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Comparison of hydrodistilation and microwave assisted hydrodistillation methods in analysis of the essential oils from aerial parts of *Thymus daenensis* celak subsp. *Lancifolius*

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Abstract

Microwave-assisted hydrodistillation (MAHD) is an advanced hydrodistillation (HD) technique utilizing a microwave oven in the extraction process. In this study, MAHD was investigated for the extraction of essential oils from aerial parts of *Thymus daenensis* celak subsp and the results were compared with those of the conventional HD. A total of 20 compounds were identified by Gas chromatography–mass spectrometry, constituting over 98.9% and 99.4% of oil composition of HD and MAHD, respectively. The two oils contained the same dominant components: carvacrol(55.7% HD; 66.0% MAHD), Thymol (23.0%; 20.0%) and Geraniol (8.0%; 2.0%). Extraction of essential oils from *T. daenensis* with MAHD was better in terms of energy saving, extraction time (15 min versus 3 h), product yield (1.2% versus 0.9%) and product quality. GC/MS analysis of the extracted essential oils indicated that the use of microwave irradiation did not adversely influence the composition of the essential oils. MAHD was found to be a green technology.

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Introduction

Thymus is one of the medicinal plants belonged to Labiatae family, and consists of about 215 species of herbaceous perennials and small shrubs in the world (Ataie Kachoie *et al*., 2013). Many of these species are typical for the Mediterranean area (Consentino *et al*., 1999). This genus is represented in Iranian flora by 14 species. four of which (*Thymus carmanicus*, *Thymus daenensis* subsp. daenensis and *T. daenensis* subsp. lancifolius, *Thymus persicus* and *Thymus trautvetteri*) are endemic (Rechinger, 1982). Infusion and decoction of aerial parts of *Thymus* species are used to carminative, digestive, antispasmodic, anti inflammatory, and expectorant and for the treatment of colds in Iranian traditional medicine (Zargari, 1990; Ghasemi-Pirbalouti, 2009). In the literature, there are many studies about the chemical composition of essential oil of *thymus* species (Bagci *et al*., 2003; Ozcan and Chalchat, 2004; Baser *et al*., 1992, 1998; Guillen & Manzanos, 1998). However, antibacterial (Karaman *et al*., 2001; Al-Bayati & Muthanna, 2009; Pavel *et al*., 2010; Imelouan, 2009;), antifugal (Pina-vaz *et al*., 2004; Pinto *et al*., 2006; Goncalvs *et al*., 2010) and antioxidant (Miguel *et al*., 2004; Hazzit *et al*., 2006) activities of the *Thymus* species has been studied. *T. daenensis* subsp. *daenensis* is an endemic subspecies of Iran that generally grows in high altitudes in Zagros mountains range (Ghasemi-Pirbalouti *et al*., 2011). The essential oil of *T. daenensis* showed high activity against a wide spectrum of bacteria and fungal strains (Ghasemi Pirbalouti *et al*., 2009a, 2009b & 2009c; Ghasemi Pirbalouti *et al*., 2010a, 2010b & 2010c). in addition, It is used as a food ingredient, as a tea,as an herbal drug for its reputed medicinal properties (Gourama & Bullerman, 1995). Due to these various usages, scientists have studied the chemical composition of extracts and essential oils isolated from the aerial parts of this plant. The essential oil of *T. daenensis* has been isolated by traditional hydrodistillation (Alavi *et al*., 2009).

Essential oils in plants are complex mixtures of volatile substances present in low concentrations. The recovery of an essential oil from plant can be achieved by hydrodistillation or steam distillation .These techniques take at least several hours and require the application of heating, which can cause the degradation of thermo labile compounds present in the starting plant material and therefore an incomplete collection of compounds responsible for its fragrance ((Khajeh *et al.,* 2004). Microwave extraction is a research area which has an impact in several fields of modern chemistry. The advantages of using microwave energy for the oil extraction are more effective heating, fast energy transfer, reduced thermal gradients, response to process heating control, reduced thermal gradients, selective heating, reduced equipment size, faster response to process heating control, faster start-up, increased production and elimination of process steps. (Stashenko *et al*., 2004; Wang & Weller, 2006; Wang *et al*., 2006). Extraction processes performed under the action of microwave radiation are believed to be affected in part by polarization, volumetric and selective heating (Chemat & Lucchesi, 2006). microwave-assisted hydrodistillation (MAHD) is an advanced hydrodistillation method based on the use of a microwave oven. This technique has also been investigated for the extraction of essential oils from several herbs (Rezvanpanah *et al*., 2008; Stashenko *et al*., 2004a). In this study, to our knowledge,we investigated for the first time, the chemical composition of *T. daenensis* subsp extract and oil obtained by MAHD. The chemical composition of *T. daenensis* subsp oil obtained by hydrodistillation (HD) was also studied and compared. Therefore,the comparison of the two techniques in terms of isolation times, yields and composition were reported.

Materials and methods

Plant material

Fresh aerial parts of *T. daenensis* celak subsp. *Lancifolius* were collected from Kerman province (south-eastern Iran) in june 2013 during the flowering period. The herbs were then dried under ambient conditions (30–40˚C) for three days on a large screened tray. Certified species was then kept in

a dark and cold room until used shortly after that for the experiments.

Conventional Clevenger or Hydrodistillation apparatus and procedure (HD)

100 g of dried aerial parts of *T. daenensis* were submitted to hydrodistillation with a Clevenger-type apparatus according to the European Pharmacopoeia, and extracted with 2l. of water for 180min (until no more essential oil was obtained). The essential oil was collected, dried under an hydrous sodium sulphate yielding and stored at 4˚C until used.

Microwave-assisted hydrodistillation (MAHD)

Microwave-assisted hydrodistillation was carried out with a Milestone MAO20-A apparatus. This is a multimode microwave reactor 2.45 GHz with a maximum delivered power of 1000 W variable in 10 W increments. Temperature was monitored by an external Infrared (IR) sensor. Based on a relatively simple principle, this method involves placing dried aerial parts of *T. daenensis* in the microwave reactor. The internal heating of the in situ water within the *T. daenensis* distends the oil glands and sacs and leads to rupture of the glands and oleiferous receptacles. This process thus frees essential oil, which is evaporated by the in situ water of the plant material. A cooling system outside the microwave oven condenses the distillate continuously.100g of dried aerial parts of *T. daenensis* were hydrodistilled with 500 mL of water by microwave energy at 600 W in the multimode reactor fixed at 2450 MHz and equipped by a Clevenger apparatus. The extraction oil was performed at atmospheric pressure for 15 min. The essential oils were dried over anhydrous sodium sulphate and stored in the dark at 5°C until analysis.

Gas Chromatography/Mass Spectrometry (GC/MS)

Essential oil composition was determined by gas chromatography coupled to mass spectrometry (GC – MS) analysis on a Hewlett-Packard 6890 gas chromatograph coupled to a 5973A mass spectrometer. The column was HP5MS (30 m ×0.25 mm×0.25µm filmthickness) with helium as carrier gas. GC oven temprature was kept at 60˚C for 5 min and programmed to 250˚C at a rate of 5˚C/mim, and then kept constant at 250˚C for 5min. MS wera taken at 70 eV. Alkans were used as reference points in the calculation of relative retention indices (RRI).

Gas chromatography

A Hewlett-Packard 6890 GC system was used for gas chromatography analysis, fitted with a fused-silica capillary column with a polar stationary phase HP5MS (30m×0.25mm×0.25µm film thickness). The column temperature progress from 60 to 280 ˚C at 2 ˚Cmin−1. Injection was performed at 250˚C in the split less mode; 1µL of sample was injected. A flow rate of 0.3 ml/min carrier gas (N2) was used. Flame ionization detection was performed at 320 ˚C.

Qualitative and quantitative analyses

Most constituents were identified by comparison of their GC Kovats retention indices (RI), determined with reference to a homologous series of C_5–C_{28} *n*-alkanes and with those of authentic standards available in the authors' laboratory. Identification was confirmed when possible by comparison of their mass spectral fragmentation patterns with those stored in the MS database and with mass spectra literature data (Adams, 1995). Component relative concentrations were obtained directly from GC peak areas obtained with GC-FID.

Results

The chemical composition of the volatile fractions obtained from *T. daenensis* celak subsp. *Lancifolius* during the HD and MAHD extraction processes are represented together with the retention indices in Table 1. The oils were investigated by capillary GC and GC/MS. Twenty constituents representing 98.9% and 99.4% of total HD and MAHD oils have been identified respectively. In both HD and MAHD essential oils, the main components were Carvacrol followed by thymol and Geraniol. However, relative concentrations of compounds differed according to the extraction method. Carvacrol, which is also an active antioxidant ingredient **(**Ruberto *et al*., 2002**)**, was the most abundant component in thyme essential oil (55.7% for HD and 66.0% for MAHD) followed by

thymol (23.0% for HD and 20.0% for MAHD) and Geraniol(8.0% for HD and 2.0% for MAHD). *p*-cymene (2.5% for HD and 1.0% for MAHD) and γ-terpinene (1.9% for HD and 0.5% for MAHD) was another main compounds. Therefore, Both essential oils were found to be rich in the active monoterpene phenols (thymol and carvacrol) and their corresponding monoterpene hydrocarbon precursors (*p*-cymene and γ -terpinene). This is in agreement with the values previously reported for the same species, but using conventional extraction methods. In the study on *T. daenensis*, 20 compounds were been identified that the major compounds were thymol, *p*- cymene, γ-terpinene and methylcarvacrol (Nickavar *et al*., 2005; Sajjadi & Khatamsaz, 2003). In another investigation on *T. daenensis*, 14 compounds were identified that formed 98.49% of essential oil and the major compounds were thymol, caryophyllene, γ-terpinene, *p*-cymene and carvacrol (Khorshidi & Rustaiee, 2011). Therefore, microwave does not involve in any deterioration of the extracted components and it can be introduced as a safe method for the extraction of essential oils. As can be seen, in Table 1 the extraction time of 15min with MAHD provided oil composition comparable to that obtained after 180 min by means of conventional HD (reference method). Extraction with MAHD started at much earlier time than that with HD. This is due to the more efficient heat flow involved with microwaves. Unlike the classical conductive heating methods, microwaves can heat the entire sample almost simultaneously and at a higher rate (Kaufmann & Christen, 2002). According to Golmakani & Rezaei (2008) for both HD and MAHD, the extraction starts at the boiling point of water (100 ºC, if the operation is performed at atmospheric pressure). But with MAHD, the boiling point was reached earlier than HD. It is interesting to note that distillation time of 15min with MAHD provided a better oil yield than that obtained after 180 min by means of HD (1.2% and 0.9%, respectively). These results indicated a substantial saving of time and energy in the extraction procedure.

Table 1. The composition of *Thymus daenensis* celak subsp. *Lancifolius* Volatile oils obtained by hydrodistillation and microwave-assisted hydrodistillation.

No	Compound	Hydrodistillation(%) (%)	Microwave (%)	RI
1	α-Pinene	0.2	0.1	938
6	β-Myrcene	0.2	0.1	991
7	-Phellandrene α	0.3	0.2	1003
8	α-Terpinene	0.3	0.2	1016
9	*P*-Cymene	2.5	1.0	1025
11	1,8-Cineole	0.5	0.3	1031
12	γ-Terpinene	1.9	0.5	1059
13	*cis*-Sabinene hydrate	0.3	0.1	1068
17	Linalool	0.9	0.4	1097
18	Borneol	1.3	0.5	1167
19	Terpinene-4-ol	0.4	0.1	1177
20	Nerol	0.4	1.2	1230
21	Pulegone	0.2	0.1	1237
22	Geraniol	8.0	2.0	1252
25	Thymol	23.0	20.0	1288
26	Carvacrol	55.7	66.0	1297
28	*trans*- Caryophyllene	1.5	1.5	1418
30	β-Bisabolene	0.5	0.5	1504
31	Spathulenol	0.3	1.6	1576
32	Caryophyllene oxide	0.5	3.0	1581
	Total	98.9	99.4	

Conclusion

Microwave-assisted hydrodistillation (MAHD) technique has been compared with the conventional hydrodistillation method, for the extraction of essential oil from aerial parts of *T. daenensis* celak subsp. *Lancifolius*. This microwave extraction method offered substantial advantages over conventional HD. A better extraction yield (1.2% against 0.9% for HD) was achieved at significantly shorter extraction time(15 min against 3 h for hydrodistillation) when using MAHD instead of HD. GC–MS results indicated that there were no significant differences between the essential oils obtained by MAHD and those obtained by HD proposing MAHD
as an excellent alternative for HD with no adverse effects on the composition of the extracted essential oils.

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