



## Screening of the profiles of the essential oils from the aerial parts of *Nepeta racemosa* using classical and microwave-based methods: Comparison with the volatiles using headspace solid-phase micro-extraction

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### ABSTRACT

**Background & Aim:** *Nepeta racemosa* is an herbal and medicinal plant and this report aims to identify chemical compositions of the essential oils and volatiles of its aerial parts through classical and advanced methods.

**Experimental:** Chemical profiles of the essential oils and volatile compounds from the aerial parts of *Nepeta racemosa* obtained through hydrodistillation (HD), solvent-free microwave extraction (SFME), microwave assisted hydrodistillation (MAHD) and headspace solid-phase microextraction (HS-SPME) methods were subsequently investigated by means of GC and GC-MS instruments.

**Results:** Totally, 25, 26, 24, and 24 components were identified in the chemical profiles, representing 98.1%, 96.6%, 97.7% and 96.4% of the total compositions when using the HD, HS-SPME, SFME and MAHD methods, respectively. In all samples, oxygenated monoterpenes were the major fractions of the chemical profiles with the exception of chemical composition of the essential oil of *N. racemosa* obtained by using the MAHD approach in which monoterpene hydrocarbons were dominant constituting compounds. In final, 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone was the most abundant compound in the chemical profiles of HD, HS-SPME and SFME approaches while 4 $\alpha$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone oxygenated monoterpene was the most frequent compound in the MAHD profile.

**Recommended applications/industries:** Using the advanced separation methods for isolation of the essential oils are economic, time saving and eminently suited compared with the classical and traditional separation methods. Moreover, the significance of this study relates to the fact that the essential oils, which are rich in monoterpene hydrocarbons and oxygenated monoterpenes could be regarded as powerful food preservatives and novel antioxidants.

### 1. Introduction

The Asteraceae or Compositae as a great family of plants involves a large number of flowering plants,

which have been classified in about 1600 genera altogether covering 23,000 species (Bessada *et al.*, 2015). It is commonly referred to as the aster, daisy, composite or sunflower family, as well. In terms of numbers of species, the plants belonging to

the Asteraceae family are only comparable with those of the Orchidaceae family. A broad spectrum of medicinal and herbal plants of the Asteraceae family has been utilized as vegetables and in folk medicine, edible oils and pesticides. As shown in the literature, diverse plants in the Asteraceae family have considerable amounts of sesquiterpenoids particularly eudesmane-type having ones (Sharma & Goyal, 2011; Wu *et al.*, 2006). The genus *Nepeta* with the local Persian name of 'Punesa' belonging to the Lamiaceae family, subfamily Nepetoideae and tribe Mentheae consists of about 300 herbaceous perennial plants mostly having beautiful flowers and a pleasant odor (Mozaffarian, 1996; Zargari, 1989). Different species of this genus are mainly found in two areas of the world, namely Southwestern Asia (Turkey and Iran) along with Western Himalayas (Hindu Kush).

According to Jamzad *et al.* (2003), about sixty-seven species of this genus could be found in different parts of the country. In the Iranian traditional and folk medicine, different species of the *Nepeta* genus are frequently used and prescribed as potent diaphoretic, antitussive, diuretic, antispasmodic, anti-asthmatic agents and proper alternatives for chemical drugs with harmful side effects. The other promising traditional uses of this genus relate to their high astringent, antiseptic, febrifuge as well as sedative characteristics. More specifically, in the cutaneous eruptions of the children and snake and scorpion bites, they have been recognized as novel tropical remedies (Formisano *et al.*, 2011). As found in the literature, nepetalactones, iridoids and respective glucosides, diterpenes, triterpenes and flavonoids have been reported as major constituents of diverse *Nepeta* species (Asgarpanah *et al.*, 2014). Approximately, most of the *Nepeta* species are good sources to produce essential oils and organic extracts having a broad spectrum of biological activities involving insecticidal (Calmasur *et al.*, 2006), acaricidal (Calmasur *et al.*, 2006), anticancer (Aloqail *et al.*, 2014), antibacterial (Sonboli *et al.*, 2009), antioxidant (Adiguzel *et al.*, 2009), antimicrobial (Sonboli *et al.*, 2004), antiviral (Abad *et al.*, 2000), anticandidal (Iscan *et al.*, 2011), antinociceptive (Ali *et al.*, 2012a, b), analgesic (Hussain *et al.*, 2015), anti-seizure (Bhat *et al.*, 2012), anti-inflammatory (Hussain *et al.*, 2015; Ali *et al.*, 2012a, b), antiprotozoal (Dua *et al.*, 2011), neuropharmacological (Galati *et al.*, 2004), antifungal (Sonboli *et al.*, 2004), antiglycation,

antiplatelets aggregation, cytotoxic and phytotoxic (Hussain *et al.*, 2010) activities. In view of many promising medical, phytochemical and pharmacological properties of the plants in the *Nepeta* genus, characterization and identification of the corresponding profiles seem highly justifiable.

The presents report aims at profiling chemical composition of the essential oils and volatiles obtained from the aerial parts of *N. racemosa* (Figure 1). Furthermore, to the best of my knowledge, there has been no report concerning the essential oil composition and volatile constituents of *N. racemosa* determined by the SFME and HS-SPME techniques up to present.



**Fig 1.** Representation of the aerial parts of *N. racemosa*.

## 2. Materials and Methods

### 2.1. Chemicals and supplies

The normal alkane mixtures were purchased from Fluka (Buchs, Switzerland). High purity helium and nitrogen carrier gases were used in the GC and GC-MS instrumentations.

### 2.2. Plant material and botanical identification

The plant material was collected while wearing polystyrene gloves during the flowering stage in April 2015, in the Afjeh, Lavasan, Tehran Province, at 51° 41' 28.00" E and 35° 51' 34.99" N. A voucher specimen was deposited at the Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran, for further authentication.

### 2.3. GC and GC-MS analyses

Gas chromatographic analyses were performed on a Shimadzu 15A gas chromatograph equipped with a split/splitless (ratio 1:30) injector and a flame ionization detector, both operating at 250 °C. In this separation process, high purity nitrogen with a flow-rate of 1 mL/min was utilized as the carrier gas across the capillary column which was of DB-5 type having dimensions of 50 m×0.2 mm and a film thickness of 0.32 µm. According to our temperature programming, the column temperature was first kept at 60°C for 3 min, then heated to 220 °C with a 5 °C/min rate and finally kept constant at 220 °C for 5 min. Relative percentage amounts were calculated from peak area using a CR5 Shimadzu CR pack without the use of correction factors.

GC/MS analysis was performed using a Hewlett-Packard 5973 instrument equipped with an HP-5MS column (30 m×0.25 mm, film thickness 0.25 µm). The effluent of the GC column was introduced directly into the source of MS. The column temperature programming was the same with the GC analysis. The flow-rate of helium carrier gas was set at 1 mL/min. Additionally, final temperature of the column was exactly 230 °C while detector temperature was set at 250 °C. All of the MS spectra were taken at 70 eV (E1) over the range 30-350 amu with an electron multiplier voltage of 1800 eV and scan times of 2 scans/ sec.

#### 2.4. Hydrodistillation method

The collected plant material was dried in the dark room at ambient temperature in the absence of light. Then, 150-g portions of the air-dried aerial parts of *N. racemosa* were subjected to hydrodistillation in a Clevenger-type apparatus for 4 h. The obtained essential oils had pale yellowish colors and were subsequently dried using minimal amounts of anhydrous sodium sulphate and stored at 4 °C in the absence of daylight in brown vials until being analyzed (Mohammadhosseini & Beiranvand, 2013).

#### 2.5. Solvent-free microwave extraction (SFME) method

The basic principles of the SFME approach have been extensively discussed in our previous reports (Mohammadhosseini, 2016; Mohammadhosseini, 2015a; Mohammadhosseini *et al.*, 2015; Mohammadhosseini & Nekoei, 2014; Nekoei & Mohammadhosseini, 2016a; Nekoei & Mohammadhosseini, 2016b). Accordingly, a Milestone

SRL microwave oven was used for isolation of the oils operating at 2450 MHz. For performance of the SFME-extraction in the absence of any solvent, the aerial parts of *N. racemosa* were placed in the microwave oven interior cavity having dimensions of 29 cm × 37 cm × 40 cm. However, prior to the extraction, 80-g portions of the dried and ground aerial parts of *N. racemosa* were soaked in appropriate amounts of distilled water in our laboratory at 25 °C for 1 h., and immediately after the excess water was drained off. The main reason of this step was to hydrate the external layers of the plant material and to condition them for better extraction of the essential oils from the respective secretory glands.

In our used set up, a full glass made Clevenger system in the outside of the extraction vessel served as the distillate condenser during the extraction process. Moreover, to maintain the same conditions of temperature and humidity within the process and to restore the *in situ* water to the plant material, the condensed water was repeatedly returned to the isolation medium. To conduct the SFME technique, the aerial parts of *N. racemosa* were heated in the microwave oven using an optimized fixed power of 800 W for an optimized time of 30 min. The chemical compositions of the isolated essential oils were then determined by the GC-MS apparatus.

#### 2.6. Microwave assisted hydrodistillation (MAHD)

The operational characteristics of the MAHD technique were described in some of our recent papers (Hashemi-Moghaddam *et al.*, 2015; Hashemi-Moghaddam *et al.*, 2014; Mohammadhosseini *et al.*, 2016a; Mohammadhosseini *et al.*, 2013; Mohammadhosseini *et al.*, 2016c). In our suggested methodology, we used a microwave oven (Samsung, South Korea) regulated at a frequency of 2450 MHz capable of maintaining a maximum power of 1000 W. To initiate the separation step, a proper power (800 W) was directly applied to the hydrodistillation apparatus. The MAHD assembly consisted of a modified microwave oven housing a 1000-mL flat bottom which was directly connected to the Clevenger apparatus via a hole located above the oven. In our proposed MAHD procedure, 75-g portions of the dried and ground aerial parts of the plant sample (*N. racemosa*) were placed in a distinct volume of distilled water (500 mL) and the extraction carried out at a power of 800 W for 30 min using the introduced system. A glass condenser in the

outside of the microwave cavity continuously condensed the vapor flow within the apparatus. The MAHD process was performed several times and was repeated until the volume of the extracted essential oil became constant. In the next step, the obtained essential oil was collected in amber brown vials. It was then dehydrated with anhydrous sodium sulfate, capped under nitrogen and kept at 4 °C until the analysis time.

### 2.7. Headspace solid-phase microextraction method

The foundations of the SPME-based procedures have been given in the literature in detail (Arthur & Pawliszyn, 1990). This method which was first introduced by Pawliszyn has considerably developed in the recent decades (Pawliszyn, 1999; Pawliszyn, 2003; Setkova *et al.*, 2007; Vuckovic *et al.*, 2010). The sequential steps by which the volatile fraction is separated from the plant materials are similar in a variety of scientific reports (Mohammadhosseini, 2015b; Mohammadhosseini *et al.*, 2016b; Nekoei & Mohammadhosseini, 2014; Nekoei & Mohammadhosseini, 2016b). In our investigation, we employed a commercially available manual SPME (Supelco, Bellefonte, USA) apparatus having 75- $\mu$ m diameter fibers and a modified syringe containing stainless steel microtubing within its syringe needle. The microtubing has an about 1-cm fused-silica fiber tip on which an organic polymer has been deposited involving polydimethylsiloxane combined with craboxene (binder) namely, PDMS-CAR. The positions of the coated silica fiber are variable in different situations inside and outside the needle through a plunger similar to a routine syringe. However, the mean diameter of the syringe needle housing the microtubing and coated silica fiber is highly restricted and miniaturized. The first experimental step corresponds to the conditioning step in which the fiber is placed in the GC injector at 250 °C for 30 min. The next step is heating step in which one gram portions of powdered plant sample are placed in a 20-mL sample vial sealed with septum-type caps (Supelco), and then heated at 70 °C for 15 min. In the third step (adsorption), the SPME needle pierces the septum immediately after the PDMS fiber is extended through the needle and exposed to the headspace above the sample for 15 min to trap the volatile compounds in the upper space of the vials. In the final step (desorption), the fiber is drawn into the needle, and then the needle is

removed from the septum and directly inserted onto the injection port of the GC in the splitless mode (250 °C) for about five minutes. Penetration from the septum of the GC injection port is possible when the fiber is withdrawn into the syringe needle. Finally, the volatile parts are desorbed and transferred to the capillary columns after again moving the fiber to the position outside the syringe.

### 2.8. Characterization of the chemical profiles

In this investigation, identification and determination of the constituents of each profile were tentatively made through comparison of their mass spectral fragmentation patterns and retention indices (RI) relative to C<sub>9</sub>-C<sub>25</sub> *n*-alkanes both with those given in the literature (Adams, 2007) as well as those stored in the MS spectral literature data (Wiley 275). In addition, complementary identification was confirmed through the comparison of the mass spectral fragmentation patterns of the constituents in the profiles with those stored in the MS database (National Institute of Standards and Technology and Wiley libraries). In some cases, when possible, we used the conformity pattern of some spectral patterns as well as consistency of Kovatz indices regarding previous findings of our research group (Akhlaghi *et al.*, 2009; Mohammadhosseini *et al.*, 2008; Mohammadhosseini *et al.*, 2011; Akhlaghi *et al.*, 2012; Mohammadhosseini *et al.*, 2010). 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 HD, SFME, MAHD and HS-SPME approaches

Although the chemical profiles of the hydrodistilled oils of *N. racemosa* have been reported previously, this study produced rather new profiles. Moreover, quantitative and qualitative analyses of the essential oils and volatile fractions from the aerial parts of *N. racemosa*, using the described methods, have resulted in recognition of several monoterpene hydrocarbons (MH), oxygenated monoterpenes (OM), sesquiterpene hydrocarbons (SH), oxygenated sesquiterpenes (OM) and non-terpene hydrocarbons (NH).

**Table 1.** Chemical composition of the essential oils from the aerial parts of *N. racemosa* obtained by using HD, SFME and MAHD approaches as well as the volatile constituents by HS-SPME method <sup>a</sup>

NO.	Compound	Class	RI <sup>b</sup>	HD (%)	HS-SPME (%)	SFME (%)	MAHD (%)
1	2-Hexenal	NH <sup>c</sup>	855	0.2	0.5	1.2	0.8
2	$\alpha$ -Thujene	MH <sup>d</sup>	930	0.1	-	1.7	1.5
3	$\alpha$ -Pinene	MH	939	<b>10.2</b>	<b>7.4</b>	<b>6.0</b>	<b>18.9</b>
4	Camphene	MH	954	2.0	0.7	1.1	<b>12.3</b>
5	$\beta$ -Pinene	MH	979	3.1	3.3	2.8	<b>8.5</b>
6	$\alpha$ -Phellandrene	MH	1003	1.1	0.2	0.8	0.7
7	$\alpha$ -Terpinene	MH	1017	0.4	5.5	1.2	0.4
8	<i>p</i> -Cymene	MH	1025	0.5	0.2	-	-
9	Limonene	MH	1029	0.2	0.1	-	-
10	( <i>Z</i> )- $\beta$ -Ocimene	MH	1037	<b>10.4</b>	<b>12.8</b>	<b>6.5</b>	<b>9.2</b>
11	Camphor	OM <sup>e</sup>	1146	-	-	-	0.5
12	$\alpha$ -Terpineol	OM	1189	2.0	0.9	2.1	1.7
13	Piperitone	NH	1221	1.8	1.2	0.4	0.9
14	Bornyl acetate	OM	1289	0.3	0.1	-	0.1
15	4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -Nepetalactone	OM	1317	<b>28.1</b>	<b>18.0</b>	<b>25.7</b>	<b>7.6</b>
16	4 $\alpha$ ,7 $\alpha$ ,7 $\beta$ -Nepetalactone	OM	1325	<b>7.4</b>	<b>4.6</b>	<b>16.1</b>	<b>20.3</b>
17	4 $\beta$ ,7 $\alpha$ ,7 $\beta$ -Nepetalactone	OM	1357	<b>20.5</b>	<b>32.4</b>	<b>25.4</b>	<b>4.3</b>
18	$\alpha$ -Copaene	SH <sup>f</sup>	1377	0.2	0.5	0.1	0.3
19	$\beta$ -Bourbonene	SH	1388	0.1	0.2	0.2	0.3
20	$\beta$ -Caryophyllene	SH	1419	3.5	1.8	1.5	3.3
21	<i>trans</i> - $\beta$ -Farnesene	SH	1457	0.5	0.5	0.4	0.6
22	<i>allo</i> -Aromadendrene	SH	1460	-	0.2	0.1	-
23	$\beta$ -Selinene	SH	1490	-	0.2	-	-
24	$\alpha$ -Muurolene	SH	1500	1.4	0.3	0.5	-
25	$\beta$ -Bisabolene	SH	1506	-	-	0.7	0.8
26	$\gamma$ -Cadinene	SH	1514	0.5	0.4	0.4	0.4
27	$\delta$ -Cadinene	SH	1523	0.4	0.4	0.7	0.4
28	Spathulenol	OS <sup>g</sup>	1578	2.6	4.0	1.8	1.8
29	$\alpha$ -Cadinol	OS	1640	-	-	-	-
30	$\alpha$ -Bisabolol	OS	1685	0.2	0.2	0.3	0.8
Total percentage				<b>98.1</b>	<b>96.6</b>	<b>97.7</b>	<b>96.4</b>
MH (%)				28.0	30.2	20.1	<b>51.5</b>
OM (%)				58.3	56.0	<b>69.3</b>	34.5
SH (%)				<b>9.2</b>	4.5	4.6	6.1
OS (%)				0.2	<b>4.2</b>	2.1	2.6
NH (%)				<b>2.4</b>	1.7	1.6	1.7
NIC <sup>h</sup>				<b>25</b>	<b>26</b>	<b>24</b>	<b>24</b>

<sup>a</sup> The compounds have been sorted according to their retention indices on an HP-5 MS capillary column.

<sup>b</sup> Kovatz retention indices given in the literature

<sup>c</sup> Nonterpene hydrocarbons

<sup>d</sup> Monoterpene hydrocarbons

<sup>e</sup> Oxygenated monoterpene

<sup>f</sup> Sesquiterpene hydrocarbons

<sup>g</sup> Oxygenated sesquiterpene;

<sup>h</sup> Number of the identified compounds

Specifically, the major components in the hydrodistilled oil of the *N. racemosa* aerial parts were found to be 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (28.1%), 4 $\alpha$  $\beta$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (20.5%), (Z)- $\beta$ -ocimene (10.4%),  $\alpha$ -pinene (10.2%) and 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (7.4%). The percentage yields of essential oils, in terms of the weight of the collected oil per gram of dried plant, were 0.17%, 0.16%, 0.13% and 0.14% (w/w) for four replicate distillations.

Using HS-SPME (Table 1), we were able to identify 26 volatile compounds, 4 $\alpha$  $\beta$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (32.4%), 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (18.0%), (Z)- $\beta$ -ocimene (12.8%),  $\alpha$ -pinene (7.4%) and 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (4.6%). In terms of general categories, eight were monoterpene hydrocarbons (30.2%), five, oxygenated monoterpenes (56.0%), nine, sesquiterpene hydrocarbons (4.5%), two, oxygenated sesquiterpenes (4.2%) and two, non-terpene hydrocarbons (1.7%).

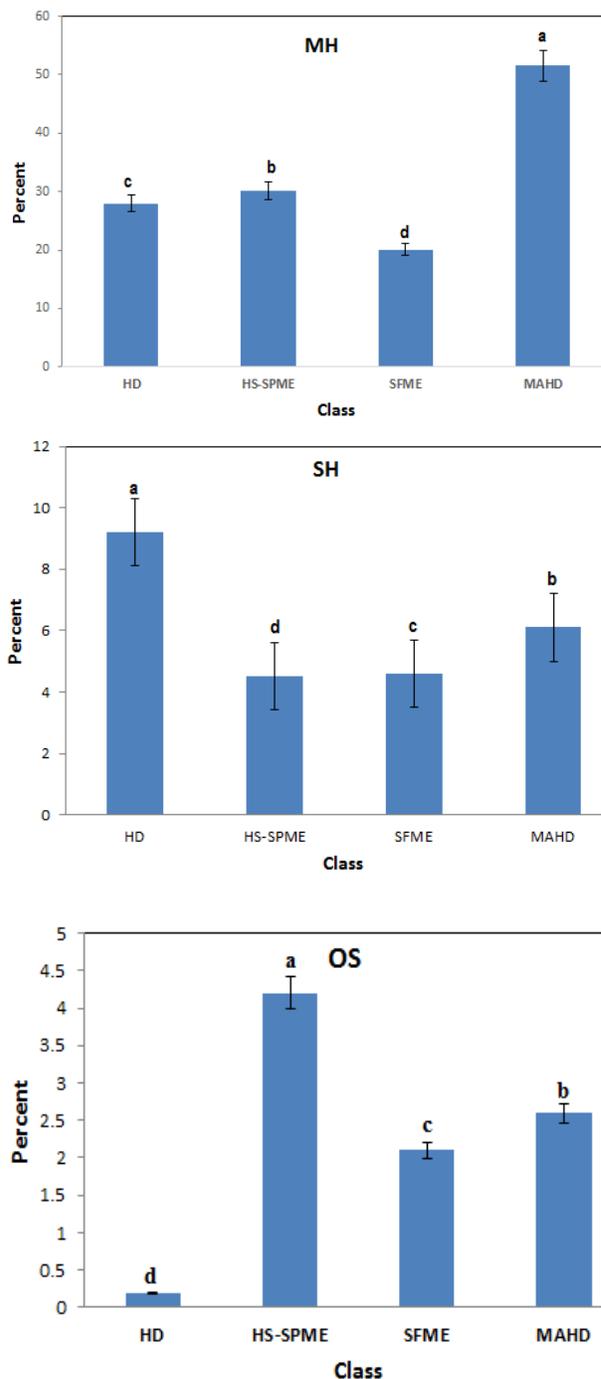
The SFME method allowed for the identification of 24 components in the isolated oil (see Table 1), of which seven were monoterpenes (20.1%), four, oxygenated monoterpenes (69.3%), nine, sesquiterpene hydrocarbons (4.6%), two, oxygenated sesquiterpenes (2.1%) and two, non-terpene hydrocarbon (1.6%). The main components included 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (25.7%), 4 $\alpha$  $\beta$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (25.4%), 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (16.1%) (Z)- $\beta$ -ocimene (6.5%) and  $\alpha$ -pinene (6.0%). The yields of four experiments done using this method were 0.21%, 0.20%, 0.18% and 0.30% (w/w).

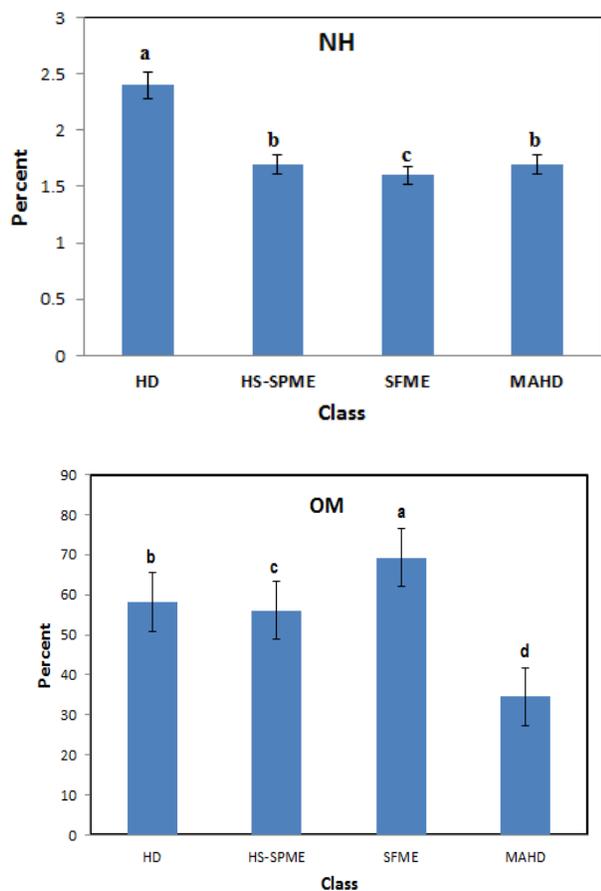
Finally, 24 components were identified in the aerial parts oils using the MAHD method (Table 1).

The major components 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (20.3%),  $\alpha$ -pinene (18.9%), camphene (12.3%), (Z)- $\beta$ -ocimene (9.2%)  $\beta$ -pinene (8.5%) and 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (7.6%), 4 $\alpha$  $\beta$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (4.3%), (See Table 1).

The names, retention indices and percentages of the constituents obtained from the aerial parts of *N. racemosa* by the aforementioned methods have been listed in Table 1. As can be seen, there are negligible differences in numerical values of Kovats retention indices between the calculated ones and those cited in the literature. Furthermore, a total of 30 components were identified, accounting for 96.4-98.1% of the components, depending on the profile.

Using the HD method (Table 1), 25 components could be identified in the oil from the aerial parts: Nine monoterpene hydrocarbons (28.0%), five oxygenated monoterpenes (58.3%), eight sesquiterpene hydrocarbons (9.2%), one oxygenated sesquiterpene (0.2%) and two non-terpene hydrocarbons (2.4%).





**Fig 2.** Comparative plots of different types of constituting class of the essential oils and volatiles from the aerial parts of *Nepeta racemosa* (As shown there are significant differences between the groups with the exception of relative percentage of non-terpene hydrocarbons in the HS-SPME and MAHD profiles regarding one-way ANOVA analysis; MH= Monoterpene hydrocarbons; OM= Oxygenated monoterpenes; SH= Sesquiterpene hydrocarbons; OS= Oxygenated sesquiterpenes; NH= Non-terpene hydrocarbons).

In terms of general categories, seven components were monoterpenes (51.5%), six, oxygenated monoterpenes (34.5%), seven, sesquiterpene hydrocarbons (6.1%), two, oxygenated sesquiterpenes (2.6%) and two, non-terpene hydrocarbons (1.7%). In addition, for three replicate extractions the yields were found to be 0.22%, 0.24% and 0.19% (w/w), respectively.

In Figure 2, the relative percentages of the various classes of the oils and volatile fractions constituting compounds are compared. From this figure, it is immediately evident that in the three the profiles (HD,

HS-SPME and SFME), oxygenated monoterpenes constituted the main components, while the largest class being monoterpene hydrocarbons in that obtained using the MAHD approach.

### 3.2. Chemical composition of the *Nepeta* oils in similar reports

In the literature there are some reports concerning the constituents of the essential oils from a variety of the *Nepeta* genus plants in different parts of the world. In a related study, Ali *et al.* (2016) reported the water-distilled essential oils from the aerial parts of four ornamental species and hybrids of *Nepeta*: *N. racemosa* Lam. hybrid, Select., *N. sibirica* L., *N. subsessilis* Maxim, and *N. xfaassenii* Bergmans ex Stearn, 'Dropmore'. In this report, *N. racemosa* hybrid, Select. and *N. xfaassenii*, Dropmore essential oils were rich in 1,8-cineole whereas *N. sibirica* and *N. subsessilis* essential oils mainly consisted of sesquiterpenes including (*Z*)- $\beta$ -farnesene,  $\beta$ -bisabolene,  $\delta$ -cadinene or  $\beta$ -caryophyllene, and caryophyllene oxide.

On the other hand, Baser *et al.* (2000) have reported a comprehensive report concerning the essential oils from 22 *Nepeta* species growing in Turkey. Accordingly, gas chromatography-mass spectrometric analyses of the essential oils revealed that four *Nepeta* species contain 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone as the major constituent component. 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -Nepetalactone was the main constituent in *N. racemosa*. In addition, seven *Nepeta* species contained caryophyllene oxide as the main constituent in their essential oils. 1,8-Cineole/linalool were the major components in the essential oils of six species, as well. On the other hand,  $\beta$ -pinene,  $\alpha$ -terpineol, germacrene-D and spathulenol were the main constituents in the essential oils from *N. phyllocllamys*, *N. viscida*, *N. sorgerae* and *N. trachonitica*, respectively.

In the work of Dabiri and Sefidkon (2003) on chemical composition of the essential oil of *N. racemosa* Lam. prepared by hydrodistillation, twenty-four components were identified constituting approximately 99.3% of the oil with major constituents being 4 $\alpha$  $\beta$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (33.6%), 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$  $\beta$ -nepetalactone (25.6%), 4 $\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (24.4%) and 1,8-cineole (9.0%) in the volatile essential oil. Kobaisy and coworkers (2005) have evaluated composition and phytotoxic activity of *N. pannonica* L. essential oil from Kazakhstan region. This study led to the characterization of sixty components from *N.*

*pannonica* with 1,8-cineole (28.9%), and 4 $\alpha$ ,7 $\beta$ ,7 $\alpha$ -nepetalactone (14.3%) as the major constituents. Rustaiyan *et al.* (2000) have characterized chemical composition of the essential oil of *N. racemosa* Lam. from Iran in 2000. In this attempt, the main constituents of the oil were found to be 4 $\alpha\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (64.9%), (*Z*)- $\beta$ -ocimene (9.5%), (*E*)-nerolidol (8.8%) and 4 $\alpha\alpha$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone (7.4%).

In the work of Safaei-Ghomi *et al.* (2006) on water distilled essential oil composition from the aerial parts of *N. gloecephala* Rech. f. from Iran, 1,8-cineole (35.2%),  $\beta$ -pinene (21.8%), sabinene (7.8%), (*E*)- $\beta$ -ocimene (7.1%),  $\alpha$ -pinene (7.1%) and (*Z*)- $\beta$ -ocimene (6.9%) were the major components of the oil. In another report of Safaei-Ghomi *et al.* (2009) dealing with the volatile constituents analysis of *N. cataria* from Central Iran, among the constituents of the volatile oil, 4 $\alpha\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone (87.1%), 4 $\alpha\alpha$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone (3.1%),  $\beta$ -caryophyllene (2.5%), and  $\beta$ -pinene (1.7%) were the major ones while the other constituents were present in relatively small amounts representing only (3.6%) of the total oil. In the study of Shafaghat *et al.* (2008) on chemical composition of the essential oil isolated from flower, leaf, stem and root of *N. sintenisii* Bornm. growing in Khalkhal, North-West Iran: Fourteen compounds representing 98.7% of the flower oil were identified; among them 4 $\alpha\beta$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone (60.3%), germacrene D (12.7%) and 1,8-cineole (8.2%) were the major ones; twenty constituents accounting for 95.7% of the leaf oil were identified of which 4 $\alpha\beta$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone (34.6%), germacrene D (14.1%), 1,8-cineole (7.9%),  $\alpha$ -cadinol (6.8%) and  $\delta$ -cadinene (5.8%) were the main components. In this work, the stem oil of *N. sintenisii* was characterized by higher amount of 4 $\alpha\beta$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone (64.2%),  $\alpha$ -cadinol (8.9%),  $\alpha$ -pinene (6.7%), 4 $\alpha\alpha$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone (5.2%) and 1,8-cineole (3.6%), among the eight components comprising 96.3% of the total oil detected. In addition, twelve compounds were characterized in the root oil representing 98.6% of the total profile with 4 $\alpha\beta$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone (61.2%), germacrene D (12.0%), 4 $\alpha\alpha$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone (8.5%), 1,8-cineole (5.7%) and  $\beta$ -caryophyllene (4.5%) as the main constituents. In terms of general categories, the oil of flower, stem and root consisted mainly of monoterpenes, but monoterpenes and sesquiterpenes of

leaf oil were approximately in equal amounts (48.2% and 47.5% respectively).

Sonboli *et al.* (2009) have also studied the antibacterial activity and composition of the essential oil of *N. menthoides* (Iran) leading to identification of twenty-nine compounds representing 97.6% of the total oil implying high frequency of oxygenated monoterpenes with 1,8-cineole (33.8%) and 4 $\alpha\alpha$ -7 $\alpha$ -nepetalactone (23.2%) as the most abundant constituents. In a complimentary report of this research group, focusing on the essential oil composition of *N. involucrate*, forty-eight components were identified in the oil, accounting for 97.2% of the total oil including 1,8-cineole (23.1%),  $\beta$ -pinene (12.2%), sabinene (6.7%) and  $\alpha$ -pinene (4.9%), germacrene-D (15.1%) and spathulenol (2.3%) (Sonboli *et al.*, 2005). Moreover, they have assessed antimicrobial activity and chemical composition of the essential oil of *N. crispa* Willd. in Iran consisting of twenty-three compounds and accounting for 99.8% of the total profile with the main constituents being 1,8-cineole (47.9%) and 4 $\alpha\alpha$ ,7 $\alpha$ ,7 $\beta$ -nepetalactone (20.3%) (Sonboli *et al.*, 2004).

#### 4. Conclusion

The main objective of this investigation was to evaluate the inherent capabilities of four different extraction techniques, namely, hydrodistillation (HD), solvent-free microwave extraction (SFME), microwave assisted hydrodistillation (MAHD) and headspace solid-phase micro-extraction (HS-SPME) in effective separation and trapping of the essential oils and volatile fractions from the aerial parts of *N. racemosa* in combination with the GC and GC-MS analyses. 25, 26, 24 and 24 components representing 98.1%, 96.6%, 97.7% and 96.4% of the total oil compositions and volatiles were recognized by using the HD, HS-SPME, SFME and MAHD methods, respectively. In most of the samples obtained by the aforementioned methods, oxygenated monoterpenes were found to be the dominant groups of constituents. On the other hand, the largest amounts of monoterpene hydrocarbons (51.5%), oxygenated monoterpenes (69.3%), sesquiterpene hydrocarbons (9.2%), oxygenated sesquiterpenes (4.2%) and non-terpene hydrocarbons (2.4%) were respectively observed in the MAHD, SFME, HD, HS-SPME and HD profiles. Among the whole identified compounds, 4 $\alpha\alpha$ ,7 $\alpha$ ,7 $\alpha$ -nepetalactone was the major

component of the chemical profiles of the HD, HS-SPME and SFME methods, while  $4\alpha,7\alpha,7\beta$ -nepetalactone was the dominant natural compound in the profile of the MAHD method

As a concluding remark, compared with the HD approach, green and environmentally friendly techniques based upon microwave beams (MAHD and SFME) permit prominent superiorities containing lower wastewater, energy and cost, and greater potential capability to separate volatile essential oils. However, the HS-SPME approach is the simplest method to use by far to trap the volatile fractions of the plant materials. The time required to obtain volatiles from a single specimen is shorter than the other methods. Moreover, it can be used to trap volatile fractions from several plant specimens simultaneously, which requires fewer samples.

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## 6. References

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