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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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| **Journal of Medicinal Plants and By-products (2020) 1: 123-131** | **124** |

in Persian) is a major member of this family that has been regularly used for medicinal purposes

1. *Nepeta* consists of about 280 speciesincluding 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
2. *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

1. 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

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]. TheEOs 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 |
|  |  |

**125**



**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 Na2SO4. 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 kgcm2 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

**Nejad Habibvash *et al.***



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., fi lm 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

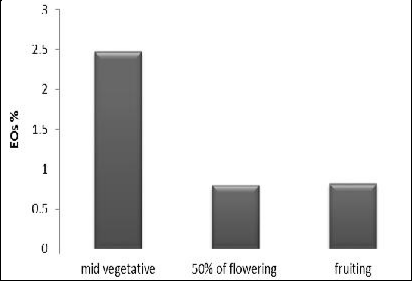
The components of the EOs were identified by comparison of their mass spectra with those a computer library by Saturn Software or with authentic compounds and confirmed by comparison of their Retention Index (RI).

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

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| **Journal of Medicinal Plants and By-products (2020) 1: 123-131** | **126** |  |
|  |  |

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

(C16H32O2) is antioxidant, pesticide, hypocholesterolemic, etc. [75]. Germacrene D (a sesquiterpene, C15H24), was observed to have cytotoxicity effect [57]. Thymol (a monoterpene phenol, C10H14O) has shown antioxidant activities [11], antibacterial [45], antiproliferative, and human liver cancer [Yin and Zhuang, 2010]. Eucalyptol or 1,8-cineole (a monoterpene ether, C10H18O10) has antioxidant [81], antitumor [76], and antimicrobial activities [60]*.* Phytol (a diterpene, C20H40O) 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].

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **127** |  |  |  |  |  |  |  |  |  |  |  |  |  |  | **Nejad Habibvash *et al.*** | | | | | | | | |  |
| **Table 2** Essential oils and chemical composition of*Nepeta kotschyi*Boiss. during different plant phenological stages | | | | | | | | | | | | | | | | | | | |  |  |  |  |  |
|  |  |  |  | |  |  |  | |  |  |  |  |  |  | |  |  |  |  |  |  |  |  |  |
|  |  |  | mid vegetative | | | | 50 % of Flowering | | |  |  |  |  | Fruiting | | | |  |  |  |  |  |  |  |
| No. | Compound | % | |  | RT | RI | Compound | | % | RT |  | RI |  | Compound |  |  | RT | % | |  | RI | | |  |
|  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  | |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 1 | Thymol | 7.29 | | 29.26 | | 1288 | Eucalyptol | | 35.82 | 15.34 | 1031 | |  | Eucalyptol or 1- | 15.34 | | | 9.32 | | 1031 | |  |  |  |
|  |  |  |  |  |  |  | or | 1-8 |  |  |  |  |  | 8 cineol |  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  | cineol |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 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 | -Caryophyllene | 6.77 | | 33.19 | | 1420 | - |  | - | - | - | |  | Caryophyllene | 39.88 | | | 45.22 | | 1570 | |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  | oxide |  |  |  |  |  |  |  |  |  |  |
|  | Germacrene D | 3.53 | | 35.79 | | 1480 | - |  | - | - | - | |  | Nonacosane | 57.35 | | | 8.69 | | 2900 | |  |  |  |
| 5 | Farnesene | 3.81 | | 34.92 | | 1485 | - |  | - | - | - | | - | | - | | | - | | - | |  |  |  |
|  | Dodecane, | 1.21 | | 32.49 | | 1503 | - |  | - | - | - | |  |  | - | | |  |  | - | |  |  |  |
|  | 2,6,11-trimethyl- |  |  |  |  |  |  |  |  |  |  |  | - | |  |  |  | - | |  |  |  |  |  |
| 6 | Nerolidol | 13.04 | | 39.31 | | 1534 | - |  | - | - | - | | - | | - | | | - | | - | |  |  |  |
|  | Caryophyllene | 10.07 | | 39.88 | | 1570 | - |  | - | - | - | | - | | - | | | - | | - | |  |  |  |
|  | oxide |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 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 | 8.72 | | 51.64 | | 1700 | - |  | - | - | - | | - | | - | | | - | | - | |  |  |  |
|  | acid |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 11 | Heptadecane, | 2.97 | | 44.33 | | 1914 | - |  | - | - | - | | - | | - | | | - | | - | |  |  |  |
|  | 2,6,10,15- |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | tetramethyl |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| 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.



|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Chemical classes | | |  |  |  |  |  |  |  |  |
| Samples |  |  |  | Monoterpene |  | Sesquiterpene |  | Non-terpene | Total |  |
|  |  |  |  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |
| Mid vegetative |  | Number | 1 | | 9 | | 11 | | 21 |  |
|  | Percentage | 7.29 | | 58.38 | | 34.33 | | 100 |  |
|  |  |  |
| 50% of |  | Number | 1 | | 1 | | 1 | | 3 |  |
| Flowering |  | 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, C15H25OH, 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*.

1. have reported that the composition of the EOs of *N. crispa* and *N. menthoides* showed differences

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| **Journal of Medicinal Plants and By-products (2020) 1: 123-131** | **128** |

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 EOsof *N. sintenisii* [84], *N. Atlantica, N. tuberosa, N.* *granatensis, N. cataria* [64], *N. cephalotes* [26], *N. nuda* [55] and *N. coerulea* [79] have beenexamined 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 fruitingstages 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 anddevelopment 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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