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Chemical characterization of volatile components of *Thymus serpyllium L*. using Microwave Distillation (MD) and Hydro-distillation (HD) methods. Green chemistry

Mohammad Hadi Meshkatalsadat*, Shabnam Shamaei, and Maysam. Hasheminejad

*Department of chemistry, Lorestan University, Koramabad Iran.
Department of Chemistry, Islamic azad University, khorramabad Iran

ABSTRACT

In this paper, Microwave distillation a new method of essential oil extraction has been compared with a conventional technique, hydro-distillation (HD). The compositions of essential oil extracted by applying kinds of extraction methods were identified by using GC–MS system. There was no obvious difference in the quality of essential oils obtained by the kinds of extraction methods. The major components of MD and HD methods are as Thymol (20.74%,21.70%), Camphene(6.60%,6.65%), Limonene(3.17%,3.26%) camphor(1.09% , 20 %) limonene (3.17% , 3.26 %) respectively.

Keywords: Essential oil, Hydro-distillation, Green chemistry, Thymol, Camphene

*Corresponding author

Fax: 0098 6614600092
Email: meshkatalsadat.m@lu.ac.ir

INTRODUCTION

The aromatic and medicinal properties of the genus *Thymus* have made it one of the most popular plants throughout the entire world. It is believed that a part of these activities is due to the volatile constituents. Therefore, there is a considerable research interest towards the compositional analysis of *Thymus* essential oils [1].

The genus Thymus L. (Labiatae) consists of about 215 species of herbaceous perennials and sub shrubs. The Mediterranean region can be described as the center of the genus [2]. 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 [3]. The Persian name of the genus is "Azorbeh" and/or "Avishan" [4]. *Thymus* species are commonly used as herbal tea, flavoring agents (condiment and spice) and medicinal plants [5]. Among the species grown in Iran, *Thymus daenensis* Celak. and *Thymus kotschyanus* Boiss. and Hohen. are more widely used for these purposes. Infusion and decoction of aerial parts of *Thymus* species are used as tonic, carminative, digestive,antispasmodic, anti-inflammatory, antitussive, expectorant and for the treatment of colds in Iranian traditional medicine [6-7]. Recent studies have showed that *Thymus* species have strong antibacterial, antifungal, antiviral, antiparasitic, spasmolytic and antioxidant activities [8-9]. A recent patent describes a new method for extracting natural products without added any solvent or water by using microwave energy [10]. The solvent free microwave extraction apparatus is an original combination of microwave heating and dry distillation at atmospheric pressure. MD was conceived for laboratory scale applications in the extraction of essential oils from different kind of aromatic plants. Based on a relatively simple principle, this method involves placing plant material in a microwave reactor, without any added solvent or water. The internal heating of the in situ water within the plant material distends the plant cells and leads to rupture of the glands and floriferous 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 condensed the distillate continuously. The excess of water was refluxed to the extraction vessel in order to restore the in situ water to the plant material. The essential oils extracted by SFME for 24 min were quantitatively (yield) and qualitatively (aromatic profile) similar to those obtained by conventional hydro-distillation for 4.5 h. The SFME method yields an essential oil with higher amounts of more valuable oxygenated compounds, and allows substantial savings of costs, in terms of time, energy and plant material. MD is a green technology and appears as a good alternative for the extraction of essential oils from aromatic plants In this paper, the potential of the MD technique has been compared with a conventional method, hydro-distillation, as the current technique and commercial situation call for research into new extracts and new extraction techniques. We have applied MD and HD techniques to extract essential oils from aerial parts of aromatic herb *thymus serpyllium L* from Iran which is a highly advanced and homogeneous family, largely used in food preparation, perfumery and medicine. We make appropriate comparisons in term of extraction yields and rates, essential oil composition, and energy consumption.

EXPERIMENTAL

Plants Material

The plant material was collected from the area near Aligoudarz lorestan state west of Iran in June 2007.

SFME apparatus and procedure

Solvent free microwave extraction has been performed in a Samsung microwave laboratory oven. This is a multimode microwave reactor 2455 MHz with a maximum delivered power of 1000 W variable in 10 W increments. The dimensions of the PTFE-coated cavity are 35 cm × 35 cm × 35 cm. During experiments, time, temperature, pressure, and power can be controlled with the "easy-WAVE" software package. Temperature was monitored by a shielded thermocouple (ATC-300) inserted directly into the sample container and by an external infrared (IR) sensor. Temperature was controlled by a feedback to the microwave power regulator.

The experimental MD variables have been optimized by the university method in order to maximize the yield of essential oil. In a typical MD procedure performed at atmospheric pressure, 50 g of fresh plant material was heated using a fixed power of 600 W for 24 min without added any solvent or water. A cooling

system outside the microwave cavity condensed the distillate continuously. Condensed water was refluxed to the extraction vessel in order to provide uniform conditions of temperature and humidity for extraction. The extraction was continued at 100 °C until no more essential oil was obtained. The essential oil was collected, dried over anhydrous Sodium Sulphate.

Hydro-distillation apparatus and procedure

Hundred grams of aromatic herb was submitted to hydro-distillation with a Clevenger-type apparatus according to the European Pharmacopoeia[11] and extracted with 600 ml of water for 4.5 h (until no more essential oil was obtained). The essential oil was collected, dried over anhydrous sodium sulphate and stored at 0 °C until used.

Gas chromatography–mass spectrometry identification

GC analyses was carried out on a Shimutzu 17A gas chromatograph and a BP-5 (non-polar and 95 % dimethyl polysiloxane) capillary column (30 m × 0.25 mm; 0.25 μm film thickness). The oven temperature was held at 60 °C for 3 min then programmed at 5°C /min to 300 °C. Other operating conditions were as follows: carrier gas He, with a flow rate of 5 ml/min; injector temperature 230°C; detector temperature 300 °C; split ratio, 1:8. A GC/MS analysis was performed on a Shimutsu 17A GC coupled with Shimutsu QGD5050 Mass system. The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. Mass range was from m/z 50–450 amu. The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions for identification of individual n-alkanes (C6–C24) and the oil on DB-5 compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 5.0) or with authentic compounds or with those of reported in the literature [18]. Quantitative data was obtained from FID area percentages without the use of correction factors.

RESULTS AND DISCUSSION

The hydro-distillation and Microwave ditillation of the aerial parts of *T. sperylium* gave pale yellow oils with a yield of 2.4% ± 0.1 (v/w) and 1.2% ± 0.1 (v/w), on dry weight basis, respectively. The general chemical profiles of the tested oils, the percentage content of the individual components and retention indices are summarized in Table 1. The chemical class distribution of the oil components is also reported in Table 2. 41compounds were identified and constituted .97.82% (MD) and 96.11% (HD) of the total oils. The essential oil of *Thymus serpyllium* was characterized by a high number of monoterpenes. Chemical composition of the essential oils of *Thymus sepyllium* extracted by MD and HD are given in Table 1.

The chemical composition, thymol and carvacrol content, insecticidal and antimicrobial effect of *T. serpyllum* oil have been the subject of previous study [6-12]. A comparison between the oil and supercritical carbon dioxide extract of Hungarian *T. serpyllum* has also been reported [13]. The published results reveal that major volatile constituents obtained from the aerial parts of the plants thymus sp.are thymol, carvacrol, *p*-cymene,γ - terpinene, β-caryophyllene, etc[15-20]. The major constituents of the essential oils were obtained by HD and MD methods thymol (20.74 ,21.70 %), β - pinene (3.39 , 2.05 %), Camphene(6.60%,6.65%), α - pinene(13.60 , 2.94%), camphor(1.09 , 20 %) limonene (3.17 , 3.26 %) and carvacrol (15.09 , 1.3%) respectively. Other components were present in amounts less than 4% (Table 1). In particular, monoterpene such as monoterpene hydrocarbons (HD: 33.67% and SFME :13.87%) and oxygenate monoterpenes (HD: 58.81% and MD: 79.51%) were the most abundant compound group of the oils . In the oils of the other species of thymus like *T. kotschyanus* , 31 compounds, constituting 98.7% of the oil, were identified. Thymol (38.6%) was the major component, followed by carvacrol (33.9%), γ-terpinene (8.2%), *p*-cymene (7.3%) [14]. Similar to *T. daenensis* subsp. *daenensis*, monoterpene phenols were also the most abundant compound group of this oil (72.7%) [20]. Therefore, both oils are rich in monoterpene and poor in other terpenoids (Table 2). From tables, it is evident that there are many qualitative similarities between the oils although the amounts of some corresponding compounds are different. In regard to the previously reported contents of the essential oils of *T serpyllium* [13], it is interesting to point out that there are no important qualitative differences between the present work and those studies but there are some quantitative differences indicating that environmental and

methods factors strongly influence its chemical composition more over a typical MD procedure appears as a good alternative for the extraction of essential oils .

Table 1

Comparison Solvent-free microwave extraction with conventional hydro-distillation of essential oil of Thymus serpyllium L.

No.	Compounds	RI[a]	HD	MD
1	α-Pinene	930	13.60	2.94
2	Camphene	943	6.60	6.65
3	Verbenene	952	0.90	–
4	β-Pinene	972	3.39	–
5	Myrcene	989	3.21	–
6	α-Phellandrene	1004	0.83	–
7	*p*-Cymene	1020	0.11	0.65
8	Limonene	1025	3.17	3.26
9	1,8-Cineole	1026	–	12.46
10	2-Propionyl Pyrrol	1026	–	0.09
11	β-Ocimene	1050	–	0.37
12	Pellandral	1080	–	0.70
13	α-Terpinolene	1086	1.88	–
14	Linalool	1097	0.50	–
15	β-Thujone	1101	–	0.81
16	Camphenol	1122	1.20	–
17	Iso-Pulegol	1130	–	0.64
18	cis-Verbenol	1139	0.53	–
19	Camphor	1140	–	20
20	Pinocarvone	1161	0.42	–
21	Borneol	1164	0.40	–
22	Terpinene-4-ol	1174	0.28	–
23	Myrtenol	1187	1.18	–
24	α-Terpineol	1189	0.43	–
25	Cis-Carveol	1203	–	13.90
26	trans-(+)-Carveol	1215	10.27	–
27	Cuminal	1233	–	0.43
28	trans-Myrtanol	1253	–	1.30
29	Bornyl acetate	1284	7.77	–
30	Thymol	1292	20.74	21.70
31	Indol	1293	–	0.93
32	Carvacrol	1301	15.09	–
33	Perillaldehyde	1310	–	6.60
34	β-Bourbonene	1382	0.28	–
35	β-Caryophyllene	1417	1.07	0.63
36	Germacrene-D	1480	–	0.36
37	Bicyclogemacrene	1490	–	0.51
38	β-Funebrene	1492	–	0.19
39	Elemol	1548	0.46	–
40	Caryophyllene oxide	1575	0.67	1.70
41	(+)-Spaththulenol	1575	1.13	–
Total identified			96.11	96.82

RI[a]=retention indices on the BP-5 column.

Table 2: class of compounds

	HD	MD
Monoterpene hydrocarbons	33.69	13.87
Oxyganated monoterpene	58.81	79.51
Sesquitepene hydrocarbons	1.35	1.69
Oxyganated sesquiterpenes	2.26	1.70
Total	96.11	96.82

CONCLUSION

Solvent free microwave extraction is an original combination of microwaves and dry distillation. The apparatus is relatively simple. The isolation and concentration of essential oils are performed in a single stage. This process thus frees essential oil which is evaporated by the in situ water of the plant material. Once the essential oils have been extracted they can be analyzed directly by GC–MS without any preliminary clean-up or solvent exchange steps. The oils of the two investigated methods are rich in monoterpene phenols (especially, thymol and carvacrol) and due to this high phenol content, they can be considered as substitutes for *Thymus vulgaris* (common thyme) oil for medicinal purposes and other applications [18]. Comparison of the volatile compounds of *T serpyllium* oil with data that have been published on the oil composition of *T. serpyllium* [13, 18] shows that there are some qualitative and quantitative differences between the two oils. These chemical differences can be most probably explained by the variability of the plant sub-species, the existence of different chemotypes and extracting methods.

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