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Analysis of the Hydrosol Aroma of Indian Oregano

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Abstract

Background: Hydrosols or aqueous distillates are herbal medicinal water obtained as a side product during the process of hydro or steam distillation of aromatic plant materials for getting essential oils. Generally, hydrosols are fragrant due to presence of a fraction of hydrophilic components of essential oils. Typically, in commercial production, the essential oils are decanted after completion of distillation process, whereas the hydrosols are discarded as waste or in few cases cohobated back to the distillation still.

Methods: In present study, Indian oregano (*Origanum vulgare* L.) populations (two chemotypes, viz. thymol, and carvacrol) were hydrodistilled and the compositions of resulted main essential oil (primary oil) and essential oil fraction dissolved in hydrosol (secondary oil) have compared using gas chromatography (GC/FID) and gas chromatography-mass spectrometry (GC/MS) techniques.

Results: The primary oils of both the investigated populations were dominated by hydrocarbons (monoterpene, sesquiterpene, benzene derivatives; 53.4-57.6%), followed by their oxygenated derivatives (38.1-44.9%). However, secondary oils were mainly dominated by oxygenated compounds (92.0-96.2%).

Conclusion: Present study concluded that the hydrosols of *O. vulgare* populations, a byproduct of essential oil industry, can be used as a potential source of phenolic compounds, viz. thymol and carvacrol.

Keywords: *Origanum vulgare* L.; Hydrodistillation; Essential oil; Hydrophilic fraction; Composition

Introduction

The essential oils or volatile oils are complex mixture of terpenoids (generally C_{10} and C_{15}) and some other classes of components, isolated from aromatic plants by physical means, e.g. steam-distillation, hydro-distillation, hydro-cum-steam distillation, and hydro-diffusion processes. These oils find extensive application in flavour, perfumery, cosmetic, and pharmaceutical industries. The process of distillation of aromatic plant material yields essential oil as a main product (primary oil or prime oil) and distillate water, also known as hydrosol as a byproduct. Typically, in commercial production, the essential oils are skimmed off and the hydrosol is discarded as waste or cohobated back to the source solution. The slightly hydrophilic part of essential oil, passes in to the aqueous phase during process of distillation, gives pleasant aroma to the resulted hydrosol. Typically, the essential oil fraction present in hydrosol is highly aromatic and its composition is often different from the primary essential oil [1,2]. However, the major components are generally the same of those present in oxygenated fraction of corresponding essential oils [3]. Biological and organoleptic properties of the hydrosols make them useful for food and cosmetic industries [2,4-9]. In addition, hydrosols also find application in biological agriculture against mushrooms, mildew, insects, and for fertilization of soils [10].

In Indian subcontinent, *Origanum vulgare* L. is locally known as 'Jungali Tulsi' or 'Oregano' or 'Himalayan marjoram'. The plant is widely used as a very popular spice. Oregano is of great economic value owing to its various traditional and modern applications. It is used as a traditional remedy to treat various ailments such as whooping and convulsive coughs, digestive disorders, and menstrual problems [11,12]. Dried *Origanum* leaves and essential oils are used by the flavouring industry in various liqueur formulations, tomato sauces, condiments, in baked goods such as pizzas and salad dressings [13]. The genus, *Origanum* is known for its huge morphological and chemical diversity [14]. The chemotypic and ontogenic variations occurring in the essential oil composition of Indian oregano (*Origanum vulgare* L.) have been explored [15-18].

Aqueous distillate volatiles of some Indian aromatic plants have been subjected to phytochemical and antimicrobial studies [1,2,19-24]. However, literature survey revealed that there were no such reports available on emerging aromatic crop, Indian oregano; hence a comparison of the composition of primary (main essential oil) and secondary (dissolved fraction of essential oil) volatile oils of two chemotypes of *O. vulgare* have been conducted in this research.

Materials and Methods

Plant materials

The fresh samples of *O. vulgare* chemotypes (I & II) were collected from experimental field of Central Institute of Medicinal and Aromatic Plants, Research Centre, Purara, Uttarakhand on 29th June, 2009 when the plants were in flowering stage. Climatologically, the site falls in temperate region (1250 m) of western Himalaya where the monsoon usually breaking in June and continuing up to September.

Isolation of essential oil

Freshly harvested plant materials were hydro-distilled for 3 hrs in a slightly modified all glass Clevenger's apparatus in which the recycling of distillate was stopped and diverted to a collection flask so that essential

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S. No.	Compound	RI[a]	RI[b]	Content (%)			
				I		II	
				PO	SO	PO	SO
1.	α-Thujene	930	924	1.5	t	1.3	t
2.	α-Pinene	936	932	0.8	0.8	1.0	t
3.	Camphene	944	946	0.1	0.3	0.2	-
4.	β-Pinene	979	974	1.7	0.5	0.2	t
5.	3-Octanone	986	979	0.7	-	-	-
6.	Myrcene	991	988	3.2	t	2.0	0.1
7.	δ-2-Carene	1000	1001	-	-	0.2	-
8.	α-Phellandrene	1006	1002	0.2	t	-	-
9.	α-Terpinene	1018	1014	2.4	0.1	2.0	t
10.	p-Cymene	1024	1020	19.0	0.8	20.9	0.7
11.	(Z)-β-Ocimene	1038	1032	1.3	-	-	-
12.	(E)-β-Ocimene	1046	1044	2.0	t	0.1	-
13.	γ-Terpinene	1058	1054	21.4	0.4	23.4	0.4
14.	Terpinolene	1089	1086	0.2	0.1	0.1	0.1
15.	Linalool	1099	1095	0.1	0.4	0.1	t
16.	*trans*-p-Menth-2 en-1-ol	1142	1136	t	0.1	t	-
17.	Camphor	1144	1141	-	-	t	-
18.	Borneol	1162	1165	0.4	0.8	0.5	0.1
19.	Terpinen-4-ol	1175	1174	0.6	0.2	0.3	0.2
20.	p-Cymen-8-ol	1183	1179	t	t	t	0.1
21.	α-Terpineol	1185	1186	0.1	0.2	0.1	0.1
22.	Thymol methyl ether	1232	1232	0.3	-	t	-
23.	Carvacrol methyl ether	1246	1241	3.2	0.1	0.1	t
24.	Bornyl acetate	1282	1284	0.1	0.3	t	0.2
25.	Thymol	1292	1289	30.8	83.4	0.6	0.3
26.	Carvacrol	1302	1298	1.0	5.9	42.3	94.7
27.	Thymyl acetate	1350	1349	t	0.1	t	t
28.	Eugenol	1352	1356	-	-	t	t
29.	Carvacryl acetate	1372	1370	t	-	0.1	-
30.	α-Copaene	1375	1374	t	-	t	-
31.	Geranyl acetate	1378	1379	-	-	t	t
32.	β-Elemene	1395	1389	t	-	-	-
33.	(E)-Caryophyllene	1418	1417	2.1	0.1	1.2	0.1
34.	β-Copaene	1427	1430	t	t	t	t
35.	Aromadendrene	1441	1439	0.1	-	0.1	-
36.	(Z)-β-Farnesene	1445	1440	t	t	0.1	-
37.	α-Humulene	1451	1452	0.1	t	0.1	-
38.	γ-Gurjunene	1472	1475	-	-	t	-
39.	Germacrene D	1481	1484	0.7	t	0.1	-
40.	α-Muurolene	1498	1500	t	t	0.1	t
41.	(E,E)-α-Farnesene	1502	1505	0.1	t	0.1	-
42.	Germacrene A	1506	1508	-	-	0.2	-
43.	γ-Cadinene	1516	1513	0.5	0.1	-	-
44.	δ-Cadinene	1524	1522	0.2	t	t	-
45.	α-Cadinene	1538	1537	-	-	t	-
46.	Germacrene-D-4-ol	1575	1574	t	t	0.2	t
47.	Caryophyllene oxide	1583	1582	0.3	t	0.3	t
48.	Viridiflorol	1595	1592	t	t	t	-
49.	*epi*-α-Cadinol	1640	1638	t	t	-	-
50.	*epi*-α-Muurolol	1644	1640	t	-	-	-
51.	α-Muurolol	1646	1644	t	t	t	-
53	β-Eudesmol	1651	1649	0.4	0.5	0.1	-
54	α-Cadinol	1652	1652	-	-	0.1	0.4
55	β-Bisabolol	1671	1674	t	-	0.1	0.1
	α-Bisabolol	1689	1685	0.1	t	-	-
	CLASS COMPOSITION						
	(1) Hydrocarbons						
	Monoterpene hydrocarbon			34.8	2.2	30.5	0.6
	Sesquiterpene hydrocarbon			3.8	0.2	2.0	0.1
	Benzenoid hydrocarbon			19.0	0.8	20.9	0.7
				57.6	3.2	53.4	1.4
	(2) Oxygenated compounds						
	Oxygenated monoterpene			1.3	2.0	1.0	0.6
	Oxygenated sesquiterpene			0.8	0.5	0.8	0.5
	Oxygenated benzenoid			35.3	89.5	43.1	95.1
	Aliphatic compound			0.7	-	-	-
				38.1	92.0	44.9	96.2
	Total identified (%)			95.7	95.2	98.3	97.6

RI[a]: experimental retention index on DB-5 column (relative to *n*-Alkane, C_8-C_{30}); **RI[b]**: retention index from literature (Adams, 2007); **I**: thymol rich population: **II**: carvacrol rich population; **PO**: primary oil; **SO**: secondary oil (recovered from hydrosol); t: trace (<0.05%).

Table 1: Chemical composition of primary and hydrosol recovered volatile oils of Indian oregano (*Origanum vulgare* L.)

oil and hydrosol can be collected simultaneously (like field distillation without cohobation) [2]. The oil collected directly in extraction burette was separated and dehydrated by anhydrous sodium sulphate and designated as 'primary oil'. The hydrosol collected simultaneously in a separate flask was used for isolation of 'dissolved' or 'secondary oil'.

Isolation of dissolved essential oil

The hydrosol collected in a separate flask was shaken vigorously with hexane (10:1×2) using separatory funnel for 30 minutes to recover the dissolved oil. The mixture was then allowed to settle and the organic layer was separated. The organic layer was then dried over anhydrous sodium sulphate, filtered and the solvent evaporated (35°C) under reduced pressure to get the 'secondary oil'. The primary and secondary oils were kept in a cool and dark place prior to analysis.

Gas chromatography (GC)

The GC analysis of the oil sample was carried out on a Nucon gas chromatograph model 5765 equipped with FID and DB-5 (30 m×0.32 mm; 0.25 μm film coating) fused silica capillary column. Oven temperature programming was done from 60-230°C at 3°C/min. Hydrogen was the carrier gas at 1.0 ml/min. The injector and detector temperatures were 220°C and 230°C, respectively. The injection volume was 0.02 μl neat (syringe: Hamilton 1.0 μl capacity, Alltech USA) and the split ratio was 1:30.

Gas chromatography-mass spectrometry (GC/MS)

GC/MS analysis of the essential oil sample was carried out on a PerkinElmer AutoSystem XL GC interfaced with a Turbomass Quadrupole Mass Spectrometer fitted with an Equity-5 fused silica capillary column (60 m × 0.32 mm i.d., film thickness 0.25 μm). The oven temperature was programmed from 60-210°C at 3°C/min using helium as the carrier gas at 1.0 mL/min. The injector temperature was 210°C, injection volume 0.1 μl prepared in *n*-hexane (dilution 10%), split ratio 1:40. MS were taken at 70 eV with a mass scan range of 40-450 amu and scan rate 1 sec with interscan delay 0.5 sec.

Identification of components

Constituents were identified on the basis of a Retention Index (RI, determined with reference to homologous series of *n*-alkanes, C_8-C_{30}, under identical experimental conditions), co-injection with standards (Aldrich and Fluka) or known essential oil constituents, MS Library search (NIST/EPA/NIH version 2.1 and WILEY registry of MS data 7th edition), by comparing with the MS literature data [25]. The relative amounts of individual components were calculated based on the GC

peak area (FID response) without using a correction factor.

Results and discussion

Primary and secondary volatile oils obtained from the hydrodistillation of fresh herbs of *O. vulgare* chemotypes (I & II) were analysed by GC/FID and GC/MS. Altogether, 55 constituents representing 95.2-98.3% of the total oil compositions were identified (Table 1). Chemotypes 'I' was rich in thymol, *p*-cymene, and *γ*-terpinene. While the chemotype II contained carvacrol, *p*-cymene, and *γ*-terpinene as major constituents. The amount of thymol and carvacrol was found to be very low in primary oil (30.8% and 1.0%, respectively) as compared to secondary oil (83.4% and 5.9%, respectively) of chemotype 'I'. On the other hand, primary oil possessed higher amounts of *γ*-terpinene (21.4%), *p*-cymene (19.0%), myrcene (3.2%), carvacrol methyl ether (3.2%), *α*-terpinene (2.4%), (*E*)-caryophyllene (2.1%), (*E*)-*β*-ocimene (2.0%), and (*Z*)-*β*-ocimene (1.3%) as compared to secondary oil of this chemotype. Furthermore, the primary oil of chemotype 'II' contained lesser amount of carvacrol (42.3%) in comparison to secondary oil (94.7%). However, the amounts of *γ*-terpinene (23.4%), *p*-cymene (20.9%), myrcene (2.0%), and *α*-terpinene (2.0%) were noticed to be higher in primary oil than secondary oil of chemotype II.

Further, the class compositions of the primary and secondary volatile oils of *O. vulgare* chemotypes also showed clear and considerable differences in their nature. The primary oils of *O. vulgare* chemotypes were dominated by hydrocarbons (chemotype I: 57.6%; chemotype II: 53.4%), followed by oxygenated compounds (chemotype I: 38.1%; chemotype II: 44.9%). However, secondary oils were dominated only by oxygenated compounds (chemotype I: 92.0%; chemotype II: 96.2%). These variations are observed due to relatively higher solubility of oxygenated compounds in water over the solubility of hydrocarbons.

Thus, on the basis of present study, it can be said that the hydrosol of *O. vulgare* populations should not be discarded as usually done with commercial distillation of aromatic crops. It could be redistilled by introducing cohobation system in field distillation unit to improve the organoleptic property of the primary oil or reused for distillation of fresh herb to minimize the loss of valuable components of the essential oil. Alternatively, hydrosols could also be used for isolation of pure compounds (thymol, and carvacrol) or it can be used as such for disinfection and cosmetic applications.

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