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Original Research Article

Chemical composition of the essential oils from the aerial parts of *Malva neglecta* Wallr. from Khorramabad, Lorestan Province, Iran using solvent free microwave extraction (SFME) method

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ABSTRACT

This study deals with the investigation of the efficacy of solvent free microwave extraction (SFME) method to extract the essential oils from the aerial parts of *Malva neglecta* Wallr.. The essential oils were then injected onto an HP-5MS column of a commercially available GC/MS (Hewlett-Packard 5973), which resulted in a chromatogram consisting of 24 compounds accounting for 99.9% of the oil composition. In terms of general categories, non-terpene hydrocarbons and sesquiterpene hydrocarbons were found to be the major fractions of the chemical profiles. Moreover, hinokione was recognized as the most abundant constituent component of the essential oil comprising 40.7% of the total oil structure. Regarding our findings in this study, the SFME can be introduced as a rapid, cost-effective, and environmentally friendly extraction method for the separation of the essential oils.

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1. Introduction

The genus *Malva* has about 10 herbal and shrub species native to Iran and in Persian and local traditional treatment is called "Panirak". Some species of this genus including *M. armeniaca*, *M. leonardi*, *M. flexuosa*, *M. rotundifolia*, *M. lljini*, *M. nicaeensis*, *M. parviflora* and *M. microcarpa*, grow in Turkmenistan, Middle Asia, Mediterranean regions, the Balkan Peninsula, Anatolia, Iraq, Afghanistan and Armenia in addition to Iran (Mozaffarian, 1996). *Malva neglecta* Wallr. belongs to Mallow Family (Malvaceae) and is an annual herb that grows to a height of 0.6 m (2 ft). This medicinal species is known as common mallow in the United States and also button or cheese weed, cheese plant, dwarf (round leaf or garden or low or round-leaved or running) mallow, malice and round dock (Zargari, 1989). Although often considered a weed, this plant is consumed as a food worldwide (Facciola, 1990). This is especially true for the seeds, that consiststs of 21% protein and 15.2% fat (Duke and Atchley, 1986). The plant (*M. neglecta* Wallr.) is shown to be an invasive species in the United States and commonly occurs in disturbed sites such as roadsides, railroads, waste places, gravel pits, nurseries, gardens, and cultivated fields. It is frequently found growing in yards around homes, buildings, and barns (Peterson and McKinney, 1968). Currently, promising phytochemical activities of different species of *Malva* genus have been reported, including potential antioxidant (Mavi et al., 2004; DellaGreca et al., 2009; Stef et al., 2009; Bouriche et al., 2010; Bouriche et al., 2011; Ghanati and Khatami, 2011; Dalar et al., 2012; Guder and Korkmaz, 2012; Tesevic et al., 2012; Turker and Dalar, 2013; Khan et al., 2016), antibacterial (Grierson and Afolayan, 1999; Keles et al., 2001; Kumarasamy et al., 2002; Shale et al., 2004; Bernardo et al., 2005; Shale et al., 2005; Cogo et al., 2010; Seyyednejad et al., 2010; Razavi et al., 2011; Walter et al., 2011; Khan et al., 2016),

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antinociceptive (Esteves et al., 2009), antiproliferative (Conforti et al., 2008), anti-inflammatory (Franzotti et al., 2000; Sleiman and Daher, 2009; Afolayan et al., 2010; Bouriche et al., 2010; Bouriche et al., 2011; Benso et al., 2015), antimicrobial (de Souza et al., 2004; Alves et al., 2009; Razavi et al., 2011; Vitullo et al., 2011; Benso et al., 2015; Khan et al., 2016), anticancer (Huang et al., 1998; Daniela et al., 2007), anti-complementary mucilage (Tomoda et al., 1989), antifungal (Wang and Bunkers, 2000; Wang et al., 2001; Andrade Pinto et al., 2010; Oshchepkova et al., 2012; Romitelli and Martins, 2013) and antiadherent properties (Alves et al., 2009). These findings account for the possibility of considering this medicinal plant as an alternative to chemical drugs that have harmful side effects. Hydrodistillation (HD) and steam distillation (SD) methods have been used for a long time to extract the essential oils from a broad spectrum of medicinal plants as conventional methods. However, these traditional procedures often need large amounts of plant samples and are also not cost-effective and time-saving (Akhlaghi et al., 2009a; Akhlaghi et al., 2011; Mohammadhosseini, 2012a, b; Mohammadhosseini, 2013; Motavalizadehkakhky et al., 2013; Shahnama et al., 2015). Therefore, development of proper alternative facile and secure techniques which are amenable to automation seems rationale and unavoidable (Nekoei and Mohammadhosseini, 2014). Over the recent decades, there has been a growing interest in using microwave radiation for the separation of the essential oils from a wide spectrum of plant organs (Hashemi-Moghaddam et al., 2015; Mohammadhosseini, 2016). The technique solvent-free microwave-assisted extraction (SFME) was developed and then patented by Chemat et al. in France (2013). This technique could be considered among the newest approaches in the extraction of the essential oils applying the microwave beams in the conditions that no solvent and/or water are being used under the atmospheric pressure. The general SFME setup involves four parts which are: i) one reactor where only matrix to be processed is placed, ii) a standard microwave oven, iii) the refrigeration system, and iv) essence container where essential oil is recovered (see Fig. 1). In fact, the extraction process using the SFME technique accounts for dry distillation assisted by microwave beams being focused on the fresh matrix in a microwave reactor without the addition of water or any other organic solvent. Water heating of the raw material breaks the essential oil containing glands. This phase releases the essential oil, which is then driven by steam produced from matrix water. A cooling system placed outside the microwave oven allows the continuous condensation of the distillate, which consists of water and the essential oil, while the excessive water returns inside the balloon allowing maintenance of the proper humidity rate of the matrix (Chemat and Cravotto, 2013; Mohammadhosseini, 2016). The extraction process is frequently continued at 100 °C until no more essential oil is obtained. In the final step, the isolated essential oil is collected, dried with anhydrous sodium sulfate and stored at 4 °C prior to the analysis using gas chromatography coupled to mass spectrometry (GC-MS). According to the literature, the SFME technique has been successfully applied to several kinds of fresh and dry plants, including spices, aromatic herbs, and citrus fruits (Lucchesi et al., 2004a, b; Ma et al., 2012; Mohammadhosseini and Nekoei, 2014; Mohammadhosseini, 2015; Mohammadhosseini et al., 2015; Nekoei and Mohammadhosseini, 2016). To the best of our knowledge, this is the first report concerning the chemical compositions of the essential oils from the aerial parts of *M. neglecta* Wallr. obtained by the SFME technique.

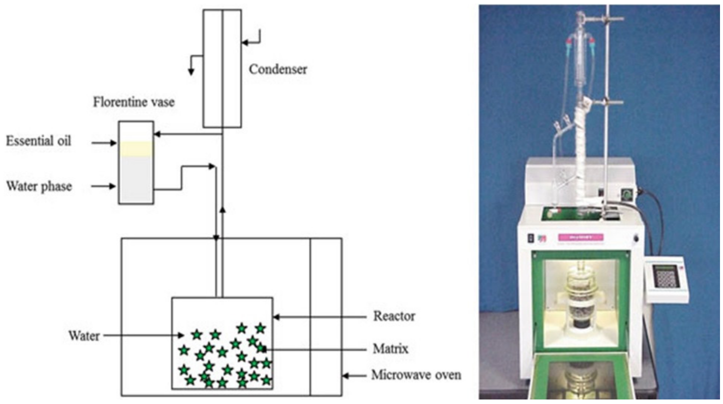

Fig. 1. The overall scheme (left) and apparatus (right) of the solvent-free microwave extraction (SFME) technique.

2. Experimental

2.1. Plant material and botanical identification

The plant material was collected during the flowering stage in April 2020 from Khorramabad region, Lorestan Province, Iran, at the geographical coordinates of 33.4878° N, 48.3558° E respectively at an altitude of 1147.8 meters above sea level. A voucher specimen was deposited at the Herbarium of the Faculty of Agriculture (Herbarium number 8894), Shahrood University of Technology, Shahrood, Iran, for further authentication.

2.2. Details of the SFME technique

The general characteristics associated with this technique were described in our previous report with slight modifications (Mohammadhosseini and Nekoei, 2014). Accordingly, 100-g portions of the powdered and air-dried aerial parts of *M. neglecta* Wallr. were soaked in 1000 mL of distilled water. This preliminary step was mainly to soak the external and outer layers of the plant organs prior to the extraction under the atmospheric pressure. At an optimized and fixed power of 800 W, the extraction continued for 30 minutes, and the mean yield of the oil obtained by this method was found to be 0.21% (w/w).

2.3. Chromatographic analyses

GC analyses were performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless (ratio 1:30) injector and a flame ionization detector (FID), both operating at 250 ˚C. High purity nitrogen was utilized as the carrier gas having a flow-rate of 1 mL/min. The capillary column used was DB-5 (50 m×0.2 mm, film thickness 0.32 μm) and its temperature was kept at 60 ˚C for 3 minutes, then heated to 220 ˚C with a 5 ˚C/min rate and finally kept constant at 220 ˚C for 5 minutes. Relative percentage amounts were calculated from peak areas using a CR5 Shimadzu CR pack without the use of correction factors. In addition, GC/MS analyses were performed using a Hewlett-Packard 5973 instrument equipped with an HP-5MS column (30 m×0.25 mm, film thickness 0.25 μm). The column temperature programming was the same with that mentioned for the GC analysis, reaching to a final temperature of 230 ˚C. The effluent of the GC column was directly transferred into the MS source producing the charged fragments. The flow-rate of helium as the carrier gas was regulated at 1 mL/min. The detector temperature (MS) was set at 250 ˚C and all the MS spectra were recorded at 70 eV (E1) over the range 30-350 amu. The optimal voltage of the electron multiplier was set at 1800 eV and scan time was fixed to be 2 scans/sec.

2.4. Qualitative and quantitative analyses

Identification and determination of the constituents of the essential oils of *M. neglecta* Wallr. (aerial parts) were tentatively made by comparison of their mass spectral fragmentation patterns and retention indices (RI) relative to C_9-C_{25} *n*-alkanes, both with those given in the literature and those stored in the MS library (Wiley 275). For further reliability, some of the spectral patterns as well as Kovats indices based on previous findings of our research group were utilized (Mohammadhosseini et al., 2008; Akhlaghi et al., 2009b; Mohammadhosseini et al., 2010; Mohammadhosseini et al., 2011a; Mohammadhosseini et al., 2011b; Akhlaghi et al., 2012; Mohammadhosseini et al., 2012; Nekoei et al., 2012; Mohammadhosseini and Beiranvand, 2013; Mohammadhosseini et al., 2013; Hashemi-Moghaddam et al., 2014; Mohammadhosseini et al., 2016a; Mohammadhosseini et al., 2016b; Rahimi et al., 2016). Relative percentages of the components were calculated from peak areas using a Shimadzu C-R4A Chromatopac on the DB-5 column without the use of a correction factor.

3. Results and Discussion

3.1. Chemical profiles by the SFME method

The volatile constituents of the essential oils obtained from the aerial parts of *M. neglecta* Wallr. are listed in Table 1, in which both the percentages and the retention indices of the components are given. As can be seen, there are negligible differences in the numerical values of the Kovats retention indices between the calculated ones and those cited in the literature. Furthermore, a total of 24 components were obtained, accounting for 99.9% of the chemical profile (see Fig. 2). The Kovats retention indices were calculated by co-injecting a mixture of normal alkanes (*n*=9-24) into the column under the same conditions with the essential oil sample. As far as we know, at this time, there is no paper in the literature dealing with the characterization of the chemical composition of the essential oils of *M. neglecta* Wallr.. Using the SFME method (Table 1 and Fig. 2), twenty-one components could be identified in the *M. neglecta* Wallr. oil from its aerial parts: three oxygenated monoterpenes (5.4%), ten sesquiterpene hydrocarbons (36.6%), five oxygenated sesquiterpenes (10.1%) and three non-terpene hydrocarbons (46.1%), while only three compounds remained unidentified, totally constituting 1.8% of the essential oil composition. Specifically, the major components in the hydrodistilled oil from the aerial parts of *M. neglecta* Wallr. were found to be hinokione (40.7%), γ-elemene (9.4%), δ-elemene (6.8%), β-ylangene (5.8%) and bicyclogermacrene (5.1%). Moreover, some components including L-linalool (3.8%), spathulenol (3.8%), viridiflorol (3.1%), *n*-tetradecane (2.9%), germacrene D (2.8%), 6,10,14-trimethyl-2-pentadecanone (2.5%), β-elemene (2.0%), γ-curcumene (1.8%), δ-cadinene (1.2%), α-cadinol (1.2%), β-damascenone (1.2%), α-muurolol (1.1%), *cis*-muurola-3,5-diene (1.0%) occurred in the oil structure in lower quantities. On the other hand, very low quantities of natural compounds involving (*E*)-nerolidol (0.9%), *cis*-cadina-1(6),4-diene (0.7%) and α-terpineol (0.4%) were recognized in the chemical profile, each contributing to less than one percent (<1.0%) of the total oil composition.

In Fig. 3, relative percentages of the various classes of the volatile oil compounds have been compared. From this Figure, it is evident that in the SFME profile of the essential oils from the aerial parts of *M. neglecta* Wallr., non-terpene hydrocarbons constituted the main components, with the second largest class being sesquiterpene hydrocarbons. Furthermore, the third, fourth, and fifth rank in the chemical profile are due to oxygenated sesquiterpenes, oxygenated monoterpenes and non-identified compounds, respectively.

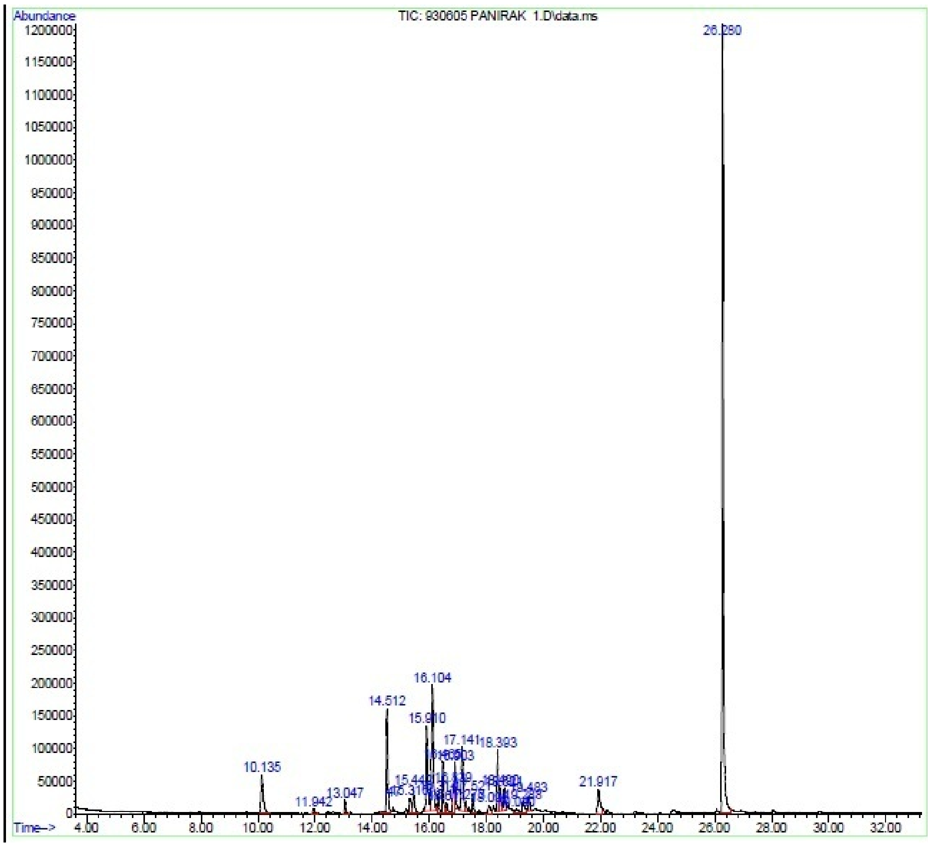

Fig. 2. The chromatogram of the volatile essential obtained from the aerial parts of *Malva neglecta* Wallr. using the SFME method.

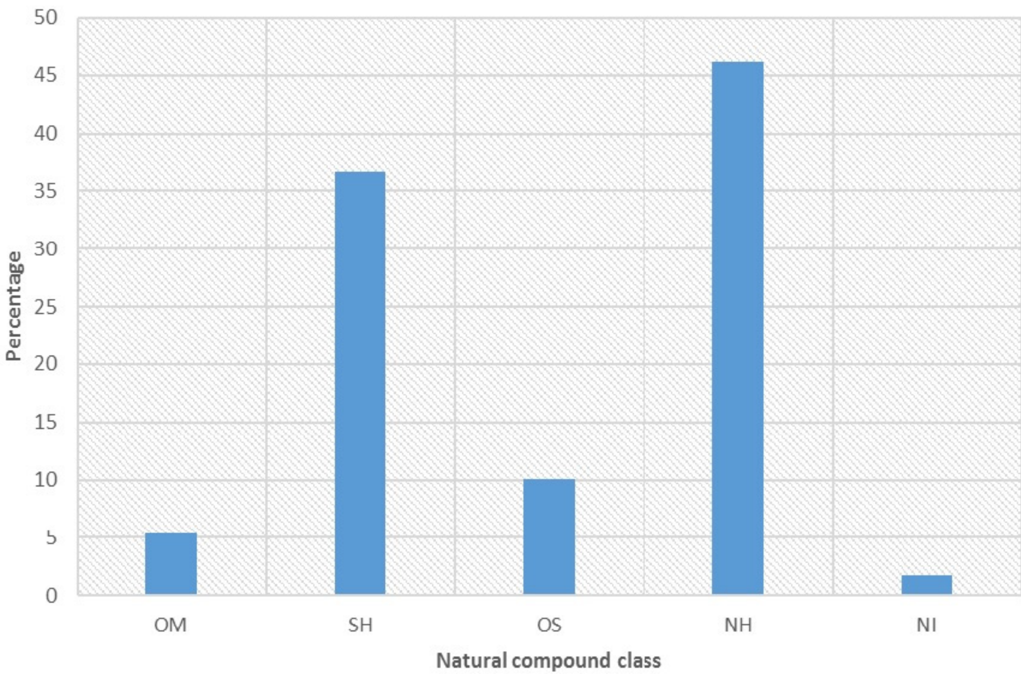

Fig. 3. Percentages of the classes of natural compounds found in the volatiles from aerial parts of *Malva neglecta* Wallr. using the SFME method (OM= oxygenated monoterpenes; SH= sesquiterpene hydrocarbon; OS= oxygenated sesquiterpenes; NH= non-terpene hydrocarbon; NI= non-identified compounds).

Table 1
Chemical compositions of the essential oils from the aerial parts of *Malva neglecta* Wallr. obtained using the SFME method. [a]

Ser. Num.	Name	Group	Molecular formula	KI (Lit.) [b]	KI (Cal.) [c]	Aerial parts (%)
1	L-Linalool	OM	$C_{10}H_{18}O$	1098	1100.5	3.8
2	α-Terpineol	OM	$C_{10}H_{18}O$	1188	1194.2	0.4
3	NI	-	-	-	1254.3	0.7
4	δ-Elemene	SH	$C_{15}H_{24}$	1337	1328	6.8
5	β-Damascenone	OM	$C_{13}H_{18}O$	1391	1386.5	1.2
6	β-Elemene	SH	$C_{15}H_{24}$	1393	1393.9	2
7	β-Ylangene	SH	$C_{15}H_{24}$	1419	1423.4	5.8
8	γ-Elemene	SH	$C_{15}H_{24}$	1433	1435.7	9.4
9	*cis*-Muurola-3,5-diene	SH	$C_{15}H_{24}$	1438	1449	1
10	*n*-Tetradecane	NH	$C_{14}H_{30}$	1400	1458.5	2.9
11	*cis*-Cadina-1(6),4-diene	SH	$C_{15}H_{24}$	1463	1467.7	0.7
12	γ-Curcumene	SH	$C_{15}H_{24}$	1482	1482.1	1.8
13	Germacrene D	SH	$C_{15}H_{24}$	1480	1486.2	2.8
14	Bicyclogermacrene	SH	$C_{15}H_{24}$	1494	1501.3	5.1
15	NI	-	-	-	1510	0.6
16	δ-Cadinene	SH	$C_{15}H_{24}$	1524	1526.5	1.2
17	(E)-Nerolidol	OS	$C_{15}H_{26}O$	1564	1564.8	0.9
18	Spathulenol	OS	$C_{15}H_{24}O$	1576	1584.5	3.8
19	Viridiflorol	OS	$C_{15}H_{26}O$	1590	1591	3.1
20	NI	-	-	-	1628.8	0.4
21	α-Muurolol	OS	$C_{15}H_{26}O$	1644	1646.1	1.1
22	α-Cadinol	OS	$C_{15}H_{26}O$	1653	1659.7	1.2
23	6,10,14-Trimethyl-2-pentadecanone	NH	$C_{18}H_{36}O$	1846	1845.4	2.5
24	Hinokione	NH	$C_{20}H_{28}O_2$	2549	2549	40.7
Oxygen-containing monoterpens (OM)						5.4
Sesquiterpene hydrocarbons (SH)						36.6
Oxygen-containing sesquiterpenes (OS)						10.1
Non-terpene compounds (NH)						46.1
None identified compounds (NI)						1.7
Total percentage						99.9
Yield (%, w/w-dry basis)						0.24

[a] Compounds are sorted in order of their elution from an HP-5MS capillary column.
[b] Kovats retention index given in the literature
[c] Kovats retention index calculated with respect to *n*-alkenes on an HP-5MS capillary column.

3.2. Chemical composition of other species of *Malva* genus

Characterization of the essential oil composition relating to another species of *Malva genus*, namely *M. sylvestris* L., has been the subject of another report. In the study of Usami and co-workers (2013), the aroma-active compounds in dry flower of *M. sylvestris* L. were characterized using gas chromatography-mass spectrometry-olfactory (GC-MS-O) analysis and odor active values (OAV) calculations. Accordingly, in the light yellow isolated oil having a sweet odor and a percentage yield of 0.039% (w/w), totally 143 volatile compounds (89.9%) were identified, in which the most abundant compounds were found to be hexadecanoic acid (10.1%), pentacosane (4.8%) and 6,10,14-trimethyl-2-pentadecanone (4.1%). In this regard, the essential oil mainly consisted of hydrocarbons (25.4%) followed by alcohols (18.8%), acids (16.7%), ethers (5.0%), ketones (7.3%), esters (12.4%), aldehydes (2.3%) and other compounds (2.0%).

4. Concluding remarks

In this report, the essential oils from the aerial parts of *M. neglecta* Wallr. from Khorramabad region, Lorestan Province, Iran were separated using the solvent free microwave extraction (SFME) method. This green method is capable of isolating essential oils, faster and more effective than the classical methods like hydrodistillation. Characterization of the corresponding chemical profiles of the isolated essences has been performed for the first time in the literature. This study led to the identification of a chemical profile consisting of hinokione (40.7%), γ-elemene (9.4%), δ-elemene (6.8%), β-ylangene (5.8%) and bicyclogermacrene (5.1%) as the major constituent components. In addition, among the 21 identified compounds in the oil from the aerial parts of *M. neglecta* Wallr., three oxygenated monoterpenes, ten sesquiterpene hydrocarbons, five oxygenated sesquiterpenes and three non-terpene hydrocarbons, respectively contributing to 5.4%, 36.6%, 10.1% and 46.1% of the total oil composition. These compounds constitute 99.9% of the oil structure, whereas only three compounds remained unidentified altogether forming about 1.7% of the oil profile.

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