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Abstract: The essential oils of the aerial parts of *Thymus kotschyanus* Boiss. obtained by hydrodistillation (HD) and microwave oven distillation (MD) were analyzed by GC and GC-MS. 39 components were identified in the essential oil of this plant (95.0 % in HD and 94.8 % in MD). The components of the essential oil extracted by MD were similar to those obtained by HD. *T. kotschyanus* essential oil was characterized by a high amount of carvacrol (64.6 % in HD and 44.7 % in MD). Other major compounds were identified as *p*-cymene, γ-terpinene and L-Borneol. Thymoquinone was absent in HD method. Although the time of MD was 12 times less than HD, but the percentage of essential oil was only 15 % less (1.2 % in MD and 1.4 % in HD). Moreover, the amount of carvacrol as the main component of the plant essential oil, in MD was 20 % less than HD.

Key words: *Thymus kotschyanus*, GC-MS, Microwave, Hydrodistillation, Essential oil.

Introduction

Thyme (Lamiaceae) is cultivated throughout the world for culinary, cosmetic and medicinal purposes. It's oil is manufactured commercially for use in cough drops, mouthwashes, liniments, toothpastes, detergents and perfumes [13]. Thyme in its crude herb form is carminative, antibiotic, anthelmintic, astringent, expectorant and antitussive [13,16]. It has been used in traditional medicine to treat heartburn, gastritis, asthma, laryngitis, pertusis and bronchitis [16]. Extracts have demonstrated anti inflammatory effects on guinea pig tracheal smooth muscle tissue [13] and the volatile oil in the herb most likely exerts spasmolytic effects on bronchial tissues in humans [25]. The herb is approved by Commission E in the treatment of bronchitis, whooping cough and upper respiratory inflammation [5]. Like the herb's infusion and extracts, thyme oil also show carminative, expectorant, antimicrobial and anthelmintic properties due to concentrated thymol and carvacrol contents [13,19], but it is

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extremely toxic. As an ingredient in toothpaste, thyme oil has been blamed for cases of inflamed lips and tongue reported in the toothpaste users. Signs of toxicity escalate from nausea to respiratory arrest [6,16].

Thymus contains fourteen species in Iran which are locally called "Avishan". One of the species which is widely grown in Iran, is *T. kotschyanus* Boiss. [15]. The aerial parts of the plant are used for gastrointestinal disturbances [28]. Phytochemical analysis of *Thymus* species have confirmed the occurrence of phenolic compounds such as thymol, carvacrol, thymonin, caffeic acid and rosmarinic acid, terpenoids, flavonoids and saponins in the plant [6]. According to the last investigations, the main component of *T. kotschyanus* essential oil is carvacrol [17,20], which is used nowadays on a large scale in the food and cosmetic industries. In addition, it has been shown to exhibit a range of biological activities such as antibacterial, antifungal, insecticidal, analgesic and antioxidant properties [2,3,7,18,24,26], but it is toxic in high doses [6]. Therefore, study of different methods for extraction of carvacrol is important. Ordinary technique for extraction of essential oil from plants is hydrodistillation (HD). Recently, microwave oven distillation (MD) has been used for obtaining essential oil of many plants. With using this method, percentage of essential oil, number and type of components and percentage of each one are different from that obtained using HD method. Although the comparison of essential oil achieved by MD and HD has been carried out during last investigations on some plants [4,8,12], but it has not been reported about *T. kotschyanus* yet. So in this paper we have compared the quality and quantity of *T. kotschyanus* essential oil components obtained by HD and MD distillation for the first time.

Experimental

Plant material

T. Kotschyanus was collected from the Yazd, province of Yazd, Iran, in Jun 2010, during the flowering stage. Voucher specimen has been deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Isolation of volatile oil

The aerial parts of *T. Kotschyanus* were air-dried and powdered. The oil was isolated from powdered plant materials (25 g) by HD method for 4 hours and by MD method for 20 minutes. The essential oils were separated from the aqueous layer, dried over anhydrous sodium sulfate and were stored in the refrigerator until analyzed.

GC and GC-MS spectrometry

The analytical gas chromatography was carried out using a Varian CP-3800 with capillary column DB-1 (60 m x 0.32 mm x 0.25 μm); carrier gas, N_2; split ratio, 20; flow rate, 1ml/min and detector, FID-300. The column temperature was programmed at 50°C for 1 min and then heated to 265°C with rate of 2.5°C/min, injector temperature 260 °C and detector temperature 300°C.

GC-MS was performed on a Thermoquest 2000 with a MS (5973 Network Mass Selective) detector with mass range of 40-400 m/z, on capillary column DB-1 (see GC). Retention indices were calculated by using retention times of *n*-alkanes that were injected after the oil at the same chromatographic conditions. The compounds were identified by comparison of retention indices (RI) with those reported in the literature and the authentic samples and by comparison of their mass spectra with the Wiley 7n.l library or with the published mass spectra [1,11,14,22]. Microwave oven instrument was from Osun company and its pulse was every one minute with 255 W power.

Results and discussion

The HD and MD of the aerial parts of *T. kotschyanus*, gave dark yellow brown oil with a strong characteristic odor. On a dry weight basis HD yielded 1.4 % (w/w) and MD yielded 1.2 % (w/w) of volatile oil.

The analysis of the essential oils isolated from the aerial parts of *T. kotschyanus*, carried out by GC-MS, lead to the identification of different components. The identified compounds and their percentages which have been listed according to their elution on the DB-1 column have been given in Table 1. 39 compounds were identified in the

oil of *T. kotschyanus* making up 95.0 % of the total composition for HD and 94.8 % for MD.

Oxygen-containing monoterpenoids represented the most abundant constituents of the volatile oils which were 76.5 % in HD and 72.8 % in MD while monoterpen hydrocarbons were detected in HD and MD about 15.6 % and 16.5 %, respectively. Sesquiterpene and non-terpene compounds were found in trace. Thymoquinone existed in oil of MD method 4.8 %, but it was absent in HD method. According to GC results, carvacrol was the main component of the oils which quantified as 64.6 % and 44.7 % in HD and MD methods, respectively; while in other researches on *T. kotschyanus,* the amount of carvacrol has been reported 22.6 %-65.9 % [17,20]. The content of carvacrol in another species of this genus was reported as 6.7 % in *Thymus daenensis* [21], 1-2 % in *Thymus vulgaris* [27] and 27-38 % in *Thymus persicus* [20].

Carvacrol is present in low concentration in human food as a flavor [6]. Moreover, it is used as oral bactericidal, anti-fungal and breath freshening compound in oral cleansing products [23]. Because of the adverse reaction of some compounds in higher doses, toxicological property of carvacrol is important. An oral LD_{50} in rats was calculated for carvacrol as 810 mg/kg body weight [10] and an IC_{50} value in Hep-2 cells was 0.32 mM [23]. It was confirmed by a set of experiments that at the doses above 10 µl/ml, carvacrol showed toxic effect against lymphocytes [9]. Therefore, quantitative determination of the compound in essential oils and finish products is necessary. Regarding to toxicity of carvacrol, methods with ability to decrease extracting of carvacrol should be mentioned. MD yielded less percentage of carvacrol (44.7 %) compared to HD (64.6 %), therefore, it is more efficient method for obtaining essential oil of *T. kotschyanus* with less carvacrol and less toxicity. Moreover, in comparison with HD, MD has some benefits such as shorter time (12 times less), energy saving and reduction of CO_2 released into the atmosphere. As it is obvious in the results, MD method yielded less percentage of essential oil compared to HD method, but it is low and regarding to benefits of this technique, it can be used as alternative method of HD in extracting of *T. kutschyanus* volatile oil.

Conclusion

In order to obtain *Thymus kotschyanus* essential oil with less carvacrol content, MD method is preferred which takes less time and is cost effective.

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Table 1. Chemical compositions of *Thymus kotschyanus* essential oil obtained by hydrodistillation (HD) and microwave oven distillation (MD)

No.	Compounds	RI[a]	RI[b]	MD%	HD%
1	α-Thujene	930	928	0.3	0.2
2	α-Pinene	939	937	0.9	0.9
3	Camphen	954	951	1.3	1.2
4	β-Pinene	979	974	0.3	0.3
5	3-Octanone	984	980	1.7	0.9
6	3-Octanol	991	985	0.5	0.2
7	β- Myrcene	991	988	0.5	0.4
8	α-Phellandrene	1003	1002	0.1	-
9	α-Terpinene	1017	1014	0.1	0.4
10	*p*-Cymene	1025	1025	8.4	8.9
11	Limonene	1029	1026	0.4	0.3
12	1,8-Cineole	1033	1028	4.3	2.1

table 1. (continued).

No.	Compounds	RI[a]	RI[b]	MD%	HD%
13	γ-Terpinen	1060	1056	3.8	2.7
14	*cis*-Sabinenehydrate	1070	1069	2.6	0.8
15	α-Terpinolene	1089	1086	0.4	0.2
16	*trans*-Sabinene hydrate	1098	1096	0.8	0.3
17	α-Thujone	1102	1099	0.6	0.3
18	*trans*-Pinocarveol	1139	1136	0.1	-
19	Camphor	1146	1140	1.3	0.6
20	L-Borneol	1165	1163	9.1	5.4
21	Terpinen-4-ol	1177	1174	1.1	0.7
22	α-Terpineol	1189	1188	0.7	0.4
23	*cis*-Dihydrocarvone	1193	1190	0.3	-
24	Thymol methyl ether	1235	1232	0.1	-
25	Carvacrol methyl ether	1245	1242	0.8	0.6
26	Thymoquinone	1253	1250	4.8	-
27	Isobornyl acetate	1286	1283	-	0.4
28	Bornyl acetate	1289	1284	0.1	-
29	Thymol	1290	1290	2.3	1.1
30	Carvacrol	1299	1298	44.7	64.6
31	α-Copaene	1375	1372	0.2	0.2
32	β-Bourbonene	1388	1385	0.1	-
33	Methyl eugenol	1410	1404	0.1	-
34	β-Caryophyllene	1419	1414	0.6	0.1
35	Germacrene-D	1483	1478	0.1	-
36	β-Bisabolen	1506	1506	0.1	0.2
37	α-Cadinol	1523	1520	0.1	0.3
38	Spatulenol	1578	1572	0.3	0.3
39	Caryophyllene oxide	1583	1577	0.8	-
	Monoterpene hydrocarbone			16.5	15.6
	Oxygenated monoterpene			72.8	76.5
	Sesquiterpene hydrocarbone			1.2	0.8
	Oxygenated sesquiterpene			1.1	0.3
	Nonterpene			3.3	1.9
	Unknown			5.2	4.9
	Total identified			94.8	95.0

[a] Retention indices as determined on a DB-1 column using the homologous series of *n*- alkanes

[b] RI reported in the literatures

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