Journal of Medicinal Plants and By-products (2016) 1: 45-50

Original Article

Comparison of Essential oil of *Ocimum sanctum* L. from Fresh and Dry Aerial Parts by Hydro-distillation and Steam Distillation

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Article History: Received: 29 April 2015/Accepted in revised form: 27 November 2015

Abstract

The use of natural products as medicinal agents presumably predates the earliest recorded history. *Ocimum sanctum* L., is a plant which is used in several traditional medicine systems to cure various diseases. In this study aerial parts of *Ocimum sanctum* were collected on august 2014 from Shahr-e-Rey in Iran. Fresh and dry partsof plants essential oils were extracted with hydro-distillation and steam distillation and then essential oils injected to GC/MS. Main component for dried leaves and flower with hydro-distillation method were chavicol (75.66%), linalool (6.69%) and c*is*-sabinene hydrate (2.84%) and 0.18% yield and for dry flower were chavicol (38.2%), linalool (32.8%), 10-epi-cubebul (3.89%) and 0.28% yield. Main component with steam distillation method for dry leaves were chavicol (60.95%), linalool (4.38%), *E*-β-farnsene (3.8%) and 0.21% yield. Main component of steam distilation for dry flower were chavicol (31.4%), linalool (24.7%), (E, E)-farnesole (20.8%), and 0.37% yield. Main component for fresh and dried leaves with hydro-distillation method with dry leaves were chavicol (75.66%), linalool (6.69%), cis-sabinene hydrate (2.84%), and 0.20% yield. Main component with fresh leaves were methyl chavicol (27.64%), spathulenol (13.92%), β-eudesmol (11.5%), and 0.18% yield. Main component for steam distillation method with dry leaves were chavicol (60.95%), linalool (4.38%), *E*-β-farnsene (3.8%) and 0.23% yield. Main component with fresh leaves were methyl chavicol (38.96%), linalool (12.19%), spathulenol (6.73%), and 0.21% yield. Main component for fresh and dry flower with hydro-distillation method with dry flower were chavicol (38.2%), linalool (32.80%), α-cadinene (4.08%), and 0.25% yield. Main component for fresh flower were methyl chavicol (26.99%), linalool (17.98%), β-eudesmol (13.12%), and 0.28% yield. Main component for steam distillation method with dry flower were chavicol (31.44%), linalool (24.70%), α-cadinene (5.28%), and 0.35% yield. Main component with fresh flower were chavicol (25.2%), linalool (17.99%), germacrene D (6.87%), and 0.37% yield. Chavicol is used as an odorant in perfumery and it is miscible with alcohol, ether, and chloroform.

Keywords: Steam distillation, Hydro distillation, *Ocimum sanctum* L., Dry parts

Introduction

Medicinal and aromatic plants (MAPs) have been used for centuries as remedies for human diseases because they contain components of therapeutic value. It has been estimated by WHO that 80% of the population, the majority of this in the developing countries, still rely on plant-based medicine for primary health care needs [1]. For this purpose, various strategies have been developed, e.g. biological screening, isolation, as well as clinical trials for a variety of plants. Following the advent of modern medicine, herbal medicine suffered a setback, but during the last two or three decades the advances in phytochemistry and in the identification of the plant compounds,

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providing effective against certain chronic diseasesand emergence of multidrug resistant bacteria. The genus *Ocimum* involves economically the most important medicinal and aromatic herbs, under shrubs or shrubs in the world. It belongs to the family Lamiaceae, subfamily Ocimoideae, and comprises more than 30 species distributed in tropical and subtropical regions of Asia, Africa, and Central and South America [2].

Essential oils are the volatile materials derived by a physical process from odorous plant material of a single botanical form and species with which it agrees in name and odour. The essential oils are mixtures of up to 200 organic compounds, many of which are either terpenes (with 10 carbon atoms) or sesquiterpenes (with 15 carbon atoms) [3]. Essential oils are fragrant, highly concentrated essences of plants which are considered to exemplify the soul or life-sourceof the plant. Essential oils are approximately 75-100 times more concentrated than dried herbs [4]. Nowadays, there are many efficient and economical methods of extraction of essential oils being developed. These methods Include-Cold pressing, Hydro distillation, Steam distillation, Solvent extraction, Supercritical CO2 extraction and enfleurage. Essential oils find wide applications in a number of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverages (hard drinks) and insecticides.

The essential oils of *Ocimum* extracted via steam distillation from the leaves and flavoring tops are used to flavor foods, dental and oral products, in fragrances, and in traditional rituals and medicines. The active compounds present volatile oil from the leaves consist mainly of eugenol, thymol, citrol, geraniol, camphor, linalooll, and methyl cinematic [5-11]. The seeds contain oil composed of fatty acids and sitosterol. The roots contain sitosterol and three triterpenes A, B, and C. Additionally, they also contain rosmarinic acid, thymol, methyl chavicol and, citral etc. [12], and vitamins C, A, and minerals like calcium, zinc, and iron [13], as well as chlorophyll and many other phytonutrients. Recent interest in *Ocimum* has resulted from its inhibitory activity against HIV-I reverse transcriptase and platelets aggregation induced by collagen and ADP (adenosine 5'-disphosphate) [14-15]. However, the antimicrobial activity of *Ocimum* essential oil against microorganisms has been investigated by some researchers [11,16-20], using different techniques and their investigations mostly covered one individual or two species. Unfortunately, the published data on the former subject are difficult to compare, because the chemical composition of essential oils is known to vary with the local climate, harvest period, and environmental conditions [21], and is also dependent on the type of solvent used inthe extraction procedure [22]. However, with our experiences in this study we reported results of essential oil of *Ocimum sanctum* L. from Shahr-e-Rey, cultivated in Iran, therefore the current study was under taken to elucidate the chemical composition.

Materials and Methods

Plant Name

Ocimum sanctum L.

Source

The aerial parts of *Ocimum sanctum* L. plant was collected during the month of Agust 2014, from Shahr-e-Rey, near capital Tehran in Iran. Samples were collected and identification of the plants was determined by Iranian Botanical Garden (IBG).

Extraction of Essential Oils

About 50g flowers and 300g leaves of *Ocimum sanctum* L. subjected to hydro-distillation (Clavenger type apparatus) for 2 hours. The essential oil was separated from aqueous layer using a 100 mL capacity separator funnel, and was dried by filtration over anhydrous sodium sulfate. Oil yield for flowers were (0.94%) and for leaves were (0.56%), respectively. For steam distillation method, about 90g flowers and 400g leaves of *Ocimum sanctum* L were used, for 45 minutes. Oil yield for flowers was (0.41%) and for leaves was (0.53%), respectively. Characteristics of aerial parts consist of leaves and flowers are different from one another. It was advisable to dry each part separately. Leaves and flowers were separated from the plants making sure no foreign matter or any other part of the plant was mixed with them. The drying experiment was done on air flow in shadow for one week.

The oils were separated from the water by decantation and were dried by filtration over anhydrous sodium sulfate and stored in sealed vials at 2 °C before analysis. The composition of essential oils was analyzed by gas chromatography (GC) and gas chromatography, coupled to mass spectrometry (GC-MS).

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

Compounds name	R.I.	Dried Flower & Leaves				Fresh & Dry Leaves				Fresh & Dry Flower			
		Steam distillation		Hydro-distillation		Steam distillation		Hydro-distillation		Steam distillation		Hydro-distillation	
		Flower	Leaves	Flower	Leaves	Fresh leaves	Dry leaves	Fresh leaves	Dry leaves	Fresh Flower	Dry Flower	Fresh Flower	Dry Flower
Champhene	950	0.21	0.33	-	0.17	-	0.33	-	0.17	0.27	0.21	-	-
Sabinene	972	-	0.14	-	0.34	0.06	0.14	0.08	0.34	0.18	-	0.08	-
β-pinene	977	0.23	0.54	0.19	0.46	-	0.54	-	0.46	0.17	0.23	-	0.19
p-cymene	1020	-	0.33	-	-	0.06	0.33	0.2	-	0.49	-	0.13	-
Limonene	1029	0.24	-	0.17	0.25	0.06	-	-	0.25	0.32	0.24	0.18	0.17
1,8 cineol	1032	-	0.46	0.13	-	0.29	0.46	0.18	-	1.61	-	1	0.13
Z-β-ocimene	1035	0.17	1.82	-	0.37	-	1.82	-	0.37	0.09	0.17	-	-
E-β-Ocimene	1048	2.29	-	1.78	1.68	0.24	-	0.09	1.68	1.74	2.29	0.17	1.78
Cis-sabinene hydrate	1065	1.60	2.69	1.66	2.84	0.18	2.69	-	2.84	0.14	1.60	0.32	1.66
Terpinolene	1089	-	-	-	0.13	0.28	-	-	0.13	0.23	-	0.39	-
Linalool	1108	24.7	4.38	32.8	6.69	12.19	4.38	5.43	6.69	17.99	24.70	17.98	32.80
Camphor	1147	0.29	0.83	0.43	1.00	4.11	0.83	3.42	1.00	1.6	0.29	1.69	0.43
Borneol	1168	-	-	-	-	0.32	-	0.36	-	0.46	-	0.31	-
Menthol	1173	0.59	0.31	0.56	0.45	2.88	0.31	0.34	0.45	0.27	0.59	0.19	0.56
α - terpineol	1194	-	-	-.	-	0.36	-	0.35	-	-	-	0.35	-
Methyl chavicol	1210	0.36	1.28	0.35	0.40	38.96	1.28	27.64	0.40	25.2	0.56	26.99	0.35
Chavicol	1239	31.4	60.9	38.2	75.6	0.24	60.95	-	75.66	-	31.44	0.12	38.2
Carvone	1244	-	-	-	-	0.76	-	0.1	-	-	-	1.42	-
Bornyl acetate	1286	-	-	-	-	0.62	-	0.67	-	0.13	-	0.16	-
Thymol	1292	-	-	-	-	3.67	-	-	-	-	-	-	-
Isomenthyl acetate	1307	-	-	-	-	0.11	-	-	-	-	-	-	-
Iso-dihydrocarveol acetate	1327	-	-	-	0.26	1.33	-	-	0.26	-	-	-	-
α-cubebene	1349	0.17	-	0.11	-	-	-	-	-	0.14	0.17	-	0.11
α-copaene	1377	-	-	-	-	0.17	-	0.15	-	0.62	-	0.29	-
β- bourbonene	1386	-	-	-	-	1.65	-	0.2	-	0.29	-	0.13	-
β- elemene	1393	-	-	-	-	0.73	-	0.35	-	2.35	-	1.02	-
α- gurjunene	1409	-	-	-	-	1.09	-	2.41	-	0.11	-	0.18	-
α-cedren	1418	-	0.10	0.44	-	-	0.10	-	-	0.09	-	-	0.44
Trans- caryophyllene	1423	0.66	0.60	3.63	0.59	2.54	0.60	4.77	0.59	3.53	0.66	0.77	3.63
β- copaene	1430	5.12	0.20	-	0.56	0.21	-	-	-	-	-	-	-
Trans-α-bergamotene	1437	-	-	-	-	3.13	0.20	4.33	0.56	5.18	5.12	2.61	-
E-β-farnesene	1456	-	3.80	1.68	2.37	1.53	3.80	2.87	2.37	2.39	-	1.39	1.68
cis-muurola-4,5-diene	1468	2.56	-	-	-	0.3	-	0.37	-	0.88	2.56	-	-
Germacren D	1480	3.98	1.22	2.64	0.85	0.34	1.22	-	0.85	6.87	3.98	-	2.64
Bicyclogermacrene	1494	0.12	-	-	-	-	-	0.09	-	-	-	0.36	-
α-muurolene	1500	0.42	-	0.30	-	0.31	0.31	0.68	-	1.39	0.42	2.22	0.30
α-bulnesene	1511	1.04	0.68	0.72	0.62	0.66	0.68	-	0.62	5.59	1.04	-	0.72
γ-cadinene	1519	0.15	0.17	-	-	1.68	0.17	2.5	-	3.75	0.15	-	-
δ-cadinene	1522	5.28	-	0.12	1.05	0.49	-	0.8	0.21	0.73	-	3.54	-
α-cadinene	1539	-	0.98	3.89		-	0.98	0.2	0.83	0.09	5.28	0.41	4.08
cis-muurol-5-en-4-α-ol	1559	3.92	0.69	2.77	0.52	-	0.69	-	0.52	-	3.92	0.49	2.77
E-nerolidol	1562	2.15	0.56	1.52	0.52	0.65	0.56	0.52	0.52	0.47	2.15	0.96	1.52
γ-undecalactone	1568	-	-	0.18	-	0.24	-	0.68	-	0.24	-	1.3	0.18
Spathulenol	1580	0.18	-	-	-	6.73	-	13.92	-	2.05	0.18	4.97	-
Humulene epoxide II	1612	-	-	-	-	1.28	-	1.98	-	0.11	-	0.92	-
β- eudesmol	1618	-	-	-	-	1.06	-	1.81	-	1.18	-	2.67	-
β-eudesmol	1649	-.	-	0.19	0.25	5.02	-	11.5	0.25	6	-	13.12	0.19
α-cadinol	1652	0.21	-	0.11	-	1.11	-	2.53	-	0.74	0.21	3.10	0.11
epi-β-basabolol	1671	-	-	0.44	0.21	-	-	-	0.21	0.16	-	1.03	0.44
Cis-14-nor-muurol-5-en-4-one	1691	0.45	0.53	-	-	0.35	0.53	-	-	0.23	-	0.71	-
(E,E)-farnesole	1724	20.8	-.	3.20	1.46	-	-	0.74	1.46	-	20.8	2.14	3.20
(E,Z)- farnesole	1740	0.22	-	-	0.16	0.19	-	0.38	0.16	-	0.22	0.37	-
n-octadecane	1796	-	-	-	-	-	-	-	-	0.26	-	0.22	-
2,7(14),10-bisabolatriene-1-ol-4-one	1842	-	-	-	-	-	-	0.5	-	-	-	0.11	-
(E)- β- santalol acetate	1865	-	-	-	-	0.13	-	-	-	-	-	0.68	-

Cubiten	1896	-	-	0.51	-	-	-	-	-	-	-	-	-
	1968	-	-			0.14	-	-	-	0.16	-	-	0.51
n- eicosane	2012	-	1.03	-	-	-	1.36	-	-	0.57	1.03	-	-
Manool	2042	0.18	-	-	-	-	-	1.05	-	-	0.18	-	-
n-octadecanol	2079	-.	0.41	-	-	-	0.41	-	-	0.29	-	-	-
n-heneicosane	2100	-	-	0.15	-	-	-	-	-	0.47	-	-	0.15
Yield%		0.37	0.21	0.28	0.18	0.21	0.23	0.18	0.20	0.37	0.35	0.28	0.25

Gas Chromatography

GC analyses were performed using a gas chromatography, Ultra-Fast Module–GC, made in Italia. Profile column machine brand Ph-5 capillary column, manufactured by Shimadzu with Length of30mmandan inner diameter of1/0 mm thick 25/0 mm, the inner surface of the stationary phase material is covered Phenyl Dimethyl Siloxane 5%. Column temperature program: initial temperature 60 °C to start the final temperature of 210° C. The initial 3 °C per minute to be added and then injected into the chamber to a temperature of 280° C. The carrier gas inlet pressure to the column: helium with a purity of99/99% of the inlet pressure to the column equal to5/1kilogramper square centimeters set.

Gas Chromatography-Mass Spectrometry

The GC/MS unit consisted of a Varian Model 3400 gas chromatograph coupled to a Saturn II ion trap detector was used. The column was same as GC, and the GC conditions were as above. Mass spectrometer conditions were: ionization potential 70 eV; electron multiplier energy 2000 V.
The identity of the oil components was established from their GC retention indices, relative to C7- C25 n-alkanes, by comparison of their MS spectra with those reported in the literature [23-25], and by computer matching with the Wiley 5 mass spectra library, whenever possible, by co-injection with standards available in the laboratories.

Results

Obtained result from dry flowers and leaves essential oils analysis with hydro and steam distillation is shown in table 1.

Discussion

The chemical composition of basil oil has been the subject of considerable studies. The use of natural products as medicinal agents presumably predates the earliest recorded history. *Ocimum sanctum* L., is a plant which is used in several traditional medicine systems to cure various diseases. In this study aerial parts of *Ocimum sanctum* were collected on august 2014 from Shahr-e- Rey in Iran. Fresh and dry parts of plants essential oils were extracted with hydro-distillation and steam distillation and then essential oils injected to GC/MS. Main component for dried leaves and flower with hydro-distillation method were chavicol (75.66%), linalool (6.69%) and c*is*-sabinene hydrate (2.84%) and 0.18% yield and for dry flower were chavicol (38.2%), linalool (32.8%), 10-epi-cubebul (3.89%) and 0.28% yield. Main component with steam distillation method for dry leaves were chavicol (60.95%), linalool (4.38%), *E*-β-farnsene (3.8%) and 0.21% yield. Main component of steam distilation for dry flower were chavicol (31.4%), linalool (24.7%), (E, E)-farnesole (20.8%), and 0.37% yield. Main component for fresh and dried leaves with hydro-distillation method with dry leaves were chavicol (75.66%), linalool (6.69%), cis-sabinene hydrate (2.84%), and 0.20% yield. Main component with fresh leaves were methyl chavicol (27.64%), spathulenol (13.92%), β-eudesmol (11.5%), and 0.18% yield. Main component for steam distillation method with dry leaves were chavicol (60.95%), linalool (4.38%), *E*-β-farnsene (3.8%) and 0.23% yield. Main component with fresh leaves were methyl chavicol (38.96%), linalool (12.19%), spathulenol (6.73%), and 0.21% yield. Main component for fresh and dry flower with hydro-distillation method with dry flower were chavicol (38.2%), linalool (32.80%), α-cadinene (4.08%), and 0.25% yield. Main component for fresh flower were methyl chavicol (26.99%), linalool (17.98%), β-eudesmol (13.12%), and 0.28% yield. Main component for steam distillation method with dry flower were chavicol (31.44%), linalool (24.70%), α-cadinene (5.28%), and 0.35% yield.
Main component with fresh flower were chavicol (25.2%), linalool (17.99%), germacrene D (6.87%), and 0.37% yield. Chavicol is used as an odorant in perfumery and it is miscible with alcohol, ether, and chloroform. The chromatogram of GC analysis

of oil obtained from the leaves of *Ocimum sanctum* L. the total no. 25 to 47 peaks were obtained out of which the peaks are the major ones. However, the qualitative and quantitative determination of the important constituents will be further done on comparison with standard chromatograms of the chemical compound.

The oil content of *Ocimum sanctum* L. in this study (0.56–0.94%) was similar to several literature reports [26, 27, 30]. A study by Marotti *et al*. [26] showed that the content of essential oil in herb of 10 Italian basil cultivars ranged from 0.3 to 0.8%. Galambosi and Szebeni [28] reported oil contents in basil herb from 0.38 to 1.29%, while Seidler-Łożykowska [29] from 0.23 to 1.67%. In a large study on 270 sweet basil accession in Germany, oil content varied from traces to 2.65% [30]. Such variations in the essential oil content of basil across countries might be attributed to the varied agroclimatic conditions of the regions. Due to the high content of Chavicol, linalool, methyl chavicol, the studied cultivars may become applied in food and perfume industries.

Acknowledgment

Support of this investigation by the Islamic Azad University Tehran South Branch through a grant is greatfully acknowledged.

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