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COMPARISON OF VOLATILE COMPONENTS OF *STACHYS LAVANDULIFOLIA VAHL* OBTAINED BY MWHD AND HD TECHNIQUES

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Microwave-assisted hydrodistillation (MWHD), hydrodistillation (HD techniques were carried out for the analysis of volatile components of *Stachys lavandulifolia Vahl* from Iran. The oils obtained were analyzed by GC–MS. The extraction time while using the MWHD is no more than 24 min using a microwave power of 300 W. The major components by two methods of HD and MWHD were carvacrol and thymol in which (1.43, 2.63%) and (10.80, 8.14%) respectively. Due to various usages of *Stachys* species or their oils and literature searches which indicated that the oil of *Stachys lavandulifolia Vahl*, have not been the MWHD in previous studies, we were interested in studying essential oil contents and compositions of *Stachys* species in Iran.

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1. Introduction

The genus *Stachy*L.comprises more than 270 species [1] and is justifiably considered as one of the largest genera of the Labiatae. In the old world area there are two main centres of diversity for the genus, as assessed by the number and distribution of the species. One is confined to South and East Antolia, Caucasia, North West Iran and North Iraq, the other to the Balkan Peninsula [2]. In Iran, 34 species of the this genus are present, among which, 13 are endemic [3] .The plant is known as Chaye-kuhi in Iran and is a native plant, which has been used as an anxiolytic and sedative in Iranian folk medicine [4]. However, the genus *Stachys* has been the subject of some phytochemical studies. Flavonoids, phenyl ethanoid glycosides, phenolic acids, iridoids, monoterpenes, sesquiterpenes, diterpenes, and triterpene saponins have been reported to be present in different *Stachys* species [5-10]. *Stachys lavandulifolia* has also been reported to contain volatile oil and a phenyl propanoid glycoside [11-12].

2. Experimental

2.1 Plant materials

About one kg of fresh aerial part of *Stachys lavandulifolia Vahl* at maturity was collected from agriculture college Garden of University, on June 2010. The dried aerial parts were stored in a dark place at 4°C.

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2.2 Chemicals and Reagents

Helium, 99.999%, used as carrier gas, was purchased from Roham Gas Company (Tehran, Iran). Alkane mixture consisting of the C8-C20 alkanes (concentration of 40 mg/mL in hexane) was purchased from Fluka. All other chemicals were of the highest purity available from Merck or Fluka. Doubly distilled deionized water was used

2.3 Instruments and GC/MS Operating Conditions and procedure

Gas chromatography was performed with a Shimadzu model GC-17A (Kyoto, Japan) instrument equipped with a Shimadzu Quadropole-MS (qMS) model QP5050 detector. Separation was performed using a 30 m × 0.25 mm I.D capillary fused silica column 6 coated with a 0.25μm film of DB5-MS (5% Phenyl-95% Polydimethyl Siloxane), and a split/splitless injector with a 1 mm internal diameter glass liner.

2.4 MWHD apparatus and procedure

MAHD was carried out with a Samsung microwave apparatus. The multimode microwave reactor has a twin magnetron (1000 W, 2455MHz) with a maximum delivered power of 1000W variable in 10W increments. A rotating microwave diffuser ensures homogeneous microwave distribution throughout the plasma coated cavity are35 cm × 35 cm × 35cm. Temperature was controlled by feedback to the microwave power regulator.

The experimental MADH variables have been optimized by the university method in Order to maximize the yield of essential oil. In a typical SFME procedure performed at atmospheric pressure, 60 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.

2.5. Hydro-distillation

The sample (100 g of dried material was charged with a particle size of about 500 μm) was submitted to hydro-distillation for 1.5 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia (1975). The volatile distillate was collected over anhydrous sodium sulphate and refrigerated until time of analysis. The yield of the oil was 3.1% v/w based on dry plant weight.

2.6 Qualitative and quantitative analyses

Most constituents were identified by gas chromatography through comparison of their retention indices (RIs) with those of the literature [13] or with those of authentic compounds available in our laboratories. The retention indices (RIs) were determined in relation to a homologous series of n-alkanes (C8–C24) under the same operating conditions. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST 98 and Wiley 5 Libraries or with mass spectra from literature [13-15]. Component relative concentrations were calculated based on GC peak areas without using correction factors.

3. Results and discussion

The yields of the oils obtained from S. lavandulifoila by two methods of extraction were 0.82% and 0.45%. The yields of the oils extracted from other species were reported as 0.18% from *S. setifera* and S. *iranica*, 0.18% from *S. chrysantha*, and 0.12% from *S.candida* [16]. The *S. lavandulifolia* oil was examined by GC and GC-MS. The list of compounds identified in the oil

of *S. lavandulifolia* can be seen in Table 1. Fifty five compounds were identified, representing 93.50%, and 92.49% by HD and MWHD of the essential oils respectively, in which the major components were germacrene-D, β-phellandrene, β-pinene , myrcene , α-pinene and Z-β-ocimene . In a previous study the main components of the oil were reported to be spathulenol (35.0%) and caryophyllene oxide (25.6%), this finding was completely different from our study. The major component of the *S. obliqua* oil was germacrene-D, which was also one of the main components of the *S. lavandulifolia* oil. The main components of the oils of *S. aegiptica* (α-pinene) and *S. glutinosa* (α-pinene and β-phellandrene) were presented as the major components of the *S. lavandulifolia oil* [19] and [20]. β-Pinene, one of the main components of *S. recta* and *S. balansae* oils, was present at an amount of 8.4% in *S. lavandulifolia* oil [21].

A qualitative comparison of the oil constituents of *S. schtschegleevii* with those of other *Stachys* species reported in the literature showed varying compositions [19, 22-24]. The oil of *S. Corsica* from France consisted of carvacryl acetate (37.5%), Iinalool (13.4%), [alpha]-terpinyl acetate (7.7%) and [alpha]-terpineol (7.8%) (6). 1-Octen-3-ol (18.7%), linalool (11.0%), [alpha]-pinene (8.3%), [delta]-cadinene (5.0%), eugenol (4.3%), [beta]-selinene (4.3%), limonene (4.2%) and [beta]-pinene (4.1%) were reported as major components of *S. athorecaltjx* from Turkey [23]. Also, the oil of S. recta from Turkey contained 1-octen-3-ol (33.8%), linalool (13.0%) and [beta]-pinene (7.5%) [23]. 1-Octen-3-ol was absent in the oil of *S. balansae*, but [beta]-caryophyllene (24.3%), [beta]-pinene (24.1%) and [alpha]-pinene (16.0%) were the major components [24]. The same group also found that the oil of *S. obliqua* contains germacrene D (25.4%), thymol (16.4%), limonene (6.2%), borneol (4.9%), [alpha]-pinene (4.7%) and isomenthol (3.4%) (9). *S. glutinosa* oil from Corsica, France, contained terpinen-4-ol (13.1%), [alpha]-pinene (10.1%), [alpha]-terpineol (8.4%), [beta]-phellanderene (6.8%) and [alpha]-terpinene (6.1%) as major compounds [17]. The major components of the oil of S. aegyptiaca from Egypt were [alpha]-pinene (54.46%), [beta]-caryophyllene (6.61%), limonene (5.35%) and myrcene (3.75%) [18].

In order to get access to the absolute mass percentage of the identified compounds, the essential oil of *Stachys lavandulifolia Vahl* was analyzed after extraction by hydro-distillation (HD) and MAHD.

3.1. Comparison of MWHD and HD for the analysis essential oil in *Stachys lavandulifolia Vahl*

The results in Table 1 show that the 26 compounds identified by HD were almost same with those by MWHD. As seen from Table 1, using the two different methods, the obtained relative contents for individual compounds (such as carvacrol) were very different. In the previous literatures [25-27], it has been demonstrated that microwave can much improve the extraction efficiencies of plant essential oil compounds [28-29]. This leads to the differences of the relative contents for individual compounds. Obviously, the HD method had good extraction efficiency. Moreover, HD required 3 h to isolate the essential oil and organic solvent to perform further extraction. Under microwave irradiation, isolation of essential oil in fresh plant materials was rapidly completed by dry distillation. Due to the isolation, extraction and concentration performed in a single step, sample preparation needed only 24 min by using, MWHD. In microwave-assisted hydrodisti, distillation time was shorter than classical hydrodistillation and also the sample reached boiling stage more rapidly. This is an advantage of MWHD when it is compared to classical HD In conclusion; the present method is simple, rapid and effective and can be used for the analysis of volatile compounds in medicinal plants. As compared to conventional technique of HD, MWHD are simple, rapid for determination of essential oils in fresh *Stachys lavandulifolia Vahl* and other plant materials.

Table 1. Chemical composition of the essential oil from Stachys lavandulifolia Vahl

No	Compound	RI*	HD (%)	MWHD(%)
1	α-Thujene	930	1.54	–
2	α -pinene	939	19.66	–
3	Sabinene	975	7.37	–
4	β-pinene	979	2.30	–
5	Myrcene	991	9.43	–
6	Para Cymene	1025	0.91	–
7	Limonene	1029	2.91	–
8	β-Phellandrene	1030	14.31	–
9	γ-Terpinene	1060	0.52	–
10	Linalool	1097	1.45	–
11	α -campholene	1120	0.48	–
12	trans-Pinocarveol	1139	0.60	–
13	TransLimonene oxide	1142	0.73	–
14	4- Terpineol	1182	0.88	–
15	Cryptone	1186	0.46	–
16	α -Terpineol	1189	0.66	5.03
17	Myrtenal	1196	0.48	–
18	Nerol	1230	–	–
19	Geraniol	1253	–	–
20	Thymol	1290	2.63	8.14
21	Carvacrol	1299	1.43	10.80
22	Cinnamyl alcohol	1304	–	–
23	γ-elemene	1338	–	–
24	α -Copaene	1377	0.67	–
25	Geranyl acetate	1381	–	7.96
26	β -Bourbonene	1388	1.05	–
27	trans-Caryophyllene	1419	–	5.12
28	Trans-α -Bergamotene	1435	0.58	–
29	β -Farnesene	1443	–	–
30	β -Caryophyllene	1455	–	–
31	β-Farnesene	1457	2.92	1.11
32	Germacrene D	1485	3.43	1.10
33	Valencene	1496	–	–
34	Bicyclogermacrene	1500	5.22	0.88
35	β -Bisabolene	1506	–	1.96
36	γ -Cadinene	1514	–	2.05
37	Myristicine	1519	–	3.56
38	δ-Cadinene	1523	0.65	–
39	elemol	1550	–	1.90
40	Spathulenol	1578	13.23	–
41	Salvial-4(14)-en-1-one	1595	0.49	–
42	Hexadecane	1600	0.59	0.56
43	10-epi-.gamma.-eudesmol	1624	–	–
44	δ-Cadinol	1636	–	–
45	Alloaromadendrene	1641	–	0.71
46	Cadinol	1650	–	0.61
47	β -Eudesmol	1651	–	0.86
48	α-Eudesmol	1654	–	–

No	Compound	RI*	HD (%)	MWHD(%)
49	Bisabolol oxide B	1658	–	–
50	Bisabolone oxide	1685	–	–
51	α -Bisabolol	1686	0.61	–
52	Heptadecane	1700	–	17.77
53	Bisabolol oxide A	1749	–	2.55
54	Benzyl benzoate	1760	0.91	–
55	Geranyl linalool isomer	2004	–	2.11

*RI= retention indices in elution order MD%=microwave distillation area %

4. Conclusions

In the work, extraction and determination of essential oil of *Stachys lavandulifolia Vahl* by HD and MWHD extraction methods were successfully performed. It has been shown that isolation; extraction and concentration of essential oil in fresh *Stachys lavandulifolia Vahl* can be done by two methods separately. Forty seven compounds were identified in the *Stachys lavandulifolia Vahl* by using the proposed methods. The experimental results demonstrate that using much less sample amount, shorter extraction time and simpler procedure, MWHD methods can achieve comparable results with those by HD for determination of essential oils in fresh materials. The major component by two methods were carvacrol and thymol in which (1.43, 2.63%) and (10.80, 8.14%), respectively. The major advantages of MWHD as compared with HD and reported HD are the low cost and highest extraction efficiency.

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