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Original Article

Essential oil Analysis of Fresh Aerial part of Iranian *Ocimum sanctum* L. by Hydro & Steam Distillation

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Abstract

The genus *Ocimum* L. belonging to family of Lamiaceae is widely distributed in Iran, *Ocimum sanctum* L. is described in many medicinal properties and a wide therapeutic range. It is used specially in the management of cough, ashtma, fever and common cold. In this research, samples were collected from Shahr-e-Rey, on August 2013, then essential oils were extracted by hydro-distillation and steam distillation methods, and their chemical composition were investigated by GC/MS. Main components obtained from hydro-distillation were methyl chavicol (26.86%) , linalool (17.76%), epi- α-cadinol (13.12%) in flowers and methyl chavicol (27.64%) , epi-α-cadinol (11.5%) in leaves, and also main components obtained from steam distillation were methyl chavicol (25.2%) , linalool (17.65%), germacrene D (6.87%) in flowers and methyl chavicol (38.96%), linalool (12.13%) in leaves, respectively. Methyl chavicol was the main constituent in all essential oils.

Key words: Essential oils, *Ocimum sanctum* L., Steam distillation, Hydro-distillation

Introduction

Application of medicinal herbs had been common in developing countries and there is a pandemic approach among the people in recent years a great number of scientists and organization turn their attention to traditional therapies in order to find and conserve important resources [1]. With the development of this science, extraction of essential oil has been considered more and they are use full as the basis of more drugs with extracts for centuries [2].

Ocimum sanctum is described in many medicinal properties and a wide therapeutic range. It is used specially in the management of cough, ashtma, fever and common cold. The herb also called holy basil has been widely known for its health promoting and medicinal value for thousands of years. Commonly called sacred or holy basil, it is a principal herb of the ancient traditional holistic health system is also known as "The Incomparable One", "The Mother Medicine of Nature", and "The Queen of Herbs". The herb is identified by botanists primarily as *O. sanctum* or more recently *O. tenuiflorum*, and *Ocimum gratissimum* Forssk. Belonging to the Lamiaceae/Labiatae mint family, these and other closely related species and varieties (e.g., *Ocimum canum* Sims) are cousins of the familiar sweet basil cooking herb *Ocimum basilicum* L., [3]. The plant, which is native to tropical Asia, is now found in most tropical parts of the world. The plant grows wild in India but is also widely cultivated in home and temple gardens and is used for household remediation [4]. The stem and leaves of holy basil contain a variety of constituents that may have biological activity, including saponins, flavonoids, triterpenoids, and tannins [5]. Different parts of *O. sanctum Tulsi* plant such as leaves, flowers, stem, root, seeds etc. are known to possess therapeutic potentials and have been used by traditional medical practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility, hepatoprotective, hypotensive, antistress, analgesic, anti-hyperlipidemic, antioxidant potentials in experimental animals [6-11]. There are two kinds of *Ocimum* (*O. sanctum* & *O. tenuiflorum* L.) available; they are red or purple variety. The percent composition of essential oil in different seasons were identified and reported that in the month of november the composition of essential oil is more [12]. The chemical composition of *O. basilicum* essential oil depends on chemotype,

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leaf and flower colors, aroma and origin of plants [13-14]. An essential oil is defined internationally as the product obtained by hydro-distillation, steam distillation, or dry distillation by a suitable mechanical process without heating (for citrus fruits) of a plant or some parts of it [15]. They are aromatic oily liquids, volatile, characterized by strongodour, rarely coloured, and generally with a lower density than that of water [16]. Essential oils of plants and their other products from secondary metabolism have had a great usage in folk medicine, food flavoring, fragrance, and pharmaceutical industries [17,18]. Essential oils are plants secondary metabolites. These metabolite shave recently been referred to as phytochemicals [19]. The total essential oils content of plants is generally very low and rarely exceed 1% [20]. Steam distillation of *O. basilicum* leaves and inflorescences from togo products a light yellow oil up to 1.8% of yield based on dried matter and it releases a savor like Thymol [21]. Essential oil and extract of *O. tenuiflorum* L. in flowering stage contains: caryophyllene 9.26%, methyl eugenol and a little percent of carvacrol and eugenol. Oumadevi Rangasamy, *et.al*, shows that inflorescences have more oily component in comparison with leaves [22]. Chemical content of *O. basillicum* due to khelifa and *et al*. study is: linalool (32.83 %), linalylacetat(16%), elemol (7.44 %), geranil acetate (6.18 %), myrcene (6.12%), allo-ocimen (5.02 %), α-terpineol (4.9%), E-β- ocimen(5.02) and nerylacetat (3.45%) [23]. While according to M.ismail from Egypt is: linalool (44.18%) , 1,8 cineol (13.65%), eugenol (8.59%) , methyl cinnamate (4.26%) , isocaryophyllene (3.10%) and α- cubebene (4.97%) as the main component [24]. Analysis of the essential oil of *O. basillicum* from Iran twelve components were characterized. Representing 99.4 % of the total oil. Methyl cavicol (40.5%), geranial (27.6%), neral (18.5%) and caryophyllene oxide (5.4%) were the major component [14]. However, there is no report available for the chemical component of essential oil obtained species cultivated in Iran, therefore the current study was under taken to elucidate the chemical composition.

Material and Methods

Plant Material

The aerial parts of *Ocimum sanctum* L. plant was collected during the month of Agust 2013, Shahr-e-Rey, from Iran. Plant materials were identified at the Research Institute of Forests and Rangelands Herbarium.

Extraction of essential oils

About 45g flowers and 80g leaves of *O. sanctum* subjected to hydro-distillation (Clavenger type apparatus) for 2 hours. The essential oil was separated from aqueous layer using a 100 mL capacity separatory funnel, and was dried by filtration over anhydrous sodium sulfate. Oil yield for flowers were (0.28%) and for leaves were (0.18%), respectively. For steam distillation method, about 150g flowers and leaves of *O. sanctum* were used, for 45 minutes. Oil yield for flowers were (0.37%) and for leaves were (0.21%), respectively.

GC-MS analysis

GC-MS analysis was carried out on a Trace MS fitted with a capillary column (30m×0.25mm); film thickness 0.25 µm. The oven temperature was programmed from 60°- 250 °C. Helium was used as carrier gas at a flow rate of 1.1 ml/min. The gas chromatograph was coupled to a quadrupole mass selective detector .The MS operating parameters were ionization voltage, 70 ev; and ion source temperature, 200 °C. Identification of components of the volatile oils were based on retention indices and computer matching with wiley library as well as by comparison percentage of area.

Results and Discussion

Interest in essential oils has reviewed in recent decades, with the popularity of aromatherapy, a branch of alternative medicine which claims that the specific aromas carried by essential oils have curative effects. Oils are volatilized or diluted in carrier oil and used in massage or burned as incense. About 300 essential oils out of 3000 known are commercially important mainly for their flavors and fragrances [25].

In the present study fresh flowers and leaves of *O. sanctum* were collected on Agust 2013, from Shahr-e-Rey city in Iran, and essential oils were extracted by hydro-distillation and steam distillation and oils analyzed for their volatile constituents by GC/MS, their compositions are given in Table 1, about fifty compounds were identified in both flowers and leaves oils by steam distilled and about fifty eight compounds were identified by hydro-distillation. The main constituents found in the oil of *O. sanctum* by hydro-distillation were methyl chavicol (26.86%), linalool (17.76%), epi- α-cadinol (13.12%), δ-cadinene (3.29%) in flowers and methyl chavicol (27.64%), epi- α-cadinol (11.5%), spathulenol (7.54%), caryophyllene oxide (6.39%) and linalool (5.43%) in leaves, and major components by steam distillation in flowers were methylchavicol (25.2%), linalool (17.65%), germacrene D (6.87%), epi-α-cadinol (6%) and in leaves were methylchavicol (38.96%), linalool (12.13%), epi-α-cadinol (5.02%), respectively.

Table 1 Percentage composition of volatile oils of *Ocimum sanctum* L.

S.No.	Compounds name	RI	Steam distillation		Hydro-distillation	
			Flower%	Leaves%	Flower%	Leaves %
1	α-pinene	934	0.14	-	-	-
2	Champhene	950	0.13	-	-	-
3	Benzaldehyde	962	-	0.01	-	-
4	Sabinene	974	0.09	-	-	-
5	1-octen-3-ol	975	0.09	0.06	0.08	0.08
6	β-pinene	978	0.17	-	-	-
7	β-myrcene	989	0.32	-	0.05	-
8	p-cymene	1024	0.17	0.06	0.08	0.2
9	Limonene	1029	0.32	0.06	0.18	-
10	1,8-cineole	1032	1.61	0.29	1	0.18
11	Z-β-ocimene	1035	0.09	-	-	-
12	E-β-ocimene	1046	1.74	0.24	0.17	0.09
13	*Cis*-sabinene hydrate	1067	0.14	0.18	0.07	-
14	*Trans*-linalool oxide	1072	-	-	0.24	-
15	Terpinolene	1089	0.23	-	0.39	-
16	Fenchone	1090	-	0.28	-	-
17	Linalool	1108	17.65	12.13	17.76	5.43
18	E-myroxide	1141	0.46	0.06	0.22	-
19	Camphore	1147	1.6	2.99	1.69	3.42
20	Menthone	1154	-	0.63	-	-
21	Menthofuran	1165	-	0.43	-	-
22	Borneol	1168	0.46	0.32	0.31	0.36
23	Menthol	1173	-	2.14	-	-
24	Terpin-4-ol	1178	0.27	0.74	0.19	0.34
25	α-terpineol	1194	-	0.36	0.35	0.35
26	N-octyl acetate	1208	-	-	0.13	-
27	Methyl chavicol	1210	25.2	38.96	26.86	27.64
28	Z- citral	1240	-	0.24	0.12	-
29	Carvone	1244	-	0.55	0.93	-
30	Geraniol	1253	-	-	0.05	-
31	Anisaldehyde	1254	-	-	0.28	0.1
32	E-citral	1268	-	0.21	0.16	-
33	Neo-menthyl acetate	1273	-	0.17	-	-
34	Bornyl acetate	1286	0.13	0.45	0.16	0.67
35	Thymol	1292	-	3.67	-	-
36	Isomenthyl acetate	1307	-	0.11	-	-
37	Iso-dihydrocarveol acetate	1326	-	1.33	-	-
38	α-cubebene	1349	0.14	-	-	-
39	α-copaene	1377	0.62	0.17	0.29	0.15
40	β-bourbonene	1386	0.29	1.65	0.13	0.2
41	β-cubebene	1391	-	-	0.24	-
42	β-elemene	1393	2.35	0.73	0.87	0.35
43	methyl eugenol	1402	0.11	1.09	0.18	1.55
44	α –gurjunene	1409	-	-	-	0.86
45	α –cedrene	1415	0.09	-	-	-
46	*Trans*-caryophyllene	1423	3.53	2.54	0.77	4.77
47	β – copaene	1430	…	0.21	…	-
48	*Trans*-α-bergamotene	1437	3.04	2.96	1.58	4.18
49	α-guaiene	1440	2.14	0.17	1.03	0.16
50	z-β -farnesene	1454	-	0.91	0.61	1.33
51	α-humulene	1456	1.39	-	0.21	-
52	E-β-Farnesene	1457	1	0.62	0.57	1.54
53	Cis-muurola-4(14),5-diene	1465	0.88	0.3	-	0.37
54	Germacrene D	1485	-	0.34	-	-
55	(E)- β-ionone	1487	6.87	1.04	-	0.44
56	δ-selinene	1493	-	-	0.36	-
57	δ- decalactone	1495	1.87	-	-	-
58	Bicyclogermacrene	1499	-	-	-	0.09
59	α-muurolene	1500	1.39	0.31	0.15	-
60	*Trans*- β- guaiene	1505	-	-	2.07	0.68
61	α-bulnesene	1511	5.59	0.66	-	-
62	γ-cadinene	1519	3.75	1.68	-	2.5

63	δ-cadinene	1525	0.73	0.49	3.29	0.8
64	Cis-calamenene	1525	-	-	0.24	-
65	10-epi-cubebol	1530	-	-	0.41	0.2
66	α-cadinene	1539	0.09	-	-	-
67	Cis-muurol-5-en-4- α- ol	1559	-	-	0.49	-
68	E- nerolidol	1562	0.47	0.65	0.96	0.52
69	γ-undecalactone	1568	0.24	0.24	1.3	0.68
70	α-cedrene epoxide	1576	-	-	0.68	-
71	Spathulenol	1581	1.63	2.23	2.46	7.54
72	Caryophyllene oxide	1586	0.42	4.5	1.93	6.39
73	Viridiflorol	1594	-	0.7	-	-
74	Geranylisovalerate	1604	-	-	-	0.22
75	Humulene epoxide II	1612	0.11	0.58	0.92	1.78
76	1,10-di-epi-cubenol	1618	1.18	1.06	2.67	1.81
77	Epi-α-cadinol	1647	6	5.02	13.12	11.5
78	α-cadinol (tau-cadinol)	1656	0.41	0.42	1.54	0.87
79	Valerianol	1658	0.33	0.69	1.54	1.64
80	Intermedeol	1665	0.16	-	0.77	-
81	Epi-β-bisabolol	1671	-	-	0.3	-
82	Epi-α-bisabolol	1685	0.21	-	0.42	-
83	Cis-14-nor-muurol-5-en-4-one	1691	0.23	0.35	0.71	-
84	(Z, Z)-farnesol	1721	-	-	1.52	0.74
85	(E,E)-farnesol	1724	-	-	0.63	-
86	Oplopanone	1740	-	-	0.17	0.38
87	(E,Z)-farnesol	1746	-	-	0.2	-
88	Aristolone	1752	-	0.19	0.24	-
89	*n*-octadecane	1796	0.13	-	-	-
90	nootkatone	1806	0.13	-	-	-
91	Iso-longifolol acetate	1815	-	-	0.22	-
92	2,7(14),10-bisabolatriene-1-ol-4-one	1842	-	-	0.11	0.5
93	(E)-β- santalol acetate	1865	-	0.13	…	-
94	n-hexadecanol	1866	-	-	0.68	-
95	Cubitene	1968	-	0.14	-	-
96	Oplopanonyl acetate	1970	0.16	-	-	-
97	*n*-eicosane	2012	0.57	-	-	-
98	Manool	2058	-	-	-	1.05
99	*n*-octadecanol	2084	0.29	-	-	-
100	*n*-heneicosane	2095	0.47	-	-	-

RI*: retention indices on DB5 column

Investigation of GC/MS results showed that if a constituent of one special method was more in an organ toward to the same constituent on other organ, about other method is too. For example linalool 17.75% in flower by hydro-distillation (Clevenger apparatus) method is more than leave's with by hydro-distillation and in steam distillation it is 17.65% that is more than leave's too. About all of constituent do too. As a result we found selected method in essential oil extraction is effective in amount of constituent not in ratio. It is logical that constituents with different percentage were seen in each special part. The yield of oil by steam distillation in flower oil were0.37% and for leaves was 0.32%, respectively. The yield of oil by hydro-distillation in flower were0.28% and for leaves was 0.23%, respectively. In general the best yield for volatile oils is in steam distillation that can confirm this note which steam distillation is better method for extraction essential oil from fresh part of plants and the best yield in part of *O. sanctum* is for flowers, so flowers with steam distillation can produce a good volatile oil.

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