent at higher altitudes. Correspondingly the percentage of Sabinene was markedly found at all three locations but was significantly higher in plant samples growing at mid altitudes (Pulwama). This difference in the essential oil composition of the said plant specimen is ascribed to the variations in the eco-edaphic or cultivation conditions of plant or may be because of structural or physiological modifications in the plant caused by specific environmental factors (phenotypic plasticity).Thus the composition differentiation and the causes thereof requires detailed analysis.

Conclusions: The essential oil composition of three plant populations appeared quite different and allows us to identify three different chemotypes of Origanum vulgare growing in the valley. The altitudinal variation has marked effect on the presence and percentage of chemical constituents of its essential oil. The study concluded that it will be useful to study the conditions of the location where aromatic plants are to be cultivated, because the geographical regions, even in the same country, affects not only the volatile oil percentage but also its composition. Moreover, for the production of a compound of interest a specific geographical location can be used for growing the plant specimens. More studies in this area of research are recommended in order to explain exactly how the environmental conditions and the altitude affect the oil composition of aromatic plants

Conflict of Interests: The authors declare that there is no conflict of interest.

Table 1: Essential Oil composition (% total) identified by GC-MS of *Origanum vulgare,* Collected from three different geographical locales of Kashmir Himalaya.

S. No	Compound name	% composition		
		Srinagar	Pulwama	Tangmarg
1	α-Thugene	0.888	1.163	0.688
2	α-Pinene	0.959	0.713	0.451
3	**Sabinine**	**15.257**	**18.083**	**6.527**
4	β-Pinene	1.218	1.261	0.817
5	α-Terpine	2.250	1.449	1.758
6	**Cymene**	**23.943**	-	-
7	Limonene	0.163	0.367	0.378
8	**Cis- ocimene**	**5.114**	**0.526**	**1.638**
9	**β-trans Ocimene**	**4.106**	**3.430**	**-**
10	**γ-Terepinene**	**38. 394**	**25.725**	**8.429**
11	Methyl carvacrol	3.495	-	-
12	**Carvacrol**	**0. 897**	**14.782**	**27.198**
13	β-Caryophyllene	1.245	2.953	0.895
14	Germacerene-D	1.248	1.701	0.587

15	Cis-α- bisabolene	0.466	-	-
16	δ-cadinene	0.358	0.941	0.169
17	**p-cymene**	**-**	**21.123**	**33.290**
18	**Cis sabinene hydrate acetate**	**-**	**-**	**8.737**
19	4-Terpineol	-	0.216	2.442
20	Borneol	-	-	1.338
21	Thymol methyl ether	-	1.764	0.393
22	7-Tetracyclo undecane	-	1.436	0.440
23	β- borbonene	-	0.260	0.398
24	α-caryophylene	-	0.582	0.331
25	Thymol	-	0.062	0.815
26	Taugurgenene	-	0.734	-
27	Alloromadendrene	-	0.234	-
28	Cardreanol.	-	0.215	-
29	α-cadinene.	-	0.212	-
30	β-cubebene.	-	0.069	-
31	3-carene.	-	-	0.968
32	α-Himachalene.	-	-	0.920
33	Terpinolene.	-	-	0.398
34	Camphene.	-	-	0.158
35	Total no of compounds	16	25	25

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