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Chemical Composition and Antibacterial Activity of the Essential Oil of *Lavandula angustifolia* Isolated by Solvent Free Microwave Assisted Extraction and Hydrodistillation

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ABSTRACT: Chemical composition of *Lavandula angustifolia* was studied by solvent free microwave extraction (SFME) and hydrodistillation (HD) methods. 1, 8-cineole (42.7% and 37.9%), Camphor (26.1% and 21.3%) and Borneol (31.2% and 21.6%) were the major compounds that obtained by SFME and hydrodistillation, respectively. The antibacterial activity of extracted oils was investigated against Gram-positive bacteria *Bacillus subtilis* (B-sub) ATCC 6633 and Gram-negative bacteria *Escherichia coli* (E-coli) ATCC 8739. The minimum inhibitory concentration (MIC) of the essential oils isolated by hydrodistillation and SFME was the same.

Keywords: *Lavandula angustifolia, Solvent Free Microwave Extraction, Hydrodistillation, Antibacterial Activity.*

Introduction

Hydrodistillation, steam distillation, supercritical fluid extraction (Bartley, 1995; Bartley & Foley, 1994), pressurized liquid extraction, pressurized hot water extraction, solvent extraction (Zancan *et al*., 2002), membrane-assisted solvent extraction, solid-phase microextraction (Chemat *et al*., 2006), stir bar sorptive extraction and ultrasounds and several other techniques are used for the extraction of essential oils from herbs. Recently, usage of microwave as a heating source affords fast heating for the extraction of volatiles (Kosar *et al*., 2005; Lucchesi *et al*., 2004a; Lucchesi *et al*., 2004b, Lucchesi *et al*., 2007; Chen & Spird, 1994). Microwave-assisted techniques have been described as time saving, energy saving, and highly efficient. In addition, they have been widely used for the extraction of plant essential oils (Bilia *et al*., 2002; Stashenko *et al*., 1999; Wang *et al*., 2006). Microwave-assisted hydrodistillation (MAHD)technique was developed for the extraction of essential oils (Silva *et al*., 2003; Silva *et al*., 2004; Lo Presti *et al*., 2005; Stashenko *et al*., 2004a; Stashenko *et al*., 2004b; Cavanagh, 2005). Solvent free microwave extraction is a new technique, which combines microwave heating with dry distillation at atmospheric pressure for the isolation and concentration of the essential oils in fresh plant materials (Lucchesi *et al*., 2004b).

Lavender oil is known for its excellent aroma, and it is extensively used in the perfumery, flavour and cosmetic industries. The oil is known to possess sedative, carminative, anti-depressive and anti-inflammatory properties. It was also found to be active against many species of bacteria, including those resistant to antibiotics (Tucker *et al*., 1984). The essential oil compositions of lavender grown in different countries were investigated (Lawrence,

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2003; Adams & Yanke, 2007; Verma *et al*., 2010; Cong *et al*., 2008).

Materials and Methods

Plant Material

The aerial parts of *Lavandula angustifolia* at the flowering stage were collected from Yazd, in June 2008. A voucher specimen was deposited in the herbarium of Shahid Beheshti University.

Isolation Procedure

- Hydrodistillation

The dried sample (100 g) was subjected to hydrodistillation by using a Clevenger apparatus for 3 h. The oils were dried over anhydrous Na_2SO_4 and stored in sealed vials at 4°C before analysis.

- Solvent-free microwave extraction (SFME)

Milestone MicroSynth microwave apparatus, 2450MHz with maximum power 1000W and ACTE0 sensor for temperature monitoring was used for SFME. The power of the oven was 500W for 10 min .The temperature was achieved at 95 °C, and the extraction was carried out for 25 min. 30 g of dried *Lavandula angustifolia* was soaked in 20 mL distilled water at room temperature (25 °C) for 1 h in order to hydrate the external layers of the plant material. The moistened plant material was placed in a flat-bottom flask combined to a Clevenger apparatus. The SFME process was performed for 35 min. The essential oils were collected in amber colored vials, dehydrated with anhydrous sodium sulphate, capped under nitrogen and kept at 4 °C until being analyzed.

Gas chromatography

GC analyses of the oils were performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless injector (250ºC). Nitrogen was used as a carrier gas (1 ml/min) and the capillary column used was DB-5 (30 m × 0.25 mm, film thickness 0.32 μm). The column temperature was kept at 60°C for 3 min, heated to 220°C with 5°C/min rates, and then kept constant at 220°C for 5 min .The relative percentage amounts were calculated from the peak area using a Shimadzu C-R4A Chromatopac without the use of correction factors.

Gas chromatography–mass spectrometry

GC–MS analyses were carried out on a Hewlett Packard (HP) 6890 GC–MS system equipped with an HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm). The oven temperature program was from 60°C to 220°C at a rate of 6°C /min, the transfer line temperature was 280°C, the injector temperature was 250°C, the carrier gas was Helium, and the flow rate was 1 ml/min. In addition, the ionization energy of MS was 70 eV.

Identification of components

The components of the oils were identified by comparison of their mass spectra with those of a computer library (Wiley 275 database) or with authentic compounds (Adams, 1995) and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature. The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes.

Antimicrobial activity

The minimum inhibitory concentrations (MICs) of the essential oil were determined by agar dilution method (EUCAST, 2000) with respect to different test microorganisms, including Gram-positive (Bacillus subtilis ATCC 6633) and Gram-negative (Escherichia coli ATCC 8739) bacteria. A series of eight dilutions of the oils was prepared in ethanol (1 ml). Each dilute was added to molten Mueller-Hinton (MH) agar (19 ml) at 50°C to give the final

concentrations of 0.05, 0.10, 0.15, 0.25, 0.50, 1.00, 1.50 and 2.00 mg/ml. The bacterial inocula were prepared by suspending overnight colonies from MH agar media in sterile saline. The inocula were adjusted photometrically at 600 nm to a cell density equivalent to approximately 107 CFU/ml. The plates were spot-inoculated with 1 µl of each prepared bacterial suspension (104 CFU/spot), including a control plate containing 1 ml ethanol without any essential oil. The plates were incubated at 35-37°C, and examined after 48 hours. The MIC was determined as the lowest concentration of the agent that completely inhibited the visible growth of the microorganisms.

Results and Discussion

Effect of microwave power on SFME procedure

30 grams of a powdered plant were subjected to various microwave power (100 to 700 W) for 30 min. As 1, 8-cineole, Camphor and Borneol were the main compounds in *Lavandula angustifolia* essential oil, these peak areas were plotted vs. the microwave power in figure 1. In 500 W power of microwave, higher values of these compounds were extracted.

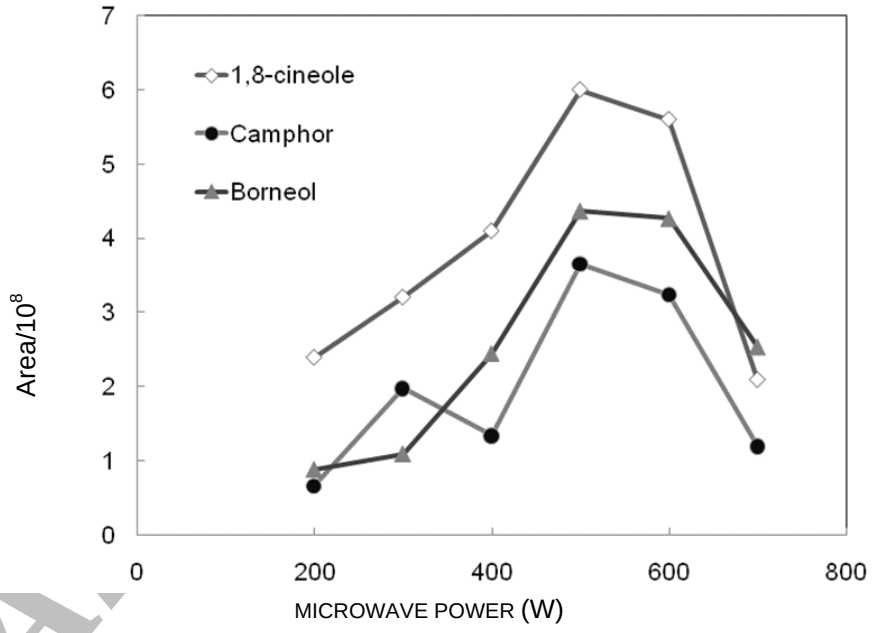

Fig. 1. The effect of microwave power in SFME method (extraction time=25 min)

Table 1. Variation of the essential oil volume (ml) in different extraction times

Extraction time (min)	Volume of extracted oil
5	Less than 0.1 ml
10	Less than 0.1 ml
15	0.2 ml
20	0.3 ml
25	0.5 ml
30	0.5 ml

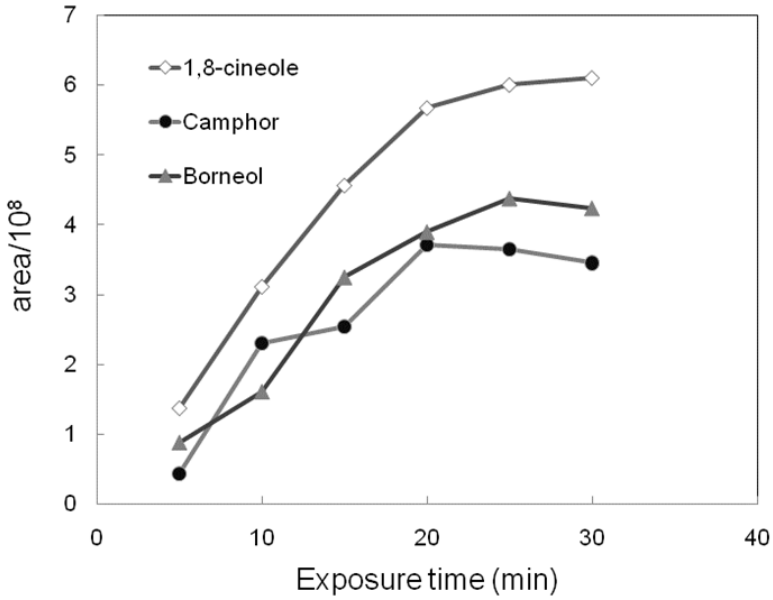

Fig. 2. the influence of extraction time in SFME method (microwave power = 500W)

Influence of the extraction time on SFME procedure

SFME method was done in 500 W power of microwave for 5, 10, 15, 20 and 25 min. The peak areas of 1,8-cineole, Camphor and Borneol as the main compounds were investigated in the different extraction times. Figure 2 shows the effect of the extraction time vs. the values of the isolated compounds. The volumes of the obtained essential oils in the different times of extraction were studied. As shown in table 1, in 25 and 30 min of the extraction time, the volumes of the obtained oils were high, and then 25 min was selected as the optimum time.

Table 2. Chemical composition of *Lavandula angustifolia* essential oils obtained by hydrodistillation and SFME

	Compound	**KI**	**HD %**	**SFME %**
1	α-pinene	939	0.9	-
2	p-cymene	1025	1.3	-
3	Limonene	1029	0.8	-
4	1,8-cineole	1031	37.9	42.7
5	Camphor	1146	21.3	26.1
6	Borneol	1169	21.6	31.2
7	Cryptone	1186	2.6	-
8	isobornyl formate	1239	1.1	-
9	cumin aldehyde	1242	2.3	-
10	Valerianol	1658	2.1	-
	total		**91.9**	**100**
Monoterpenes			3.03	-
Oxygenated compounds			88.84	99.98

Antibacterial activity

The antibacterial activities of the essential oils obtained by both methods (hydrodistillation and SFME) were examined against *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 8739. They showed the same antibacterial activity. The results showed MIC of 0.5 mg/ml for *Bacillus subtilis* and MIC of 1.0 mg/ml for *Escherichia coli*, for both essential oils.

Conclusion

The SFME method was more able to extract oxygenated compounds than HD. The antibacterial activities of the essential oils isolated by both methods against *Bacillus subtilis* and *Escherichia coli* were the same.

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