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Short Communication

Essential Oil Content and Composition of *Nepeta kotschyi* Boiss. (Lamiaceae) from Iran during Different Phenological Stages

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Abstract

Essential oils (EOs) because of being natural compounds and having antibacterial properties are important for health. The chemical composition of EOs might be affected by environmental conditions and plant growth and development stages. In this study, the essential oils and chemical compositions of aerial parts of *Nepeta kotschyi* Boiss. (Lamiaceae) were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) at different plant phenological (in mid vegetative, 50% of flowering and fruiting) stages. The results showed that the EOs of *Nepeta* was affected by plant growth and development stages. So, both of content and their constituents were different in the plant phenological stages. The average percentage of EOs was ranged from 2.48 (in vegetative), 0.8 (in 50% of flowering), and 0.82 (in fruiting) stages. According to the results, 21 compounds with the range of 0.84-13.04 % in vegetative, 3 compounds with the range of 10.93-53.25% in 50% of flowering and 6 compounds with the range of 8.51-45.22% in fruiting stages were identified. The compounds 1,6,10-Nerolidol in vegetative (13.04%), Spathulenol in 50% of flowering (53.25%) and Caryophyllene oxide in fruiting stages (45.22%) had the highest value. Based on the results, the highest percentage of essential oils and compounds were related to the vegetative stage. The present study is the first report of the essential oil content of *Nepeta* during different plant phenological stages. The results of this study can be useful to understand the proper harvest time in *Nepeta*.

Keywords: Essential oils, GC/MS, Medicinal plants, *Nepeta,* Growth, Developmental stages.

Abbreviations: AMU: Atomic mass unit; EOs: Essential oils; FID: Flame ionization detector; GC: Gas chromatography; GC/MS: Gas chromatography-mass spectrometry; RT= Retention Time; RI: Retention Index.

Introduction

Medicinal and aromatic plants are important and valuable natural compounds [42] that their quality is high compared to other crops [4]. Medicinal plants have secondary metabolites [83] and some of them have essential oils (EOs) that are volatile compounds in different organs of plants. Cellular damages made by free radicals may cause some diseases such as cancer, heart disease, and immune system decline can be prevented by EOs [7, 36]. EOs are also used for flavoring food and drinks. Also, these plants are essential components in health and cosmetic industries such as shampoos, soaps, skin creams, etc. [15]. Moreover, medicinal plants have been used as a source of biologically active drugs for treating different diseases for many years. Nowadays, scientists believe these plants are a good choice [83] against micro-organisms [51]. So, to increase the product shelf life there is a great tendency towards using natural products in food industries [62].

Lamiaceae is a very important family among the various medicinal plants. The *Nepeta* ("Pune-say"

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in Persian) is a major member of this family that has been regularly used for medicinal purposes [61]. *Nepeta* consists of about 280 species including a large number of volatile oil plants that are widely distributed in Asia, Europe, North America, and the mountains of tropical Africa [17]. *Nepeta* has beautiful flowers and pleasant odor [12]. *Nepeta kotschyi* Boiss. growing wild in Iran. This species is hairy perennial herbs having dense leaves that are covered with soft hairs. Floral leaves are bract-like; Calyx is tubular; teeth 5, Corolla has 2 lips; The stamens are 4, nearly parallel, glabrous, ascending under the upper lip of the corolla, posterior 2 longer than anterior, fertile; Leaves have an oval shape and heart-shaped at the base. The petiole is shorter than the lamina. The flowers are purple, pink or blue. The fruit is tetrachene [19]. Most *Nepeta* species are rich in essential oils and various active compounds such as lactones, iridoids, glucosides, diterpenes, triterpenes and flavonoids [80, 82]. Different parts of the plant are widely used in traditional medicine [5, 63, 2] and anti-bacterial, fungicidal and anti-viral activities of some species have been reported [8, 31].

The chemical compounds of EOs affect their antibacterial activity [38]. It has been reported that their antimicrobial activity is related to the presence of compounds such as alcohols, aldehydes, alkenes, esters and ethers [13]. It is well established that phenolic and secondary metabolites with conjugated double bonds usually show substantial anti-oxidative properties [40]. Antioxidant activity of essential constituents such as thymol is related to their phenolic structure. These phenolic compounds have redox properties, so they play an important role in neutralizing free radicals and also in peroxide decomposition [10]. Generally, EOs cytotoxicity mainly correlates to the presence of phenols, alcohols, and monoterpene aldehydes [71]. Zengin and Baysal (2014) reported that eucalyptol, a terpene compound, causes permeability alteration of the outer membrane of bacteria, alteration of cell membrane function, and leakage of intracellular materials. This leads to the antimicrobial action of EOs specially monoterpene [85].

There are several reports on the chemical composition of EOs from the *Nepeta* species such as *N. persica* Boiss [32], *N. ispahanica* Boiss and *N. binaludensis* Jamzad [63], *N. daenensis* [68], *N. sibirica* [46], *N. sintenisii* [70], *N. involucrate* [77], *N. pannonica* [39], *N. satureioides* [25], *N. hellotropifolia* [65], and *N. meyeri* [14, 16, 35]. The EOs and chemical composition of medicinal plants and their biological activities are influenced significantly by both intrinsic and external factors such as cultivation area, climatic conditions, genetic modification, type of plant part, plant phenological stages and collection time [18, 49, 67], processing of plant materials and method of oil extraction [23, 28, 54, 56, 73]. EOs chemical composition may be affected by plant growth stages and environmental conditions [10, 22, 86]. In this case, the plant growth stage is one of the important factors in the quantity and quality of essential oil components [1]. Some studies have reported that the chemical composition of EOs in medicinal plants as affected by plant phenological stages and harvest time [20, 50, 67]. Therefore, it is necessary to determine the proper harvest time and plant growth stages by analyzing the EOs and their compositions during various growth and developmental stages.

Considering the importance of *Nepeta*, in this study EOs components of *N. kotschyi* were evaluated in western regions of Iran during plant growth and development stages by GC and GC/MS to understand the proper harvest time of *N. kotschyi*.

Material and Methods

Plant Material

The aerial parts of *Nepeta kotschyi* Boiss. were collected in May, June and July 2017 at different plant phenological stages including mid vegetative (beginning of May), 50 % of flowering (beginning of June) and fruiting stages (beginning of July) from its natural habitat in the North of West Azerbaijan, the region of Qushchi valley, Iran (Fig. 1; Table 1). The collected plants were identified in the Department of Medicinal Plant, Urmia University, Iran based on the botanical reference of Ghahreman, 1979-1992 [19].

Table 1 Characteristics of the studied region

Altitude (m)	1483-2716
Longitude (E)	44˚ 51’10”-44˚ 57’52”
Latitude (N)	37˚56' 1"-38˚0' 53"
Climate	cold semi-dried
Average temperature (˚C)	8.1
Average annual rainfall (ml)	303.3

Qushchi region

Latitude: 37° 56' 1"-38° 0' 53" N

Longitude: 44° 51’ 10”-44° 57’ 52” E

Altitude: 1483-2716 m

Fig. 1 The studied region in West Azerbaijan

Essential Oil Extraction

The parts of plants that were collected during different stages of growth were dried in Herbarium Laboratory, in Urmia University, Iran and then powdered. The samples (50 g) were hydro-distilled for 3 hours by using an all-glass Clevenger-type apparatus, to extract EOs, according to the method recommended by the European Pharmacopoeia. The extracted EOs samples were dried over anhydrous Na_2SO_4. Then the efficiency of EOs was calculated as the following formula [33]:

% Essential oils= Weight of essential oil/dry weight of plant ×100

Finally, the extracted samples stored in sealed vials at low temperatures (4 ˚C) until analyzing by gas chromatography (GC) and gas chromatography/mass spectrometric (GC/MS).

GC and GC/MS Analysis

The analysis of EOs was carried out using gas chromatography (GC) that was performed using a Shimadzu model A9 equipped with a DB-5 (dimethylsiloxane, 5% phenyl) fused silica capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). EOs (100 µl) were injected while Helium with the purity of 99.999 % was used as a carrier gas at a pressure of 1.5 kgcm^2 and a flow rate of 31.5 cms^{-1}. The thermal planning of the column was started at 60 ˚C and then programmed to rise to 210 ˚C at a rate of 3 ˚C min^{-1}. After raising the temperature to 210-240 ˚C at a rate of 20 ˚C min^{-1}, stop at this temperature for 8.5 min. The flame ionization detector (FID) and the injector temperature was 280 and 300 ˚C, respectively. EOs were also subjected to gas chromatography/mass spectrometric (GC/MS) analyses by using a Varian 3400 GC/MS system. The GC/MS was equipped with a DB-5 column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was the same as the previous one. The final temperature of the injection chamber was adjusted 10 ˚C higher than column temperature. The carrier gas was helium with a flow rate of 31.5 cm s^{-1}, scan time was 1 s, the ionization energy was 70 eV, and mass range 40-340 AMU (atomic mass unit).

Identification of EOs Components

The components of the EOs were identified by comparison of their mass spectra with those a computer library by Saturn Software or with authentic compounds and confirmed by comparison of their Retention Index (RI).

Results and Discussion

The results showed that the EOs of "Persian *Nepeta*" were affected by plant growth and development stages and there were significant differences among the *Nepeta* essential oil in both content and compounds in plant phenological stages. Average percentage of EOs was 2.48 (in vegetative), 0.8 (in

50 % of flowering) and 0.82 % (in fruiting) stages. The EOs percentage was the highest in the vegetative stage (Fig. 2).

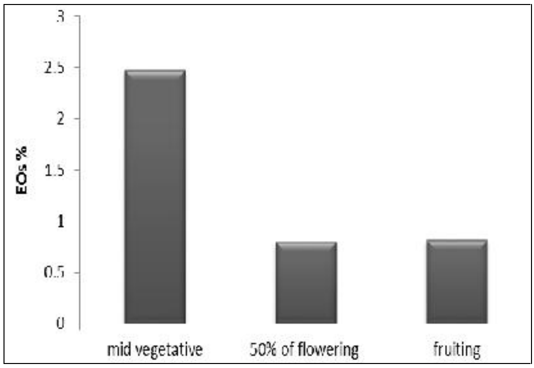

Fig. 2 EOs percentage in different plant growth stages of *N. kotschyi*

As shown in Table 2, the yield of EOs ranged from 0.84-13.04 % in vegetative, 10.93-53.25 % in 50 % of flowering, and 8.51-45.22 % in fruiting stages. The highest compounds were related to the vegetative stage, so 21 compounds were identified in this stage. The constituents including Verbenol acetate (1.30 %), Hexadecane (1.21 %), Thymol (7.59 %), Copaene (0.84 %), Dodecane, 2,6,11-trimethyl- (1.21 %), Caryophyllene (6.77 %), Germacrene D (3.53 %), Docosane (3.62 %), Nerolidol (13.04 %), (-)-Spathulenol (7.84 %), Caryophyllene oxide (10.07 %), tau.-Cadinol (6.73 %), tau.-Muurolol (5.75 %), Heptadecane, 2,6,10,15-tetramethyl (2.97 %), Eicosane (1.53), Dotriacontane (4.18), n-Hexadecanoic acid (8.72), Tetratetracontane (2.51), Phytol (2.83) and Heptacosane (4.27 %) were found which Farnesene in RT 39.31, had the highest compound in the vegetative stage. In 50 % of the flowering stage, 3 compounds were identified that the highest was related to Spathulenol with a percentage of 53.25 in RT 39.88. In fruiting stages, 6 compounds were identified that the highest was related to Caryophyllene oxide with a percentage of 45.22 in RT 39.88.

The largest number of identified compounds was 21, that they were related to the mid vegetative stage. These compounds contained 100 % of essential oil content. The only monoterpene compound in this stage was thymol (7.29 %). Sesquiterpene compounds with 9 compounds had the highest percentage (58.38 %) of essential oil content, which the major sesquiterpene components were nerolidol (13.04 %), caryophyllene oxide (10.07 %) and (-)-spathulenol (7.84 %) in this stage. The number of non-terpene compounds was 11 that contained 34.33% of essential oil content, and the major non-terpene components were n-hexadecanoic acid (8.72 %), heptacosane (4.25 %) and dotriacontane (4.18 %). The stage of 50 % of flowering just had 3 number identified compounds, which they had a high percentage. The only identified monoterpene was 1,8-cineol (35.82 %), the only identified sesquiterpene was spathulenol (53.25 %) which was the highest compound in this stage, and the only non-terpene identified compound was docosane (10.93 %). 5 compounds were identified in the fruiting stage, in which the only identified monoterpene was 1,8-cineol, that compound was (9.32 %), three sesquiterpene components identified in this stage were caryophyllene oxide (45.22 %), isocaryophyllene (21.67 %) and germacrene D (8.51 %). As can be seen at this stage most of the identified compounds are related to sesquiterpene terpenes (75.4 %). The only identified non-terpene compound was nonacosane (8.69 %) (Table 3).

The identified compounds have many biological properties. For example, n-Hexadecanoic acid ($C_{16}H_{32}O_2$) is antioxidant, pesticide, hypocholesterolemic, etc. [75]. Germacrene D (a sesquiterpene, $C_{15}H_{24}$), was observed to have cytotoxicity effect [57]. Thymol (a monoterpene phenol, $C_{10}H_{14}O$) has shown antioxidant activities [11], antibacterial [45], antiproliferative, and human liver cancer [Yin and Zhuang, 2010]. Eucalyptol or 1,8-cineole (a monoterpene ether, $C_{10}H_{18}O_{10}$) has antioxidant [81], antitumor [76], and antimicrobial activities [60]. Phytol (a diterpene, $C_{20}H_{40}O$) has antibacterial activities against *Staphylococcus aureus* [30].

Phytol plagiarized key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies. Also, they have antimicrobial, anticancer, cancer preventive, diuretic and anti-inflammatory effects [75].

Table 2 Essential oils and chemical composition of *Nepeta kotschyi* Boiss. during different plant phenological stages

No.	mid vegetative				50 % of Flowering				Fruiting			
	Compound	%	RT	RI	Compound	%	RT	RI	Compound	RT	%	RI
1	Thymol	7.29	29.26	1288	Eucalyptol or 1-8 cineol	35.82	15.34	1031	Eucalyptol or 1-8 cineol	15.34	9.32	1031
2	Verbenol acetate	1.30	26.39	1346	Spathulenol	53.25	39.88	1575	Isocaryophyllene	33.2	21.67	1407
3	Copaene	0.84	31.36	1375	Docosane	10.93	44.33	2200	Germacrene D	35.8	8.51	1485
4	β-Caryophyllene	6.77	33.19	1420	-	-	-	-	Caryophyllene oxide	39.88	45.22	1570
	Germacrene D	3.53	35.79	1480	-	-	-	-	Nonacosane	57.35	8.69	2900
5	Farnesene	3.81	34.92	1485	-	-	-	-	-	-	-	-
	Dodecane, 2,6,11-trimethyl-	1.21	32.49	1503	-	-	-	-	-	-	-	-
6	Nerolidol	13.04	39.31	1534	-	-	-	-	-	-	-	-
	Caryophyllene oxide	10.07	39.88	1570	-	-	-	-	-	-	-	-
7	(-)-Spathulenol	7.84	39.78	1575	-	-	-	-	-	-	-	-
8	.tau.-Cadinol	6.73	42.21	1640	-	-	-	-	-	-	-	-
9	.tau.-Muurolo	5.75	42.71	1674	-	-	-	-	-	-	-	-
10	n-Hexadecanoic acid	8.72	51.64	1700	-	-	-	-	-	-	-	-
11	Heptadecane, 2,6,10,15-tetramethyl	2.97	44.33	1914	-	-	-	-	-	-	-	-
12	Phytol	2.83	54.07	1950	-	-	-	-	-	-	-	-
13	Hexadecane	1.21	27.19	1954	-	-	-	-	-	-	-	-
14	Eicosane	1.53	49.43	2000	-	-	-	-	-	-	-	-
15	Docosane	3.62	36.39	2200	-	-	-	-	-	-	-	
16	Heptacosane	4.27	57.33	2700	-	-	-	-	-	-	-	-
17	Dotriacontane	4.18	50.1	3200	-	-	-	-	-	-	-	-
18	Tetratetracontane	2.51	53.68	4395	-	-	-	-	-	-	-	-

RT= Retention Time; RI= Retention Index.

Table 3 The percentage and number of different chemical classes of *Nepeta kotschyi* Boiss. essential oil.

Samples \ Chemical classes		Monoterpene	Sesquiterpene	Non-terpene	Total
Mid vegetative	Number	1	9	11	21
	Percentage	7.29	58.38	34.33	100
50% of Flowering	Number	1	1	1	3
	Percentage	35.82	53.25	10.93	100
Fruiting	Number	1	3	1	5
	Percentage	9.32	75.4	8.69	93.41

Nerolidoll (3,7,11-trimethyl-1,6,10-dodecatrien-3-ol, $C_{15}H_{25}OH$, sesquiterpene alcohol), also known as peruviol, is aliphatic sesquiterpene alcohol present in essential oils of several plants. It is frequently used in cosmetics (e.g., shampoos and perfumes) and non-cosmetic products (e.g., detergents and cleansers) [44]. In medicinal fields, nerolidol has shown antioxidants [57], antinociceptive [43] and antiulcer [37] activities. Nerolidol is active against bacteria and fungi [9, 59, 34]. Concerning the antiparasitic effect of nerolidol, it has shown antileishmanial [6], antitrypanosomal [29], and antimalarial [47] activities as well as inhibitory effect on the growth of *Babesia* parasites [3].

There are some studies about the EOs of *Nepeta*. [52] have reported that the composition of the EOs of *N. crispa* and *N. menthoides* showed differences in the quantitative and qualitative patterns of the samples. The major component of two oil was1,8-cineole.

In other reports, 1,8-cineol was also the major compound in *N. crispa* [77, 74; 52], *N. ispahanica,* and *N. binaludensis* [63], *N. denudata* [64], *N. meyeri* [72], *N. heliotropifolia* [69]. Also, the EOs of *N. sintenisii* [84], *N. Atlantica, N. tuberosa, N. granatensis, N. cataria* [64], *N. cephalotes* [26], *N. nuda* [55] and *N. coerulea* [79] have been examined and are characterized by the presence of one or more of the nepetalactone isomers. So, the various nepetalactone isomers such as 4aα,7α,7aα-nepetalactone, 4aα,7α,7aβ-nepetalactone and 4aβ, 7α,7aβ-nepetalactone, have been labeled as the biochemical markers of the *Nepeta* EOs that are very useful in chemotaxonomic studies [27]. In other studies, the major EOs compounds were β caryophyllene in *N. daenensis* [70], caryophyllene oxide in *N. Cilicia* [41], linalool in *N. satureioides* [25]. In other studies, the EOs of *N. kotschyi* were evaluated. Hadi *et al*. (2016) have reported 4aα,7α,7aα-nepetalactone, cubenol, geranyl acetate and cubenol were the highest components in some Iranian endemic species of *N. kotschyi*. Also, the main EOs components in *N. kotschyi* were 4aβ,7α,7aα-nepetalactone, and 1,8-cineole in as study of Nori-Shargh *et al*. (2006) [58]. In the present study, we identified 1-8 cineol for *N. kotschyi* just in 50 % of flowering and fruiting stages with a 9.86 and 4.46 percent, respectively.

There is a little information about the effect of phenological stages on EOs of *Nepeta*. Abdoli *et al*. (2016) have reported that the *N. crispa* EOs in before flowering and flowering stages were different in content and presence [1]. So, 32 and 31 constituents were detected respectively. So, the component 1,8-cineol was the major compound in two stages. According to their results, before the flowering stage EOs content was high that are matched with our results. 1,8-cineol [66] and alpha-citral [21] as the most abundant compounds of *N. cataria*.

Conclusion

In this study the EOs content and compounds of *Nepeta* were varied in different plant growth and development stages. So, 21 compounds in vegetative, 3 compounds in 50 % of flowering, and 6 compounds in fruiting stages were identified. The compounds Nerolidol in vegetative, "Spathulenol " in flowering, and Caryophyllene oxide in fruiting stages had the highest value. Based on our results, the highest EOs content and compounds were observed in the vegetative stage. The results of this study can be useful to understand the proper harvest time in *Nepeta*.

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References

1. Abdoli P, Moradkhani SH, Dastan D. Comparative analysis of *Nepeta crispa* essential oil composition in flowering and vegetative stages. AM J Phytomedi and Clinical Therapeutics (AJPCT). 2016;4:106-112.
2. Aberoomand-Azar P, Zare K, Saber-Tehrani M, Jafari-Kokhedan A, Vafaei A, Nekoei M, Larijani K. Chemical composition of the essential oil from *Nepeta macrosiphon* Boiss. growing wild in Iran by different extraction methods and studies on the quantitative relationship between the retention indices of essential oils and their molecular structures. Asian J Chem. 2013;25:4741-4746.
3. AbouLaila, M, Sivakumar T, Yokoyama N, Igarashi I. Inhibitory effect of terpene nerolidol on the growth of *Babesia* parasites. Parasitol. Int. 2010;59:278-282.
4. Ahmadian A, Tavassoli A, Amiri E. The interaction effect of water stress and manure on yield components, essential oil, and chemical compositions of cumin (*Cuminum cyminum*). Afr J Agr Res. 2011;6:2309-2315.
5. Amin GR. Popular medicinal plants of Iran. Ministry of Health Publisher, Iran. 1991
6. Arruda, DC, D-Alexandri FL, Katzin AM, and Uliana SR. Antileishmanial activity of the terpene nerolidol. Antimicrobe Agents Chemother. 2005;5:1679-1687.
7. Aruoma OI. Free radicals, oxidative stress, and antioxidants in human health and disease. J. Am. Oil Chem. Soc. 1998; 75:199-212.
8. Bourrel C, Perineau F, Michel G, Bessiere JM. Catnip (*Nepeta cataria* L.) essential oil: analysis of chemical constituents, bacteriostatic, and fungistatic properties. J Essent Oil Res. 1993;5:159-167.
9. Brehm-Stecher BF, Johnson EA. Sensitization of *Staphylococcus aureus* and *Escherichia coli* to antibiotics by the sesquiterpenoids nerolidol, farnesol, bisabolol, and apritone. Antimicrob Agents Chemother. 2003; 10:3357-3360.
10. Burt S, Essential oils: their antibacterial properties and potential applications in food, a review. Int J Food Micro. 2004;94:223-253.

11. Caldefie-Chézet, F, Guerry M, Chalchat JC, Fusillier C, Vasson MP, Guillot J. Anti-inflammatory effects of *Malaleuca alternifolia* essential oil on human polymorph nuclear neutrophils and monocytes. Free Radic Res. 2004;38:805-811.
12. Cantino PD, Harley RM, Wagstaff SJ. Genera of Labiatae: status and classification. In: Harley, R. M., and T. Reynolds (eds.) Advances in Labiate science, Kew: Royal Botanic Gardens, UK. 1992;511-522.
13. Chalchat JC, Garry RP, Menut C, Lamaty G, Malhuret R, Chopineau J. Correlation between chemical composition and antimicrobial activity. VI. The activity of some African essential oils. J Essent Oil Res. 1997;9:67-75.
14. Chizzola R, Volatile compounds from some wild growing aromatic herbs of the Lamiaceae from southern France. Plant Biosys. 2006;140:206-210.
15. De-Almeida RN, De-Fátima Agra M, Souto Maior FN, and De-Sousa DP. Essential oils and their constituents: anticonvulsant activity. Molecules. 2011;16:2726-2742.
16. Esmaeili A, Rustaiyan A, Masoudi S, Nadji K. Composition of the essential oils of *Mentha aquatica* L. and *Nepeta meyeri* Benth. from Iran. J Essent Oil Res. 2006;18:263-265.
17. Evans WC. Trease and Evans' Pharmacognosy. W.B. Saunders Company Ltd., London. 1996.
18. Franz C. Genetics, Volatile oil crops: their biology, biochemistry and production; In: Hay, R.K.M., and P.G. Waterman (eds.), Harlow: Longman Scientific and Technical, England. 1993:63-96.
19. Ghahreman A, Colorful flora of Iran. Research Institute of Forests and Rangelands, Iran. 1979-1992.
20. Ghani A, Saharkhiz MJ, Hassanzadeh M, Msaada K. Changes in the essential oil content and chemical compositions of *Echinophora platyloba* DC. during three different growth and developmental stages. J Essent Oil Bear. 2009;12:162-171.
21. Gilani AH, Shah AJ, Zubair A, Khalid S, Kiani J. Chemical composition and mechanisms underlying the spasmolytic and bronchodilatory properties of the essential oil of *Nepeta cataria* L. J of Ethnopharmacol. 2009;121:405-411.
22. Gkinis G, Tzakou O, Iliopoulou D, Roussis V. Chemical composition and biological activity of *Nepeta parnassica* oils and isolated Nepetalactones. Zeitschriftfür Naturforschung. 2003;58:681-686.
23. Gudaityt O, Venskutonis PR. Chemotypes of *Achillea millefolium* transferred from 14 different locations in Lithuania to the controlled environment. Biochem Syst Ecol. 2007;35:82-592.
24. Hadi N, Sefidkon F, Shojaeiyan A, Jafari AA. Essential oil diversity of 21 populations from Iranian endemic species *Nepeta kotschyi* Boiss. Iranian Journal of Medicinal and Aromatic Plants. 2016;32:189-202.
25. Hadian J, Sonboli A, Nejad-Ebrahimi S, Mirjalili MH. Essential oil composition of *Nepeta satureioides* from Iran. Chem Nat Comp. 2006; 42:175-177.
26. Handjieva NV, Popon SS. Constituents of essential oils from *Nepeta cataria* L., *N. grandiflora* MB. and *N. nuda* L. J Essent. Oil Res. 1996;8:639-643.
27. Hassan T, Rather MA, Shawl AS. Chemical composition of the essential oils of *Nepeta laevigata* and *Nepeta elliptica* from India. Chem Nat Comp. 2011;47:456-458.
28. Heywood VH. The conservation of genetic and chemical diversity in medicinal and aromatic plants. In: Sener B (ed) Biodiversity: biomolecular aspects of biodiversity and innovative utilization, Kluwer Academic/Plenum Publishers, New York. 2002:13-22.
29. Hoet S, Stévigny C, Hérent MF, Quetin-Leclercq J. Antitrypanosomal compounds from the leaf essential oil of *Strychnos spinosa*. Planta Med. 2006;5:480-482.
30. Inoue Y, Hada T, Shiraishi A, Hirore K, Hamashima H, Kobayashi S. Biphasic effects of Geranylgeraniol, Terpenone and Phytol on the growth of *Staphylococcus aureus*. *Antimicrobial agents and Chemother*. 2005;49:1770-1774.
31. Jamzad Z, Grayer RJ, Kite GC, Simmonds MSJ, Ingrouille M. Leaf surface flavonoids in Iranian species of *Nepeta* L. (Lamiaceae) and some related genera. Biochem Syst Ecol. 2003;31:587-600.
32. Javidnia K, Miri R, Safavi F, Azarpira A, Shafiee A. Composition of the essential oil of *Nepeta persica* Boiss. from Iran. Flav Fragr J. 2002;17:20-22.
33. Jaimand K, Rezaee MB. Investigation on chemical constituents of essential oils from *Achillea millefolium* L. subsp. *millefolium* by distillation methods. Med and Aroma Plants Res of Iran. 2004;20:181-190.
34. Johann S, Oliveira FB, Siqueira EP, Cisalpino PS, Rosa CA, Alves TM, Zani CL, Cota BB. Activity of compounds isolated from Baccharis dracunculifolia D.C. (Asteraceae) against *Paracoccidioide brasiliensis*. Med Mycol. 2012;8:843-851.
35. Kalpoutzakis E, Aligiannis N, Mentis A, Mitaku S, Charvala C. Composition of the essential oil of two *Nepeta* species and *in vitro* evaluation of their activity against *Helicobacter pylori*. Planta Med. 2001;67:880-883.
36. Kamatou GPP, Viljoen AM. A review of the application and pharmacological properties of α-Bisabolol and α-Bisabolol-rich oils. J. Am. Oil Chem. Soc. 2010;87:1-7.
37. Klopell FC, Lemos M, Sousa JP, Comunello E, Maistro EL, Bastos JK, De-Andrade SF. An antiulcer constituent from the essential oil of *Baccharis dracunculifolia* DC (Asteraceae). Z Naturforsch. C. 2007;62:537-542.
38. Knobloch K, H Weigand, N Weis, HM Schwarm, H Vigenschow. Action of terpenoids on energy metabolism. In Progress in Essential Oil Research: 16th International Symposium on Essential Oils; Brunke EJ, Ed.; De Walter de Gruyter: Berlin, Germany. 1986:429-445.
39. Kobaisy M, Tellez MR, Dayan FE, Mamonov LK, Mukanova GS, Sitpaeva GT, Gemejieva NG.

Composition and phytotoxic activity of *Nepeta pannonica* L. essential oil. J Essent Oil Res. 2005;17:704-707.

40. Koh KJ, Pearce AL, Marshman G, Finlay-Jones JJ, Hart PH. Tea tree oil reduces histamine-induced skin inflammation. Br. J. Dermatol. 2002;147:1212-1217.
41. Kökdil G, Tanker M, Kurucu S, Topcu G. Essential oil analysis of *Nepeta cilicia* Boiss. Flavour Frag J. 1997;12:99-101.
42. Koocheki A, Sabet-Teimouri M. Effects of fertilizer types and irrigation intervals of on quantity criteria of Lavender (*Lavandula angustifolia*), Rosemary (*Rosemarinus officinalis*) and Hyssop (*Hyssopus officinalis*). Iran J Field Crop Res. 2011;9:78-87.
43. Koudou J, Abena AA, Ngaissona P, Bessière JM. Chemical composition and pharmacological activity of essential oil of *Canarium schweinfurthii*. Fitoterapia. 2005;76:700-703.
44. Lapczynski A, Bhatia SP, Letizia CS, Api AM. Fragrance material review on nerolidol (isomer unspecified). Food Chem. Toxicol. 2008;46:247-250.
45. Lambert RJW, Skandamis PN, Coote P, Nychas GJE. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. J Appl Microbiol. 2001;91:453-462.
46. Letchamo W, Korolyuk EA, Tkachev AV. Chemical screening of essential oil-bearing flora of Siberia IV. composition of the essential oil of *Nepeta sibirica* L. tops from Altai region. J Essent Oil Res. 2005;17:487-489.
47. Lopes NP, Kato MJ, Andrade EH, Maia JG, Yoshida M, Planchart AR, Katzin AM. Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol.) Warb. by Waiãpi Amazon Indians. J. Ethnopharmacol. 1999;67:313-319.
48. McGinty D, Letizia CS, Api AM. Addendum to Fragrance material review on Nerolidol (isomer unspecified). Food Chem. Toxicol. 2010;48:43-45.
49. Miliauskas G, Venskutonis PR, Van-Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004;85:231-232.
50. Mohammadi S, Saharkhiz MJ. Changes in essential oil content and composition of catnip (*Nepeta cataria* L.) during different developmental stages. J Essent Oil Bear Pl. 2011;14:396-400.
51. Mohammadi A, Malek F, Dehpour A, Nazari H. Chemical composition and antibacterial activity of the essential oils of some medicinal plants. Afr J Agric Res. 2013;8:3151-3158.
52. Mojab F, Nickavar B, Hooshdar H. Essential oil analysis of *Nepeta crispa* and *N.menthoides* from Iran. Iran J Pharm Sci. 2009;5:43-46.
53. Mozaffarian VA. dictionary of Iranian plant names: Latin-English-Persian. Farhang Moaser, Iran. 2006.
54. Msaada K, Hosni K, Taarit MB, Chahed T, Kchouk ME, Marzouk B. Changes on essential oil composition of coriander (*Coriandrum sativum* L.) fruits during three stages of maturity. Food Chem. 2007;102:1131-1134.
55. Negueruela AV, Rico MM, Benito PB, Perez-Alonso MJ. Composicion de los aceites esenciales de *Nepeta nepetella* subsp. aragonensis, *Nepeta coerulea* subsp. coerulea *Nepeta cataria* Giorn, Bot. Ital. 1998;122:295-302.
56. Nemeth E, Essential oil composition of species in the genus *Achillea*. J Essent Oil Res. 2005;17:501-512.
57. Nogueira-Neto JD, De-Almeida AA, Da-Silva Oliveira J, Dos-Santos PS, De-Sousa DP, De-Freitas RM. Antioxidant effects of nerolidol in mice hippocampus after open field test. Neurochem Res. 2013;38:1861-1870.
58. Nori-Shargh D, Baharvand B, Raftari S, Deyhimi F. The volatile constituents' analysis of *Nepeta kotschyi* Boiss. from Iran. J Essent Oil Res. 2006;18:237-238.
59. Park MJ, Gwak KS, Yang I, Kim KW, Jeung EB, Chang JW, Choi IG. Effect of citral, eugenol, nerolidol and alpha-terpineol on the ultrastructural changes of *Trichophyton mentagrophytes*. Fitoterapia. 2009;80:290-296.
60. Penfold AR, Willis JL. The eucalypts, botany, cultivation, chemistry and utilization, Interscience Publishers, New York. 1961.
61. Rechinger KH, *Nepeta*. In: Rechinger KH (ed) Flora Iranica, No.150. Akademische Druck and Vertagsanstatt, Graz, Austria. 1982.
62. Ricke SC, Kundinger MM, Miller DR, Keeton JT. Alternatives to antibiotics: chemical and physical antimicrobial interventions and foodborne pathogen response. Poultry Sci. 2005;84:667-675.
63. Rustaiyan A, Nadji K. Composition of the essential oils of *Nepeta ispahanica* Boiss. and *Nepeta binaludensis* Jamzad from Iran. Flavour Frag J. 1999;14:35-37.
64. Rustaiyan A, Komeilizadeh H, Monfared A, Nadji K, Masoudi SH, Yari M. Volatile constituents of *Nepeta denudate* Benth. and *N.Cephalotes* Boiss. from Iran. J Essent Oil Res. 2000;11:459-461.
65. Rustaiyan A, Jamzad M, Masoudi S, Ameri N. Volatile constituents of *Nepeta hellotropifolia* Lam., *Mentha mozaffarianii* Jamzad and *Ziziphora persica* Bunge. three labiatae herbs growing wild in Iran. J Essent Oil Res. 2006;18:348-51.
66. Saeidnia S, Gohari AR, Hadjiakhoondi A. Trypanocidal activity of oil of the young leaves of *Nepeta cataria* L. obtained by solvent extraction. J Med Plants. 2008;7:54-57.
67. Saharkhiz MJ, Ghani A, Hassanzadeh-Khayyat M. Changes in essential oil content and composition of Clary sage (*Salvia sclarea*) aerial parts during different phenological stages. Med Aromat Plant Sci Biotechnol. 2009;3:90-93.
68. Sajjadi SE. Analysis of the essential oil of *Nepeta sintenisii* Bornm. from Iran. Daru. 2005;13:61-64.
69. Sajjadi SE, Khatamsaz M. Volatile constituent of *Nepeta heliotropifolia* Lam. J Essent Oil Res. 2001;13:204-205.

70. Sajjadi SE, Mehregan I. Chemical constituents of the essential oil of *Nepeta daenensis* Boiss. J Essent Oil Res. 2005;17:563-564.
71. Santoro GF, Das-Gracas Cardoso M, Guimaraes LG, Salgado AP, Menna-Barreto RF, Soares MJ. Effect of Oregano (*Origanum vulgare* L.) and Thyme (*Thymus vulgaris* L.) essential oils on Trypanosoma cruzi (Protozoa: Kinetoplastida) growth and ultrastructure. Parasitol. Res. 2007;100:783-790**.**
72. Sefidkon F, Shaabani A. Essential oil composition of *Nepeta meyeri* Benth. from Iran. Flavour Fragr J. 2004;19:236-238.
73. Sefidkon F, Abbasi K, Bakhshi-Khaniki G. Influence of drying and extraction methods on yield and chemical composition of the essential oil of *Satureja hortensis*. Food Chem. 2006;99:19-23.
74. Sefidkon F, Jamzad Z, Mirza M. Chemical composition of the essential oil of five Iranian *Nepeta* species (*N. crispa*, *N. mahanensis*, *N.ispahanica*, *N. eremophila* and *N. rivularis*). Flavour and Frag J. 2006;21:764-767.
75. Sermakkani M, Thangapandian V. GC-MS analysis of Cassia italicia leaf methanol extract. Asian Journal of Pharmaceutical and Clinical Research. 2012;5:90-94.
76. Sobral MV, Xavier AL, Lima TC, Sousa DP. Antitumor activity of monoterpenes found in essential oils. Hindawi Publishing Corporation. 2014:35.
77. Sonboli A, Salehi P, Yousefzadi M. Antimicrobial activity and chemical composition of the essential oil of *Nepetacrispa* Willd. from Iran. Zeitschriftfür Naturforschung. 2004;59:653-656.
78. Sonboli A, Salehi P, Allahyari L. Essential oil composition of *Nepeta involucrate* from Iran. Chem Nat Comp. 2005;41:683-685.
79. Thappa RK, Agarwal SG, Srivastava TN, Kapahi BK. Essential oils of four Himalayan *Nepeta* species. J Essent Oil Res. 2001;13:189-191.
80. Tucker AO, Tucker SS. Catnip and the catnip response. Econ Bot. 1988;42:214-231.
81. W-Dhifi L, Bellili S, Jazi S, Bahloul N, Mnif W. Essential oils' chemical characterization and investigation of some biological activities: a critical review. Medicines. 2016;3:1-16.
82. Wagner H, Wolf P. New natural products and plant drugs with pharmacological, biological, and therapeutical activity. Springer Verlag, New York. 1977.
83. Zargari A. Medicinal Plants. Tehran University, Iran. 1996.
84. Zenasni L, Bouidida H, Hancali A, Boudhane A, Amzal H, Idrissi AI, Aouad RE, Bakri Y, Benjouad A. The essentials oils and antimicrobial activity of four *Nepeta* species from Morocco. J Med Plant Res. 2008;2:111-114.
85. Zengin H, Baysal AH. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. Molecules. 2014;19:17773-17798.
86. Zomorodian K, Saharkhiz MJ, Rahimi MJ, Bandegi A, Shekarkhar G, Bandegani A, Pakshir K, Bazargani A. Chemical composition and antimicrobial activities of the essential oils from three ecotypes of *Zataria multiflora.* Pharma Magazine. 2011;7:5.