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Research Article

Comparison of two method of essential oil extraction: microwave-assisted hydrodistillation and hydrodistillation methods from *Stachys lavandulifolia* (Chai-E-Koohi)

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ABSTRACT

The essential oils isolated from aerial parts of *Stachys lavandulifolia* by hydrodistillation (HD), and microwave-assisted hydrodistillation (MWHD) techniques, were analyzed by means of GC and GC-MS methods. The oil extracted by MWHD method contained 12 com- pounds with a yield of 0.12% (w/w), representing 77.78% of the total oil. 12 compounds were identified in the volatilesfrom the aerial parts of *S. lavandulifolia* by HD method comprising 37.9% of the total oil in 0.12% (w/w) yield. The main components of the water distilled oil were found to Phenyl glyoxime (12.84 %), Isopulegone (7.17%), p-Mentha-2,4,(8)-diene (5.67%) while the oil extracted by MWHD method was mainly composed of dihydro indol-4- ol-2-one,5,7-dibromo-3,3-dimethyl(34.03), Benzene, (1-methyl-2-propenyl- 3-ol) (21.52%) and 7-hydroxy-bicyclo [3.3.1] non-2-en-9-one (7.55%).Also the anticlerical activities of the oils were compared by using MIC method.

Keywords: Essential oil; GC-MS; MWHD; Stachys lavandulifolia

1. Introduction

Stachys lavandulifolia or Chai-E-Koohi (in Persian) is a biennial medicinal plant belonging to the family Lamiaceae. Essential oil of *S. lavandulifolia* is used as flavoring agents in food products such as beverages, bread, pickles, pastries, and cheese. It is also used as a constituent of cosmetic and pharmaceutical products [1]. Herbal drugs and essential oils of *S. lavandulifolia* have hepatoprotective effects [2], as well as antispasmodic effects [3]. They are also known for their diuretic, anti-inflammatory, analgesic and antioxidant activities [4]. Anand and his co-workers [5] reported that *S. lavandulifolia* seed possesses anticancer activity. Recently it was shown that *S. lavandulifolia* essential oil possesses emmenagogue and galactagogue properties [6] and is a cure for pediatric colic and respiratory disorders due to its antispasmodic effects [7,8]. Many phytochemical studies have been conducted to investigate the chemical composition of the essential oil of *S. lavandulifolia* from different origins and have shown that the major components are phenylpropanoid derivatives and monoterpenoids [9-10]. Ethnobotanical data currently available on wild useful plants in Egypt highlight the importance of *S. lavandulifolia*. culinary and medicinal uses [11]. Moreover, *S. lavandulifolia* has been used for centuries in the Mediterranean area as an aromatic herb and also in folk medicine, due to the aforementioned pharmacological properties of its essential oil Essential oil composition depends upon internal, environmental and ag cultural practices as well as factors affecting the plant such as genetics, and ecological conditions [12,13]. Microwave heating has an incontestable place in analytical and organic laboratory practices as a very effective and non-polluting method of activation. The main reason for this increased interest lies in the much shorter operation times achievable. Microwave-assisted extraction of natural compounds is also an alternative to conventional techniques. Essential oils are among the products which have been extracted efficiently from a variety of matrices by this method and many microwave- assisted essential oil extractions

from several plants and subsequent product analyses have been reported [14-20]. The objective of the work described in this communication was to investigate the components and Antibacterial activity of the essential oil from the aerial parts of *Stachys lavandulifolia*. Obtained by microwave-assisted hydro-distillation, as compared with the normal hydro-distillation.

2. Materials and methods

2.1. Plant materials

The dried aerial parts of *S. lavandulifolia* were obtained in July 2021 from the Zagros Mountains, lying in the Fars province (Southern Iran). The *Stachys* genus was certified by top experts from the Medicinal Chemistry Research Center of Shiraz, Shiraz Iran. Certified specimens were then kept in a dark and cold room until used shortly afterwards for the experiments.

2.2. Isolation and analysis of the essential oil

Leaves (600 g) were homogenized and hydrodistilled for 4 h using a Clevenger-type apparatus to yield about 0.12%of yellowish-colored oil with a strong odor. Fresh leaves (200 g) were also homogenized and hydrodistilled at 800Wfor 30min using an adapted microwave distillation apparatus which consists of a microwave oven connected to a Clevenger type apparatus, to yield 0.12 %of yellowish-colored oil with a strong odor.

2.3. Identification of components

For identification of components, an analytical HP 5890 gas chromatograph (Hewlett Packard, Palo Alto, CA) was used with a DELSI 121 C apparatus fitted with a flame ionization detector and a CPWAX 51 fused silica column (25m_0.3mm; 0.25_mfilmthickness). The temperature was set at 50°C for 5 minutes and programmed to reach 220°C at a rate of 3°C/ minute. ACPWAX51 fused silica WCOT column (60 m _ 0.3mm) for gas chromatography was used with helium as carrier gas. For gas

chromatography/mass spectrometry (GC/MS) a CPWAX52 fused silica CB column (50m_ 0.25mm) was used with helium as the carrier gas (flowrate 1mL/minute) and coupled to an HP mass spectrometer with an ionization energy of 70 eV. Temperature programming was from50° to 240°C at a rate of 3°C/minute. The samples were injected at an injector temperature of 240°C. The components were identified by comparing linear Kovats indices, their retention times, and mass spectra with those obtained from the authentic samples and/or the mass spectrometry library.

The percentage composition of the essential oils was computed from 6°C peak are as without correction factors. Qualitative analysis was based on a comparison of retention times and mass spectra with corresponding data in the literature.

2.4. Antibacterial analysis

To determine the antibacterial activities of each tested essential oil, the MIC method was utilized [21]. Using cultures of *Escerichia coli*, *Psedumon asaeruginosa*, *Staphylococcus aureus, Bacillus pumilus*. The test microorganisms used in this experiment were obtained from the culture collections section of Iran Institute of Medical Research Tehran. Those were further re-cultured for this experiment. The antibacterial activities of the *Stachys lavandulifolia* oil were investigated by MIC method on Muller-Hinton broth. It was performed using a 24 h old bacterial culture at 37°C, reseeded on Nutrient Broth. Bacterial species were cultured on nutrient agar media nutrient broth inoculated with each bacterial species was incubated for 24 h at 37 °C The agar plates or malt and yeast oil agar previously seeded with 100 ìL of inoculum suspension of each bacterial species respectively. The cultures were incubated either at 35 °C for 72 h for filamentous fungus or at 37 °C for 24.48 h for yeasts and bacteria. Each experiment was replicated three times.Antibioticswere used as positive and negative control; ampicillin and gentamaicinewas used as antibacterial standard.

Fig. 1 *Stachys lavandulifolia* (Chai-E-Koohi) In the flowering season

3. Result and discussion

3.1. Essential oil

The compositions of the oils from both methods were analyzed using GC/MS. Identification of all components was performed by a comparison of their mass spectra with literature data (NIST and WILEY) and by comparison of their retention indices (RI)with those in the literature [22,23]. The gas chromatograms of the essential oils from both methods are presented in and TABLE 1 lists the identified components of the oils. The oil extracted by MWHD method contained 12 compounds with a yield of 0.12%(w/w), representing 77.78%of the total oil. 12 compounds were identified in the volatiles from the aerial parts of *S. lavandulifolia* by HD method comprising37.9% of the total oil in 0.12% (w/w) yield. The main components of the water distilled oil were found to Phenyl glyoxime (12.84%), Isopulegone (7.17%), p-Mentha-2,4,(8)-diene (5.67%) while the oil extracted by MWHD method was mainly composed of dihydro indol-4-ol-2-one,5,7-dibromo- 3,3-dimethyl (34.03),Benzene, (1-methyl-2-propenyl- 3-ol) (21.52%) and 7-hydroxy-bicyclo[3.3.1]non-2-en-9-one (7.55%). As can be seen, there is some difference between the amounts and number of these components in MAHD oil, compared with the HD oil. There were some minor

constituents which appeared only in the MAHD oil. A possible reason for these minor differences may be the different heat sources used in the two methods. It is known that a microwaved solution is sometimes superheated, with the temperature of the solution being as much as 20 degrees higher than normal.

Table 1. Chemical composition of the essential oils of *Stachys lavandulifolia*

No	Constituent	RI	% *Hydrodistilation*	% *Microwave*	KI-MS
1	Santolina-triene	909	3.85	1.95	KI-MS
2	2,5-cyclohexadiene-1-one,3,4,4-trimethyl	972	-	0.5	MS
3	α-pinene	979	0.5	0.38	KI-MS
4	Octahydrocyclobuta [c] pentalene	1023	-	3.86	MS
5	p-Cymene	1025	0.46	-	KI-MS
6	Cis-Ocimene	1037	0.63	-	KI-MS
7	Trans-Ocimene	1050	-	0.68	KI-MS
8	α -Terpinen	1060	0.6	-	KI-MS
9	Isopulegone	1067	7.17	-	MS
10	7-hydroxy-bicyclo[3.3.1]non-2-en-9-one	1070	-	7.55	KI-MS
11	p-Mentha-2,4,(8)-diene	1088	5.67	-	KI-MS
12	Camphor	1146	-	0.64	KI-MS
13	Phenyl glyoxime	1176	12.84	-	MS
14	Benzene,(1-methyl-2-propenyl-3-ol)	1178	-	21.52	MS
15	m-Anisaldehyde	1196	-	3.09	KI-MS
16	5-Caranol, (1s,3R,5s,6R) -(-)-	1221	-	2.07	MS
17	exo-Fenchyl acetate	1233	0.5	-	KI-MS
18	p-Anis aldehyde	1250	3.1	-	KI-MS
19	2,3-dihydroindol-4-ol-2-one,5,7-dibromo-3,3-dimethyl	1270	-	34.03	MS
20	Anis ketone	1337	0.5	-	MS
21	5-oxo-1,3a,4,5,6a,hexahydropentalen-1-yl)-acetaldehyde	1342	1.27	-	MS
22	α-Thujaplicin	1477	-	1.51	KI-MS
	Total	-	37.09	77.78	-

3.2. Antimicrobial activity

The anticlerical activities of the oils were evaluated using MIC method and the results are shown in TABLE 2. It can be seen that both oils had more or less of the same activity. Due to antimicrobial activity, eseential oils of *S. lavandulifolia* extracted by both methods can be used as supplement in pharmaceutical industries. It can also be used in stabilizing food against oxidative deterioration. It was reported by Nenad et al. (2007) [24] that the main advantage of natural agents is that they do not enhance the antibiotic resistance, a phenomenon commonly encountered with the long-term use of synthetic antibiotics. There

are reports of the active principles of essential oils from various plants with antibacterial or antifungal activity. The antimicrobial activity of essential oils is assigned to a number of small terpenoids and phenolic compounds, which also in pure form demonstrate high antibacterial activity [25]. The essential oils and their components are known to be active against a wide variety of microorganisms, including Gram-negative and Gram-positive bacteria. Gram-negative bacteria were shown to be generally more resistant than Gram-positive ones to the antagonistic effects of essential oils because of the lipopolysaccharide present in the outer membrane, but this was not always true.

Table 2. Antibacterial activity of *Stachys lavandulifolia*

	Tested Bactria	*S. lavandulifolia* Oil (HD) µg/ml	*S. lavandulifolia* Oil (MWHD) µg/ml
Gram-negative	*Escherichia coli*	256	256
	Pseudomonas aeruginosa	256	256
Gram-positive	*Staphylococcus*	64	128
	Bacillus pumilus	128	256

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