Chemistry of Natural Compounds, Vol. 39, No. 5, 2003

ISOLATION OF SOME SOLUBLE AND DISPERSED MATERIALS OF OREGANO WATER

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UDC 547.913

Recent results obtained on determining the extraction efficiency and composition of organic volatiles in Oregano Water obtained from Origanum onites *L. are described. Oregano Water was subjected to liquid-liquid extraction with a range of solvents with different polarities and each extract was analyzed by GC/MS.*

Key words: *Origanum onites*, distillation water, Oregano water, *p*-menthene diols.

The term Kekik (Oregano in English) is a Turkish word to describe plants which smell like carvacrol or thymol. *Thymus*, *Origanum*, *Thymbra*, *Coridothymus*, and *Satureja* species are used as kekik in Turkey. Five *Origanum* species are widely traded in Turkey. *Origanum onites* L. is the most commonly exported species. This is known in Turkish as Bilyali Kekik or Izmir Kekigi and cultivated in the Aegean region exceeding 6000 da [1–4].

Origanum onites L. is used as a spice and a source of *Origanum* oil and *Origanum* Water (Kekik Suyu). Distilled *Origanum* Water is used as an oral remedy to reduce blood glucose and cholesterol levels after the complete removal of essential oil [5].

Oregano water contains water-soluble components of the oil which mainly consist of the oxygenated components. Some of the polyoxygenated components may not even be present in the oil. Therefore, the distillate may have different biological properties than the oil [5].

The volatile organic compounds may be extracted from the aqueous phase by distillation, extraction with organic solvents, solid phase microextraction (SPME), and poroplast and supercritical fluid extraction (SFE) techniques [6–8].

In this work, the traditional solvent extraction technique was used. Dispersed and soluble materials in the distillate were extracted by using organic solvents. The extracts were then analyzed by GC/MS.

The oil used in this study turned out to be a material poor in carvacrol content. Nevertheless, this did not affect the results of our experiments since our main concern was on the distillate.

The plant material yielded 2.3% oil by steam distillation and 2.9% oil by hydrodistillation. The oils contained carvacrol (28–34%), thymol (12–14%), γ-terpinene (8–10%), linalool (7–10%), *cis*-sabinene hydrate (5–9%) and *p*-cymene (4–5%) as the main constituents.

The best yield of volatiles from the distillates was obtained with diethyl ether (0.1%) in all the three experiments conducted (Table 1). Carvacrol and thymol were the main constituents of all four extracts obtained with ethyl acetate, diethyl ether, methylene chloride, and *n*-hexane. While all the extracts showed the occurrence of oxygenated components the ether extracts also showed the presence of alkane and alkene hydrocarbons (Table 1).

An interesting observation was the selective extraction of *p*-menthendiols by chloroform both after extraction of the distillate with hexane as well as following redistillation. All the chloroform extracts showed *cis*-*p*-mentha-4-en-1,2-diol (58–69%) as the main constituent. It was followed by *cis*-*p*-mentha-3-en-1,2-diol (8.0–9.5%) and *trans*-*p*-mentha-3-en-1,2-diol (2.1–2.3%).

Redistillation of the distillate using a Clevenger apparatus yielded 0.2% oil which contained carvacrol (53%), thymol (22%), terpinen-4-ol (12%), and linalool (7%) as the main components. *cis*-*p*-Mentha-4-en-1,2-diol (0.2%) was a minor constituent, which was the main constituent (55%) of the chloroform extract obtained after redistillation. The $CHCl_3$ extract also contained 2,3-dihydroxy-*p*-cymene (14%).

Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy , 26470, Eskisehir, Turkey. Pablished in Khimiya Prirodnykh Soedinenii, No. 5, pp. 380-383, September-October, 2003. Original article submitted July 9, 2003.

0009-3130/03/3905-0465$25.00

TABLE 1. The Percentage Yields and the Results of Analysis of the Extracts Obtained by Using Various Solvents

Compounds	Florentine flask										Separatory funnel				
	overflow					distillate									
	EA	DE	DC	H	Ch	EA	DE	DC	H	Ch	EA	DE	DC	H	Ch
Linalool	3.0	-	4.2	4.0	-	5.3	-	6.0	6.7	-	2.1	1.9	2.5	2.9	-
1,8-Menthadien-4-ol	6.2	-	Tr.	8.5	-	-	-	-	-	-	-	-	-	-	-
3,7-Dimethyl-1,5-octadien-3,7-diol	1.0	-	-	-	6.4	0.9	-	-	-	6.6	-	0.5	-	-	3.4
Terpinen-4-ol	-	-	7.9	0.1	-	9.6	-	11.4	12.8	-	3.6	3.8	4.4	5.6	-
3,7-Dimethyl-octa-1,7-dien-3,6-diol	0.5	-	-	-	6.1	-	-	0.4	-	7.0	-	0.2	-	-	1.8
Dodecane	-	-	-	-	-	-	2.5	-	-	-	-	-	-	-	-
Thymol	19.4	4.7	21.1	3.6	-	8.2	13.5	18.6	19.9	-	8.9	7.3	9.0	10.3	-
Carvacrol	52.7	64.8	42.9	3.5	3.0	6.8	56.0	49.0	50.6	4.6	60.0	62.4	60.2	72.5	2.0
trans-p-Mentha-3-en-1,2-diol	0.8	-	0.9	0.4	2.1	0.7	-	0.5	-	2.3	0.8	0.6	0.8	Tr.	0.8
Carvacryl acetate	-	4.6	-	-	-	-	-	-	-	-	-	-	-	-	-
cis-p-Mentha-4-en-1,2-diol	7.0	-	8.7	1.3	8.4	5.8	-	6.2	0.7	67.8	14.2	12.5	14.2	2.3	69.2
cis-p-Mentha-3-en-1,2-diol	1.1	-	1.3	0.1	8.0	0.1	-	0.8	-	9.3	1.9	1.3	1.8	0.1	9.5
1-Tetradecanol	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Tetradecane	-	2.9	-	-	-	-	3.6	-	-	-	-	-	-	-	-
2,3-Dihydroxy-*p*-cymene*	0.4	-	2.3	2.2	3.1	-	-	1.4	-	2.7	1.2	-	1.7	1.6	2.3
Hexadecene	-	1.5	-	-	-	0.7	1.9	-	-	-	-	-	-	-	-
Hexadecane	-	2.9	-	-	-	-	3.7	-	-	-	-	-	-	-	-
Octadecane	-	1.9	-	-	-	-	2.2	-	-	-	-	-	-	-	-
Palmitic acid	-	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl palmitate	-	-	-	-	-	-	2.4	-	-	-	-	-	-	-	-
Eicosane	-	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethyl stearate	-	-	-	-	-	-	3.0	-	-	-	-	-	-	-	-
Stearic acid	-	1.8	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexacosane	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-
Yield (%)	**0.06**	**0.1**	**0.06**	**0.05**	**0.07**	**0.05**	**0.1**	**0.04**	**0.04**	**0.005**	**0.1**	**0.1**	**0.1**	**0.09**	**0.01**

EA: Ethyl acetate, DE: Diethyl ether, DC: Dichloromethane, H: *n*-Hexane, Ch: Chloroform, Tr.: Traces; *Tentative identification.

p-Mentha-3-en-1,2-diol (a) and *p*-mentha-4-en-1,2-diol (b) were previously reported as biotransformation products of *Corynespora cassiicola* from γ-terpinene [9].

The same compounds were later isolated and characterized from the essential oil of *Ferula jaeschkeana* [10, 11]. The occurrence of these compounds in Oregano oil was reported earlier [5].

As expected, Oregano Water proved to be rich in oxygenated compounds. It has been shown that some oxygenated monoterpenes can be selectively isolated by using different organic solvents. It will be interesting to study the biological activities of *p*-menthendiols.

TABLE 2. The Composition of Essential Oils Obtained by Steam and Hydrodistillation

Compounds of volatile oil	Steam distillation		Hydrodistillation
	Florentine flask	separatory funnel	Clevenger apparatus (plant material)
α-Thujene	0.8	0.8	0.5
α-Pinene	0.6	0.6	0.4
Camphene	0.2	0.2	0.1
1-Octen-3-ol	0.3	0.2	0.3
Sabinene	1.3	1.3	0.7
β-Pinene	0.2	0.2	0.1
3-Octanol	0.6	-	0.1
Myrcene	1.0	1.5	1.1
α-Phellandrene	0.3	0.3	0.2
α-Terpinene	2.7	2.4	2.1
p-Cymene	4.5	4.7	3.8
β-Phellandrene	1.2	1.2	0.9
(Z)-β-Ocimene	0.4	0.4	0.3
(E)-β-Ocimene	0.2	0.1	0.1
γ-Terpinene	10.0	8.8	8.3
trans-Sabinene hydrate	1.6	1.6	1.1
cis-Linalool oxide	-	-	0.1
trans-Linalool oxide	-	-	0.1
Terpinolene	0.5	0.5	0.4
cis-Sabinene hydrate	8.7	8.3	5.0
Linalool	7.0	7.2	9.7
trans-*p*-Mentha-2-en-1-ol	0.5	0.4	0.5
cis-*p*-Mentha-2-en-1-ol	0.3	0.3	0.3
Borneol	0.8	0.9	0.8
Terpinen-4-ol	5.0	3.9	5.9
α-Terpineol	1.2	0.8	0.8
Thymol methyl ether	0.3	0.3	0.3
Carvacrol methyl ether	1.0	1.0	0.9
Geraniol	-	Tr.	0.2
Linalyl acetate	1.2	1.4	0.5
Isothymol	-	-	0.1
Thymol	11.9	12.0	14.0
Isocarvacrol	-	-	0.2
Carvacrol	28.2	30.2	33.8
cis-*p*-Mentha-4-en-1,2-diol	0.2	0.1	-
Thymyl acetate	-	0.1	0.1
β-Caryophyllene	3.2	3.2	2.1
Aromadendrene	0.1	0.2	0.1
α-Humulene	0.2	0.2	0.2
Bicyclogermacrene	0.8	0.9	0.5
β-Bisabolene	1.1	1.2	0.7
γ-Cadinene	0.1	0.1	-
δ-Cadinene	0.1	0.1	0.1
Spathulenol	0.3	0.4	0.5
Caryophyllene oxide	0.4	0.5	0.6
Total	**99.0**	**98.5**	**98.6**

Tr.: Traces.

TABLE 3. The Composition of the Volatiles Obtained from the Distillate by Re-distillation and $CHCl_3$ Extraction of the Spent Distillate

Compounds	Clevenger apparatus (*Origanum* Water)	Chloroform extract
Furfural	0.1	-
(E)-2-Hexenal	0.1	-
1-Octen-3-ol	0.3	-
α-Phellandrene	0.1	-
Benzyl alcohol	-	0.6
trans-2-Hexenoic acid	-	0.6
Phenylacetaldehyde	0.2	-
α-Terpinene	0.1	-
p-Cymene	0.2	0.3
β-Phellandrene	0.2	-
γ-Terpinene	0.3	-
p-Cresol	-	0.2
cis-Linalool oxide	0.3	-
trans-Linalool oxide	0.4	-
Phenyl ethyl alcohol	-	0.7
Linalool	6.6	-
trans-p-Mentha-2-en-1-ol	0.3	-
cis-p-Mentha-2-en-1-ol	0.2	-
Borneol	0.9	-
1,8-Menthadien-4-ol	0.3	-
3,7-Dimethyl-1,5-octadien-3,7-diol	-	1.9
Terpinen-4-ol	11.8	-
α-Terpineol	0.8	-
γ-Terpineol	1.0	-
3,7-Dimethyloct-1-en-3,7-diol	-	0.3
3,7-Dimethyl-octa-1,7-dien-3,6-diol	-	3.6
Isothymol	0.1	-
Cumin alcohol	-	0.3
Thymol	21.6	0.2
Isocarvacrol	0.4	-
Carvacrol	52.6	3.0
trans-p-Menta-3-en-1,2-diol	-	1.6
cis-p-Mentha-4-en-1,2-diol	0.2	55.4
cis-p-Menta-3-en-1,2-diol	-	3.2
2,3-Dihydroxy-*p*-cymene*	-	13.7
Dihydroactinidiolide	-	0.5
Spathulenol	0.3	-
Total	99.4	86.3

*Tentative identification.

EXPERIMENTAL

Plant Material. Commercial, dried herb of *Origanum onites* L. (August, 2000) supplied by Turer Tarim in Izmir was used.

Steam Distillation Studies. A 5 L capacity S/S steam distillation apparatus was used for steam distillation of the herbal material. Three experiments were carried out with 1.5 kg charge each. The distillate was directed to the Florentine flask. Oregano water was collected in a vessel connected to the overflow pipe of the Florentine flask. The flow of water was controlled to achieve the desired flow rate of the steam. Distillation was continued for 3 h. The percentage yield of the oil was 2.3% on a moisture-free basis. The composition of the oil is shown in Table 2.

Liquid-Liquid Extraction Studies. The liquid-liquid extraction experiments were realized with the distillate. Various organic solvents with different polarities were used. These solvents are as follows: ethyl acetate, diethyl ether, dichloromethane, *n*-hexane, and chloroform (following extraction with *n*-hexane, the distillate was re-extracted with chloroform).

The oil-free aqueous distillate was shaken to homogenize it. 250 ml of Kekik Water was transferred to a separatory funnel (500 ml). Equal amounts of the organic solvent were added to the aqueous distillate and shaken gently successively three times. The organic layers separated were combined, filtered through anhydrous sodium sulfate, and evaporated using a rotary evaporator at temperatures not exceeding 50°C. Yields and the results of GC/MS analysis of extracts are shown in Table 1.

In a separate study, a separatory funnel was used instead of a Florentine flask to collect the distillate. Again, the experiment was repeated three times with a charge of 1.5 kg plant materials each time. The distillate was collected in a separate vessel. After 3 h distillation the oil was obtained in 2.3% yield. The composition of the oil is shown in Table 2.

Liquid-liquid extraction of the distillate was carried out as before. The yields of extracts and their compositions are given in Table 1.

Clevenger Apparatus. The plant material (50 g) was distilled using a Clevenger apparatus for 3 hours. The percentage yield of the oil on a moisture-free basis was 2.9%. The composition of the oil is shown in Table 2.

The water content of the plant material was determined as 8.5% according to the EP method (12).

Clevenger Distillation of *Origanum* Water. The distillate (500 ml) obtained from the steam distillation studies was shaken to homogenize it, charged to a Clevenger apparatus, and distilled for 3 h. The experiment was repeated three times and 1 ml of essential oil was obtained in each case. The oil was analyzed by GC/MS. The results are shown in Table 3.

The spent distillate (250 ml) was extracted with chloroform to yield an extract (0.03 g) after evaporation of the solvent *in vacuum*. Its composition is shown in Table 3.

GC/MS Conditions. The volatile oils and the extracts were analyzed by the Gas Chromatography/Mass Spectrometry system. GC/MS analysis was carried out using a Shimadzu GC/MS-QP 5050 A system. A CP Sil 5CB (25 mm × 0.25 mm *i.d.*) column was used with helium as a carrier gas. The GC oven temperature was kept at 60°C and programmed to 260°C at a rate of 5°C /min and then kept constant at 260°C for 20 min. The split ratio was adjusted at 50:1. The injector temperature was at 250°C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 400. Library search was carried out using the Wiley Library and the BASER Library of Essential Oil Constituents. The relative percentage amounts of the separated compounds were calculated from Total Ion Chromatograms by the computerized integrator.

ACKNOWLEDGMENT

The authors thank Assist. Prof. Dr. B. Demirci of TBAM for her help in one of the GC/MS analyses.

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