American Journal of Essential Oils and Natural Products

Available online at www.essencejournal.com

AJEONP

American Journal of Essential Oils and Natural Products

ISSN: 2321-9114
AJEONP 2019; 7(2): 15-22
© 2019 AkiNik Publications
Received: 09-02-2019
Accepted: 13-03-2019

Seyed Mostafa Goldansaz
Department of Natural resources, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran

Zeinab Jafarian Jeloudar
Department of Natural resources, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran

Roja Safaeian
Department of Natural Resources and Environment, Shiraz University, Shiraz, Fars, Iran

Ali Sonboli
Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Evin, Tehran, Iran

Correspondence:
Seyed Mostafa Goldansaz
Department of Natural resources, Sari Agricultural Sciences and Natural Resources University, Sari, Mazandaran, Iran

Comparison of the chemical constitutions, antibacterial, anti-*Candida*, and antioxidant activity of *Nepeta asterotricha* Rech. F. essential oil

Seyed Mostafa Goldansaz, Zeinab Jafarian Jeloudar, Roja Safaeian and Ali Sonboli

Abstract
Essential oils of eight populations of *Nepeta asterotricha* Rech. f. were isolated by hydro-distillation method. The obtained oil varied between 1.92-2.87% and 2.23-3.44% before and during full flowering stages, respectively. The GC-FID[1] and GC–MS[2] analyses identified 31 compounds among which 4a*α*, 7*β*, 7a*α*-nepetalactone (20.6-35.8%) was the major component in all populations. Bioassay results identified weak to acceptable activity against *S. aureus* and *E. coli* however; the oil exhibited a remarkable anti-*Candida* activity. Minimum inhibitory concentration (MIC) values for the tested bacteria varied between 1-2 mg/mL while for Cefixime, the value fluctuated from 1 to 64 µg/mL. Antioxidant activity of the oil was carried out with DPPH[3] that showed suitable property. The strongest activity was exhibited by NaF-8 with an IC_{50} value of 5.06 µg/mL, which was as effective as BHT[4].

Keywords: 4aα, 7β, 7aα-nepetalactone; DPPH; disk diffusion; MIC

1. Introduction

Nepeta L. is represented more than 280 species around the world, which elucidates a significant diversity in growth forms, pollination biology, floral morphology, and secondary metabolites [1]. The genus is demonstrated by 79 species in the Iranian flora, 42 of which are endemic such as *Nepeta asterotricha* Rech. f. [1, 2]. A literature survey identified *Nepeta* as a source of nepetalactone, which led to categorized into two groups. The first group consists of different stereoisomers of nepetalactone while, the other group has different sesquiterpenes as the main compound [3-8].

The chemical composition of oil depends on both biotic (genetic, ontogenic, morphogenic) and abiotic (Climate, soil, temperature, etc.) factors. Briefly, different plant populations are affected by environmental factors that might lead to change in pharmaceutical properties and biological activities [9]. The essential oil compositions have been studied to characterize the level of variation in several medicinal plant species such as *Ziziphora clinopodioides* ssp. *rigida* [10], *Satureja khuzestanica* jamzad [11], *Stachys lavandulifolia* VAHL [12], *Salvia tebesana* Bunge [13], *Mentha* × *rotundifolia* (L.) Huds. [14], and *Salvia verbenaca* L. [15]. Furthermore, essential oils have attracted a great deal of scientific interest due to being a potential source of natural remedies. In other words, many researchers have studied the pharmacologic values of essential oils [14, 16-20]. In following, examination of the oil from *N. asterotricha* showed an interesting effect on Gram-positive bacteria [3]. Antimicrobial activity of *N. ucrainica* L. spp. *kopetdaghensis* was done and the result showed a good activity against Gram-positive bacteria particularly *Staphylococcus aureus*, MIC = 14 µg/mL [21]. Ezzatzadeh *et al*. (2014) explored antimicrobial activity and essential oil of root, leaf, and aerial part of *N. asterotricha* in Tezerjan at flowering time and discovered the effect of the oil on *Bacillus cereus* and *Staphylococcus aureus* to be significant [8]. The chemical composition, antioxidant, and antimicrobial activity of *N. hindostana* (Roth) Haines were studied and the outcomes showed the oil had some positive activity in antioxidant and antimicrobial properties [22].

In addition, the present study aims to investigate the chemical variability in the essential oil

[1]. Gas Chromatography – Flame Ionization Detector
[2]. Gas Chromatography–Mass Spectrometry
[3]. 2,2-Diphenyl-1-picrylhydrazyl
[4]. Butylated hydroxytoluene

composition of *N. asterotricha* in all known areas of Iran, antibacterial and anti-*Candida* activities of the oil with disk diffusion and MIC test, and antioxidant activity of the oil in all populations. To the best of the present knowledge, antioxidant activity and MIC have not been previously studied.

2. Materials and Methods

2.1 The study areas

The study areas were located in eight dissimilar locations in the province of Yazd, Iran, the only habitat of this species in the world. Table 1 shows the geographical location of each area.

2.2 Plant material

The plant materials were prepared in two stages (before and during full flowering). All samples were collected from eight different natural areas. The samples were identified and placed in Herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.

2.3 Isolation of Essential Oil

All samples were dried in laboratory situation. Then, 100 g of each community was powdered and the oils were obtained by hydro-distillation using a Clevenger for 3 hours. The resulting oil was dried with sodium sulfate and kept in tightly closed vials at 4°C for chemical analyses. The oil yields [%(w/w)] were calculated by taking into account the weight of the dried material. The quantitative and qualitative analyses of the oils were conducted using GC-FID and GC-MS.

2.4 Gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analysis

The GC-FID analyses were performed using a Thermoquest-Finnigan instrument equipped with a DB-5 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μm). Nitrogen was used as the carrier gas at a flow rate of 1.1 ml/min. Planning thermal column was raised from 60ºC to 250 ºC with a rate of 5ºC/min and finally held isothermally for 10 min. The split ratio was 1:50. The injector and detector (FID) temperatures were kept at 250 ºC and 280 ºC, respectively. The Thermoquest-Finnigan Trace GC-MS instrument was same as GC. The injector and detector temperatures were kept at 250 ºC and 300 ºC, respectively. Helium with ionization voltage of 70 eV was used as the carrier gas at a flow rate of 1.1 mL/min, with a split ratio equal to 1/50. Ion source and interface temperatures were 200 ºC and 250 ºC, respectively. Mass range was from 35 to 456 amu. The oven temperature program was the same as given above for the GC.

2.5 Identification and quantification of the oil components

Identification of oil components was based on the comparison of retention indices (RI) under temperature-programmed conditions for *n*-alkanes (C_6 – C_{24}) and the oil on a DB-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of the mass spectra with those of the internal reference mass spectral library (NIST, Adams 2001 and Wiley 7.0) or with authentic compounds confirmed by comparison of the retention indices with authentic compounds or with those reported in the literatures.

2.6 The antioxidant activity

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) analysis is one of the tests used to assess the ability of the components to act as donors of hydrogen atoms. Antioxidant properties have been analyzed by inhibition assay of free radicals of DPPH (0.08 mg/mL in methanol). Because of some sediment, every oil was dissolved in dimethyl sulfoxide (DMSO; 930 µL/mL). Different volumes of the new solution (10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 µL) were mixed with 50 µL of DPPH, increasing the volume to 200 µL by using methanol. Each mixture was shaken for 30 min at room temperature in dark situation. The absorbance was measured at 517 nm with a UV-vis spectrophotometer against blank. Similarly, experiment with BHT as control was also ran parallel for the purpose of comparison. All experiments were replicated three times. There were different stocks such as control (DPPH/methanol), methanol (methanol), sample (DPPH/methanol/sample), and blank (methanol/sample). The radical scavenging activities of each sample were calculated according to the following formula for inhibition percentage (Ip) of DPPH:

$$\text{Ip} = \frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}} * 100$$

Abs C (Control-methanol) and Abs S (Sample-blank) indicate the absorbance values of the control and the sample, respectively. Analyses were used for estimation of IC_{50} value. In other words, the oil concentration providing 50% inhibition (IC_{50}) were calculated from the graph plotting inhibition percentage against oil concentration.

2.7 The antibacterial and anti-*Candida* activities

In vitro antibacterial and anti-*Candida* properties of essential oil samples were assessed against *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* PTCC1023, vancomycin resistant strain of *Enterococus faecium* (VRE) and *Enterococcus faecalis* ATCC29212 as Gram-positive bacteria, *Escherichia coli* ATCC 25922 as a Gram-negative bacterium, and a pathogenic yeast; *Candida albicans* ATCC 10231. All microbial strains were generously provided by Professor Mohammad Mehdi Feizabadi, Tehran University of Medical Sciences. Initially, the disk diffusion method was carried out against three models of Gram-positive, Gram-negative, and yeast. In other words, disk diffusion method was done to evaluate the antimicrobial activity of all samples. Briefly, normal saline was used for preparation of inoculants having turbidity equal to 0.5 McFarland standards. Mueller Hinton agar plates were also inoculated with normal saline by soaked swabs. Subsequently, 6-mm sterile disks, each containing 20 µL of essential oil were placed on the microbial lawns. For better absorption, the prepared plates were cooled at 4 °C for 30 min and then incubated at 37 °C for 22 h. Finally, the diameters of inhibition zones were measured and reported in mm. Cefixime and Nystatin were used as control for bacteria and yeast, respectively. It is noteworthy that triplicate tests were prepared in all experiments. According to the results, the essential oil divided into different categories. One candidate from each category was selected for determination of minimum inhibitory concentration (MIC) against six selected microorganisms. All steps were prepared as recommended by CLSI (Clinical Laboratory and Standard Institute) with some modifications [23]. According to broth micro-dilution method, MIC values were determined with multi-well. A serial dilution of the essential oil with 32 to 0.25 mg/mL concentration was made in Mueller-Hinton Broth containing 0.5% Tween 80 for bacteria and RPMI pH 7 contained 2%

Dextrose Broth with 0.5% Tween 80 and MOPS for yeast. The inoculants of the microbial strains were prepared from freshly cultured bacteria that were adjusted to 0.5 McFarland standard turbidity and were further diluted (1:100 and 1:1000 for bacteria and yeast, respectively) using corresponding medium right before adding to the serially diluted samples. The plate was incubated for 20 and 24 h at 37 °C for bacteria and yeast, respectively. Minimum inhibitory concentration (MIC) values were recorded as the lowest concentrations that could have inhibited visible growth of microorganisms.

3. Results & Discussion

3.1 Essential oil outcome

As shown in Table 2, essential oil yields varied between 1.92 - 2.87% and 2.23 - 3.44% before and during full flowering stages, respectively. The highest oil yield was obtained from NaF-6 while, the lowest one was obtained from NaB-2. GC-FID and GC/MS analyses revealed 31 constituents with the identified rang of 99.6% up to 99.9% of total essential oil compounds. It is speculated that the major constituent of the monoterpene hydrocarbons, oxygenated monoterpene, and sesquiterpene hydrocarbons was *γ*-terpinene (2.4 – 5.8%), 4a*α*,7*β*,7a*α*-nepetalactone (20.6 – 35.8%), and *(E)-β*-farnesene (0.4 – 1.6%), respectively. All populations identified the oxygenated monoterpenes as the predominate constituents with a variation of 81.4 – 87.8% before flowering and 79.2 – 88.0% during full flowering stage. Sesquiterpene hydrocarbons were also present in lower concentrations in both stages and in all population. In the following, NaF-1 and NaB-7 have the highest quality of monoterpene hydrocarbons (19.8%) and sesquiterpene hydrocarbons (1.7%), respectively. Six populations such as NaB-1, NaB-2, NaF-2, NaB-6, NaF-6, and NaF-8 had 5 isomers of nepetalactone. In addition, 4a*α*,7*β*,7a*α*-nepetalactone was found as the main component in the studied populations. More specifically, the highest levels of the other main components such as 1,8-cineole, *cis*-sabinene hydrate, terpinen-4-ol, 4a*α*,7*α*,7a*α*-nepetalactone, and linalool were obtained from NaF-5 (19.1%), NaB-2 (23.3%), NaF-1 (16.0%), NaF-4 (14.1%), and NaF-3 (10.2%), respectively. There are some differences in the main chemical compositions between the present study and the previous research. Rustaiyan's team revealed terpinen-4-ol (22.8%) and 4a*β*,7*α*,7a*β*-nepetalactone (14.8%) as the main constitutions [24]. Ezzatzadeh's team discovered that 1,8-cineole (26.12%), terpinen-4-ol (14.83%), 4a*α*,7*α*,7a*β*-nepetalactone (8.65%) and *cis*-sabinene hydrate (8.58%) were the main components in areal part during flowering stage [8], while the current investigation showed that 4a*α*,7*β*,7a*α*-nepetalactone, 1,8-cineole, *cis*-sabinene hydrate, and terpinen-4-ol were the main constituents. These differences may be because of the collection time, drying conditions, geographic or climatic factors, and mode of distillation.

In order to categorize and confirm the variation of the obtained essential oil from *N. asterotricha*, the oil compositions were submitted to cluster analysis (CA). The CA was based on the eight populations during full flowering time for 31 selected compounds. As can be seen in Figure 1, the eight populations could be divided into two chemotypes. 4a*α*, 7*β*, 7a*α*-nepetalactone was found as the key constituent in separating the population into two distinct chemotypes. Chemotype-I consists NaF-1, NaF-5, and NaF-7, which have lower amounts of 4a*α*,7*β*,7a*α*-nepetalactone than chemotype-II NaF-2, NaF-3, NaF-4, NaF-6, and NaF-8.

3.2 Antioxidant activity

Each essential oil was individually assessed for the possible antioxidant activities by DPPH test. The outcomes for antioxidant properties of each oil, which are the first DPPH test on *N. asterotricha* have been summarized in the Figure 2. In contrast with BHT (butylated hydroxytoluene) as a control, the antioxidant activity of the obtained essential oil from various populations of *N. asterotricha* showed valuable activity, which is associated to the main constituents. Generally, the antioxidant activity of the essential oil of some populations of *N. asterotricha*, which were collected before flowering stage such as NaB-6 and NaB-7 were higher than full flowering stage. Statistical analysis showed significant difference between various populations. The reason why different populations showed diverse activity can be attributed to the high percentages of the main components, synergy among the different oil constituents or to micro components acting as pro-oxidants. Based on statistical analysis, the IC_{50} value of NaF-8 was as effective as BHT. This valuable activity of the essential oil might be ascribed to their high content of nepetalactone isomers. The other main components of NaF-8 were 4a*α*, 7*β*, 7a*α*-nepetalactone, *cis*-sabinene hydrate, linalool, terpinen-4-ol, and 1, 8-cineole. Meanwhile, many researchers have studied the antioxidant activity of *Nepeta* essential oil. A literature survey on previous studies showed that different isomers of nepetalactone, 1, 8-cineole, terpinen-4-ol had a key role in antioxidant activity [22, 25-32]. A research group stated that 1, 8-cineole exhibited marked antioxidant activity, whereas linalool did not show any activity [26, 33]. These main components resemble the current research. In addition, it is noteworthy that most populations showed high-quality activity, which could be directly attributed to the main components. The antioxidant activity could be affected by test system, concentrations, emulsion system, oxidation time, and the test method used [34].

3.3 Antibacterial and anti-*Candida* activities

According to disk diffusion method, antibacterial and anti-*Candida* activities of all samples against three selected microorganisms were done and the results have been shown in Figures 3, 4, and 5. Based on inhibition zone (IZ) value, each essential oil showed valuable anti-*Candida* activity against *C. albicans*. Each oil showed reasonable and weak antibacterial activity against *E. coli* as a Gram-negative bacterium and *S. aureus* as a Gram-positive bacterium, respectively. The anti-*Candida* activity at the first step was excellent, too. These results are related to the main components such as 4a*α*, 7*β*, 7a*α*-nepetalactone, 1, 8-cineole, terpinen-4-ol, *cis*-sabinene hydrate, and linalool. The low antibacterial activity of isolated linalool, terpinen-4-ol and 1, 8-cineole are in accordance with the literature [35-37]. On the other hand, the antimicrobial activities of isolated essential oil from *Nepeta* species such as *N. leucophylla* Benth. (Iridodial *β*-monoenol acetate-25.4%, dihydroiridodial diacetate-18.2%, and iridodial dienol diacetate-7.8%), *N. discolour* Royle ex Benth. (1,8-cineole-25.5%, *β*-caryophyllene-18.6%, and *p*-cymene-9.8%), *N. govaniana* Benth. (isoiridomyrmecin-35.2% and pregeijerene-20.7%), *N. clarkei* Hook. f. (iridodial *β*-monoenol acetate diastereomers-25.3%, *β*-sesquiphellandrene-22%, and germacrene D-13%), *N. elliptica* Royle ex Benth. ((7*R*)-*trans,trans*-nepetalactone-83.4%), and *N. erecta* Benth. (isoiridomyrmecin-66.7%) were revealed to be effective against *Pseudomonas aeruginosa*, *Escherichia coli*, *Pasteurella multocida*, *Proteus vulgaris*, *Serratia marcescens*, *Staphylococcus aureus*, *Candida albicans*, and *Trichophyton*

rubrum [38]. Screening of the essential oil of *N. atlantica* Ball, *N. tuberosa* L. subsp. *reticulata* (Des f.) Maire, *N. cataria* L., *N. granatensis* Boiss with 4a*α*, 7*α*, 7a *β*-nepetalactone as the marker component in all of them has shown pronounced variable antibacterial activity against *E. coli*, *S. aureus*, and *Ps. aeruginosa* [39]. The antimicrobial activities of root, leaf and aerial part of the obtained essential oils from *N. asterotricha* were tested with disk diffusion method against seven bacteria and three yeasts. Results revealed that the oil from root and leaf has inhibitory activity against all the tested bacteria, especially *Bacillus cereus*, and three tested yeasts. The oil from the aerial part of *N. asterotricha* was active against all tested microorganisms, except *Pseudomonas aeruginosa* and *Klebsiella pneumonia* as Gram-negative strains [8].

Based on dick diffusion results, five samples (NaB-1, NaF-2, NaF-4, NaB-5, and NaF-6) were selected for MIC research against a larger collection of pathogenic microorganisms. As shown in Table 3, the MIC value of the essential oil varied between 1.0-2.0 and 0.5-2 mg/mL for antibacterial and anti-*Candida* activities, respectively. In addition, these results show low efficacies against the tested microorganism while earlier researches revealed that the oil of *Nepeta* damages the cell membrane structure of *E. coli*, *S. aureus*, and *C. albicans*. In other words, the present findings are not in accordance with the oil of *N. hindostana* (MIC ranged from 2.0 to 8.0 µL/mL), *N. granatensis* (22.5 to 80 µL/mL), *N. tuberosa* (4.37 µL/mL), *N. atlantica* (7.50 µL/mL), and *N. cataria* (5.00 µL/mL) [22, 39]. From the present results, the low antimicrobial activity is suspected to be associated with the chemical composition.

4. Conclusions

The present results indicate that the variation in the chemical compositions are very much limited. This research identified weak to acceptable antimicrobial activities and perfect antioxidant properties of the obtained essential oil from *N. asterotricha*. Because of the variation in constituents, the present results have some differences with the previous studies on *N. asterotricha*. Furthermore, the marker compositions in the current investigation had low antibacterial activity. These results can be considered as the first information on MIC and antioxidant properties of *N. asterotricha*. In addition, the best population in terms of yield, nepetalactone, antioxidant, antibacterial, and anti-*Candida* activities is in regions of Taghi Abad, Manshad, Zardein, Deh Bala, and Zardein during full flowering stage, respectively. This study also supports the primary knowledge of all populations of *N. asterotricha* for future investigations on biological activity and pharmacologic efficacies.

Appendix

Table 1: Geographical location of study areas

Study area \ Geography	Herbarium code	Latitude	Longitude	Altitude (m)	Code	
Darreh shir	MPH-2568	31° 38' 05" N	54° 01' 44" E	2463	Before flowering	NaB-1
					Full flowering	NaF-1
Deh Bala	MPH-2569	31° 34' 53" N	54° 05' 22" E	2791	Before flowering	NaB-2
					Full flowering	NaF-2
Khames Abad	MPH-2570	31° 36' 22" N	53° 54' 21" E	2591	Before flowering	NaB-3
					Full flowering	NaF-3
Manshad	MPH-2571	31° 30' 50" N	54° 12' 30" E	2399	Before flowering	NaB-4
					Full flowering	NaF-4
Sanij	MPH-2572	31° 35' 29" N	54° 00' 29" E	2492	Before flowering	NaB-5
					Full flowering	NaF-5
Taghi Abad	MPH-2573	31° 34' 24" N	54° 07' 14" E	2736	Before flowering	NaB-6
					Full flowering	NaF-6
Tezerjan	MPH-2574	31° 34' 28" N	54° 09' 30" E	2532	Before flowering	NaB-7
					Full flowering	NaF-7
Zardein	MPH-2575	31° 30' 04" N	54° 14' 17" E	2610	Before flowering	NaB-8
					Full flowering	NaF-8

Table 2: Essential oil compositions of the eight *Nepeta asterotricha* Rech. f. populations in two stages

No	Compound	RI	NaB-1	NaF-1	NaB-2	NaF-2	NaB-3	NaF-3	NaB-4	NaF-4
1	*α*-Thujene	926	0.8	1.0	1.4	0.7	0.8	0.7	0.6	0.6
2	*α*-Pinene	934	0.5	0.8	0.7	0.6	0.6	0.6	0.5	0.5
3	Sabinene	972	1.3	1.6	2.1	1.2	1.3	1.2	1.1	1.0
4	*β*-Pinene	978	0.7	1.2	0.9	1.2	0.9	1.0	1.1	0.9
5	*β*-Myrcene	989	0.5	0.6	0.7	0.5	0.5	0.5	0.5	0.4
6	*α*-Phellandrene	1005	0.0	0.1	0.1	tr	0.1	0.1	0.0	tr
7	*α*-Terpinene	1017	1.7	3.0	1.9	1.7	2.1	1.7	0.9	1.8
8	*p*-Cymene	1024	1.3	3.1	2.0	1.5	1.2	1.2	2.1	1.2
9	Limonene	1029	1.8	2.1	3.4	2.8	0.7	1.4	1.0	1.2
10	1,8-Cineole	1032	7.6	12.6	7.5	13.2	8.9	10.1	12.1	10.0
11	(*Z*)-*β*-Ocimene	1035	0.3	0.4	0.6	0.6	0.1	0.3	0.2	0.2
12	*γ*-Terpinene	1058	3.5	5.8	3.7	3.3	3.8	3.3	2.4	3.4
13	*cis*-Sabinene hydrate	1068	21.4	10.5	23.3	12.7	15.6	13.3	10.5	7.8
14	*cis*-Linalool oxide	1072	0.6	1.3	0.8	0.7	0.5	0.0	0.6	0.7
15	*α*-Terpinolene	1089	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1
16	Linalool	1101	7.2	6.4	6.9	4.8	10.0	10.2	8.1	7.4
17	*cis*-*p*-Menth-2-en-1-ol	1122	1.3	1.3	1.4	1.2	1.3	1.1	0.9	0.9

18	*trans-p*-Menth-2-en-1-ol	1140	0.6	1.0	0.5	0.5	0.6	0.5	0.5	0.5
19	Rose furan epoxide	1175	0.3	0.4	0.2	0.5	0.3	0.3	0.4	0.4
20	terpinen-4-ol	1179	8.3	16.0	7.8	8.8	8.3	8.1	7.2	8.5
21	*α*-Terpineol	1192	1.3	2.0	0.9	2.0	1.3	1.6	1.7	2.0
22	Geranial	1271	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.2
23	Geranyl formate	1302	0.2	0.1	0.1	0.2	0.1	0.1	0.2	0.2
24	4a*β*,7*α*,7a*α*-nepetalactone	1354	0.1	0.1	0.1	0.1	0.2	0.1	1.4	0.2
25	4a*α*,7*α*,7a*α*-nepetalactone	1363	5.2	3.9	5.4	5.5	4.5	5.5	8.9	14.1
26	4a*β*,7*α*,7a*β*-nepetalactone	1378	0.3	0.2	0.2	0.3	0.4	0.2	0.2	0.3
27	4a*α*,7*α*,7a*β*-nepetalactone	1392	1.0	tr	0.1	0.1	0.0	tr	0.0	tr
28	4a*α*,7*β*,7a*α*-nepetalactone	1401	30.8	23.3	26.1	33.9	34.5	35.8	35.0	34.8
29	*trans*-Caryophyllene	1423	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
30	(*E*)-*β*-Farnesene	1457	0.9	0.7	0.7	0.7	0.9	0.4	1.3	0.5
31	(*Z*)-*α*-Bisabolene	1504	0.0	0.1	0.0	0.1	0.0	0.1	0.1	tr
	Monoterpene hydrocarbons		12.5	19.8	17.5	14.2	12.2	12.1	10.5	11.3
	Oxygenated monoterpenes		86.3	79.2	81.4	84.6	86.6	87.1	87.8	88.0
	Sesquiterpene hydrocarbons		1.0	0.9	0.8	0.9	1.0	0.6	1.5	0.6
	Total identified		99.8	99.9	99.7	99.7	99.8	99.8	99.8	99.9
	Yield%		2.4	2.85	1.92	2.23	2.67	2.94	2.54	3.23

Table 2: Essential oil compositions of the eight *Nepeta asterotricha* Rech. f. populations in two stages

No	Compound	RI	NaB-5	NaF-5	NaB-6	NaF-6	NaB-7	NaF-7	NaB-8	NaF-8
1	*α*-Thujene	926	0.7	0.9	0.9	0.8	0.8	0.8	1.0	0.8
2	*α*-Pinene	934	0.8	0.7	0.6	0.5	0.7	0.8	0.9	0.6
3	Sabinene	972	1.3	1.7	1.3	1.2	1.2	1.6	1.4	1.3
4	*β*-Pinene	978	1.7	1.7	0.9	0.7	1.4	1.8	1.4	0.7
5	*β*-Myrcene	989	0.6	0.7	0.5	0.4	0.6	0.7	0.6	0.5
6	*α*-Phellandrene	1005	0.0	0.1	0.1	tr	0.0	tr	0.0	tr
7	*α*-Terpinene	1017	1.2	2.0	2.2	1.9	1.8	2.5	2.5	2.2
8	*p*-Cymene	1024	1.8	1.7	2.6	1.4	2.6	2.9	2.5	1.2
9	Limonene	1029	1.1	1.1	1.8	2.2	2.1	2.1	1.5	2.2
10	1,8-Cineole	1032	18.7	19.1	8.5	6.6	15.6	18.9	13.2	7.1
11	(*Z*)-*β*-Ocimene	1035	0.2	0.2	0.4	0.5	0.4	0.4	0.3	0.4
12	*γ*-Terpinene	1058	2.6	4.0	4.3	3.6	4.0	4.9	4.7	4.0
13	*cis*-Sabinene hydrate	1068	13.9	12.2	9.3	12.3	8.8	9.0	8.8	14.4
14	*cis*-Linalool oxide	1072	0.6	0.9	0.8	0.7	0.9	1.1	0.8	0.0
15	*α*-Terpinolene	1089	0.1	0.1	0.1	tr	0.1	0.1	0.0	tr
16	Linalool	1101	4.4	5.2	6.4	8.9	8.7	7.5	6.6	8.5
17	*cis-p*-Menth-2-en-1-ol	1122	1.0	1.0	1.1	1.2	1.0	1.0	1.0	1.2
18	*trans-p*-Menth-2-en-1-ol	1140	0.5	0.5	0.8	0.5	0.6	0.7	0.7	0.6
19	Rose furan epoxide	1175	0.7	0.7	0.3	0.2	0.6	0.6	0.4	0.2
20	terpinen-4-ol	1179	7.4	9.4	12.8	9.0	11.4	12.7	11.6	8.1
21	*α*-Terpineol	1192	2.4	2.2	1.5	1.1	1.9	2.3	2.0	1.3
22	Geranial	1271	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
23	Geranyl formate	1302	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2
24	4a*β*,7*α*,7a*α*-nepetalactone	1354	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1
25	4a*α*,7*α*,7a*α*-nepetalactone	1363	4.9	5.4	5.9	8.1	6.8	4.9	7.1	7.7
26	4a*β*,7*α*,7a*β*-nepetalactone	1378	0.3	0.2	0.3	0.2	0.2	0.2	0.2	0.3
27	4a*α*,7*α*,7a*β*-nepetalactone	1392	0.0	tr	0.2	0.3	0.0	tr	0.0	0.1
28	4a*α*,7*β*,7a*α*-nepetalactone	1401	31.7	27.1	34.4	35.8	25.4	20.6	29.4	35.1
29	*trans*-Caryophyllene	1423	0.1	0.1	0.1	0.1	0.0	tr	0.1	0.1
30	(*E*)-*β*-Farnesene	1457	0.8	0.6	1.1	0.9	1.6	1.2	0.7	0.9
31	(*Z*)-*α*-Bisabolene	1504	0.1	0.1	0.1	0.1	0.1	tr	0.1	tr
	Monoterpene hydrocarbons		12.1	14.9	15.7	13.2	15.7	18.6	16.8	13.9
	Oxygenated monoterpenes		86.8	84.2	82.6	85.4	82.2	79.8	82.1	85.0
	Sesquiterpene hydrocarbons		1.0	0.8	1.3	1.1	1.7	1.2	0.9	1.0
	Total identified		99.9	99.9	99.6	99.7	99.6	99.6	99.8	99.9
	Yield%		2.87	3.23	2.39	3.44	2.79	2.6	2.64	2.89

Table 3: MIC values of selected samples against bacteria and yeast.

	NaB-1 (mg/mL)	NaF-2 (mg/mL)	NaF-4 (mg/mL)	NaB-5 (mg/mL)	NaF-6 (mg/mL)	Cefixime (µg/mL)	Nystatin (µg/mL)
E. coli	2	2	2	2	1	16	-
S. aureus	2	2	1	2	1	4	-
B. subtilis	1	1	1	1	1	1	-
E. faecalis	2	2	2	2	2	8	-
E. faecium	1	1	1	2	2	64	-
C. albicans	1	0.5	2	0.5	0.5	-	128

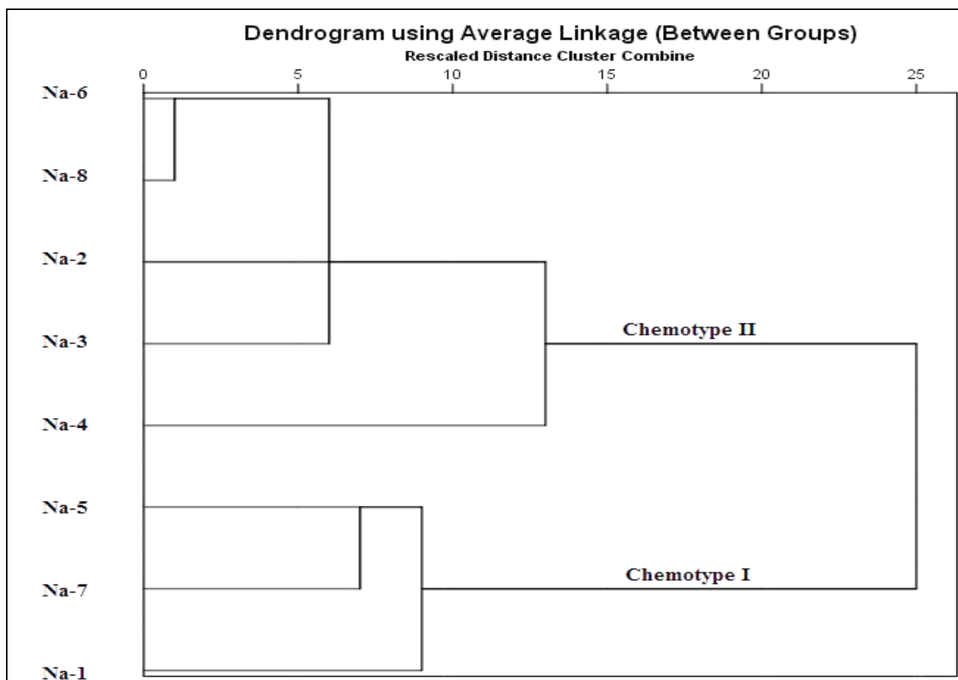

Fig 1: Ward dendrogram of the eight *Nepeta asterotricha* Rech. f. population (Na-1-Na-8), based on the squared Euclidean distance and resulting from the cluster analysis (CA) of essential-oil components (>0.1%).

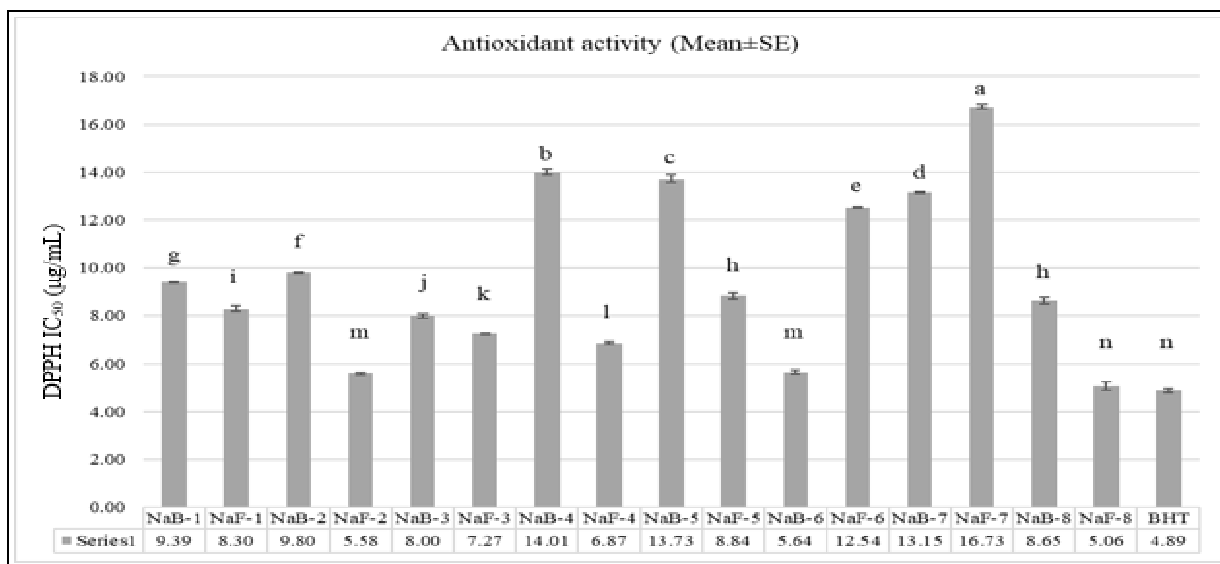

Fig 2: DPPH IC_{50} of *Nepeta asterotricha* rech. f.

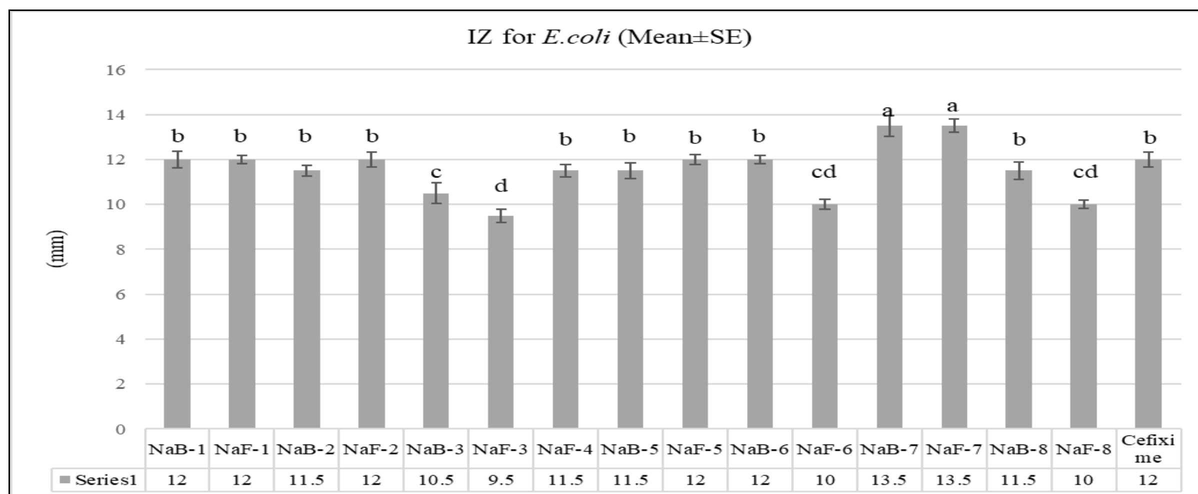

Fig 3: Inhibition Zones (mm) for *E. coli*.

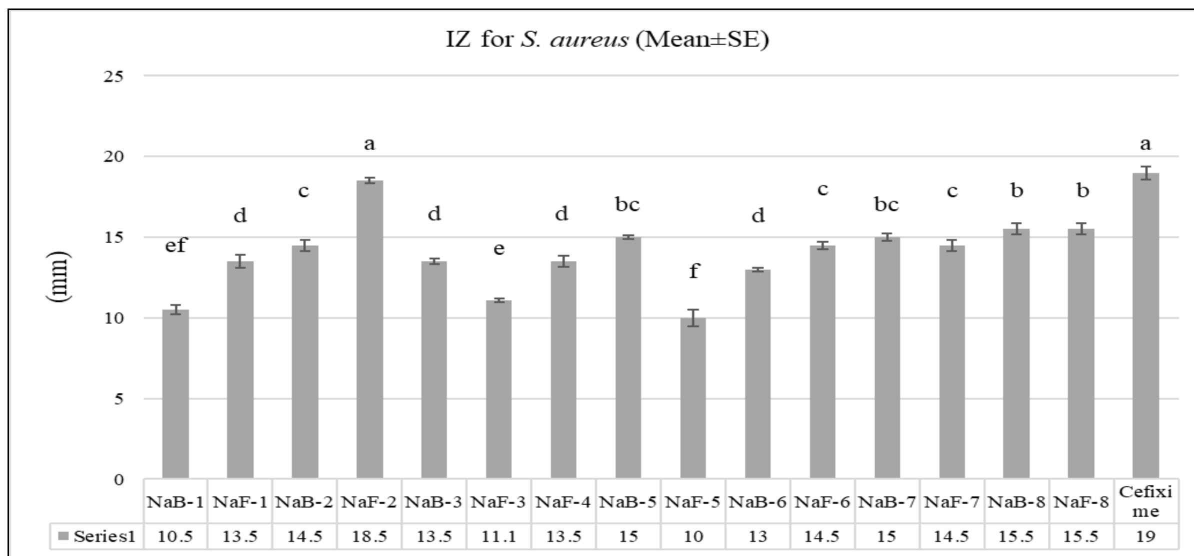

Fig 4: Inhibition Zones (mm) for *S. aureus*.

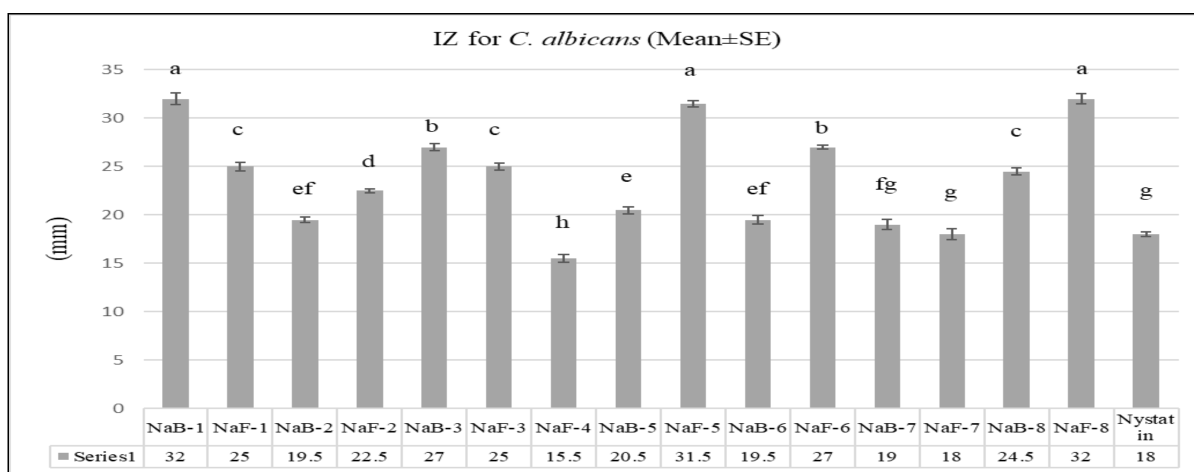

Fig 5: Inhibition Zones (mm) for *C. albicans*

5. Acknowledgments

Part of financial funding of this research was supported by Shahid Beheshti University (600/1186) that is gratefully appreciated. Furthermore, all tests were done at Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.

6. References

1. Mozaffarian V. Identification of medicinal and aromatic plants of Iran. Tehran, Iran: Farhang Moaser. 2013, 523-529.
2. Jamzad Z. A survey of Lamiaceae in the flora of Iran. Rostaniha. 2013; 14(1):59-67.
3. Masoudi S, Rustaiyan A, Mohebat R, Mosslemin M. Composition of the essential oils and antibacterial activities of *Hymenocrater yazdianus*, *Stachys obtusicrena* and *Nepeta asterotricha* three Labiatae herbs growing. Natural Product Communications. 2012; 7(1):117-120.
4. Salehi P, Sonboli A, Khaligh P, Mirzajani F. Essential oil composition and antioxidant activity of different extracts of *Nepeta betonicifolia* C.A. Meyer and *Nepeta saccharata* Bunge. Natural Product Research. 2012; 26(8):736-743.
5. Joshi Rakesh, Mathela C. Chemical constituents of leaf essential oil from *Nepeta laevigata* (D. Don) Hand.-Mazz from Kumaun Himalaya. American Journal of Essential Oils and Natural Products, 2013; 1(1):7-10.
6. Sharma A, Cannoo D. Phytochemical composition of essential oils isolated from different species of genus *Nepeta* of Labiatae family: a review. Pharmacophore, 2013; 4(6):181-211.
7. Asgarpanah J, Sarabian S, Ziarati P. Essential oil of *Nepeta* genus (Lamiaceae) from Iran: a review. Journal of Essential Oil Research. 2014; 26(1):1-12.
8. Ezzatzadeh E, Fallah Iri Sofla S, Pourghasem E, Rustaiyan A, Zarezadeh A. Antimicrobial Activity and Chemical Constituents of the Essential Oils from Root, Leaf and Aerial Part of *Nepeta asterotricha* from Iran. Journal of Essential Oil Bearing Plants, 2014; 17(3):415-421.
9. Sener B. Biodiversity: biomolecular aspects of biodiversity and innovative utilization. Berlin: Springer. 2012, 77-94.
10. Sonboli A, Atri M, Shafiei S. Intraspecific Variability of the Essential Oil of *Ziziphora clinopodioides* ssp. *rigida* from Iran. Chemistry & Biodiversity. 2010; 7(7):1784-1789.
11. Hadian J, Mirjalili MH, Kanani MR, Salehnia A, Ganjipoor P. Phytochemical and Morphological Characterization of *Satureja khuzistanica* Jamzad Populations from Iran. Chemistry & Biodiversity. 2011; 8(5):902-915.
12. Aghaei Y, Mirjalili MH, Nazeri V. Chemical Diversity

among the Essential Oils of Wild Populations of *Stachys lavandulifolia* Vahl (Lamiaceae) from Iran. Chemistry & Biodiversity. 2013; 10(2):262-273.
13. Goldansaz SM, Hakimi Meybodi MH, Mirhosseini A, Mirjalili MH. Essential Oil Composition of *Salvia tebesana* Bunge (Lamiaceae) from Iran. Records of Natural Products. 2017; 11(3):310-314.
14. Yahia I, Jaouadi R, Trimech R, Boussaid M, Zaouali Y. Variation of chemical composition and antioxidant activity of essential oils of *Mentha x rotundifolia* (L.) Huds. (Lamiaceae) collected from different bioclimatic areas. Biochemical Systematics and Ecology. 2019; 84:8-16.
15. Farhat M, Sotomayor J, Jordán M. *Salvia verbenaca* L. essential oil: Variation of yield and composition according to collection site and phenophase. Biochemical Systematics and Ecology. 2019; 82:35-43.
16. Ghasemi Pirbalouti A, Firoznezhad M, Craker L, Akbarzadeh M. Essential oil compositions, antibacterial and antioxidant activities of various populations of *Artemisia chamaemelifolia* at two phenological stages. Revista Brasileira de Farmacognosia. 2013; 23:861-869.
17. Nikolić M, Glamočlija J, Ferreira I, Calhelha R, Fernandes A, Marković T *et al*. Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential. Industrial Crops and Products, 2014; 52:183-190.
18. Linde J, Combrinck S, Vuuren S, Van Rooy J, Ludwiczuk A, Mokgalaka N. Volatile constituents and antimicrobial activities of nine South African liverwort species. Phytochemistry Letters. 2016; 16:61-69.
19. Hamdaoui AEl, Msanda F, Boubaker H, Leach D, Bombarda I, Vanloot P *et al*. Essential oil composition, antioxidant and antibacterial activities of wild and cultivated *Lavandula mairei* Humbert. Biochemical Systematics and Ecology. 2018; 76:1-7.
20. Halla N, Heleno SA, Costa P, Fernandes IP, Calhelha RC, Boucherit K *et al*. Chemical profile and bioactive properties of the essential oil isolated from *Ammodaucus leucotrichus* fruits growing in Sahara and its evaluation as a cosmeceutical ingredient. Industrial Crops and Products. 2018; 119:249-254.
21. Shakeri A, Khakdan F, Soheili V, Sahebkard A, Rassam G, Asili J. Chemical composition, antibacterial activity, and cytotoxicity of essential oil from *Nepeta ucrainica* L. spp. *kopetdaghensis*. Industrial Crops and Products, 2014; 58:315-321.
22. Pandey K, Mohan M, Singh P, Tripathi N. Chemical composition, antioxidant and antimicrobial activities of the essential oil of *Nepeta hindostana* (Roth) Haines from India. Records of Natural Products. 2015; 9:224-233.
23. Jorgensen J, Turnidge J. Susceptibility test methods: dilution and disk diffusion methods. Manual of Clinical Microbiology. Washington DC: ASM Press. 2015, 1152-1172.
24. Rustaiyan A, Monfared A, Masoudi S. Composition of the Essential Oil of *Nepeta asterotricha* Rech. f. from Iran. Journal of Essential Oil Research. 1999; 11:229-230.
25. Salehi P, Sonboli A, Allahyari L. Antibacterial and Antioxidant Properties of the Essential Oil and Various Extracts of *Nepeta ispahanica* from Iran. Journal of Essential Oil Bearing Plants. 2007; 10(4):324-331.
26. Tepe B, Daferera D, Tepe A, Polissiou M, Sokmen A. Antioxidant activity of the essential oil and various extracts of *Nepeta flavida* Hub.-Mor. from Turkey. Food Chemistry. 2007; 103(4):1358-1364.
27. Adiguzel A, Ozer H, Sokmen M, Gulluce M, Sokmen A, Kilic H *et al*. Antimicrobial and antioxidant activity of the essential oil and methanol extract of *Nepeta cataria*. Polish Journal of Microbiology. 2009; 58(1):69-76.
28. Alim A, Goze I, Cetin A, Duran Atas A, Cetinus SA, Vural N. Chemical composition and in vitro antimicrobial and antioxidant activities of the essential oil of *Nepeta nuda* L. subsp. *albiflora* (Boiss.) Gams. African Journal of Microbiology Research. 2009; 3(8):463-467.
29. Giamperi L, Bucchini A, Cara P, Fraternale D, Ricci D, Genovese S *et al*. Composition and antioxidant activity of *Nepeta foliosa* essential oil from Sardinia (Italy). Chemistry of Natural Compounds. 2009; 45:554-556.
30. Gkinis G, Bozin B, Mimica-Dukic N, Tzakou O. Antioxidant Activity of *Nepeta nuda* L. ssp. *nuda* Essential Oil Rich in Nepetalactones from Greece. Journal of Medicinal Food. 2010; 13(5):1176-1181.
31. Hasimi N, Kizil S, Tolan V. Essential Oil Components, Microelement Contents and Antioxidant Effects of *Nepeta italica* L. and *Achillea filipendulina* LAM. Journal of Essential Oil Bearing Plants. 2015; 18(3):678-686.
32. Joshi R. Antioxidant activity of essential oil of *Nepeta laevigata* (D. Don) Hand.-Mazz from Himalayan region of Uttarakhand. American Journal of Essential Oils and Natural Products. 2014; 2(1):15-18.
33. Formisano C, Rigano D, Senatore F. Chemical Constituents and Biological Activities of *Nepeta* Species. Chemistry & Biodiversity. 2011; 8(10):1783-1818.
34. El-Ghorab A, Mansour A, El-massry K. Effect of extraction methods on the chemical composition and antioxidant activity of Egyptian marjoram (*Majorana hortensis* Moench). Flavour and Fragrance Journal. 2004; 19(1):54-61.
35. Friedman M, Henika P, Mandrell RE. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*. Journal of Food Protection. 2002; 65(10):1545-1560.
36. Friedman M, Henika P, Levin C, Mandrell R. Antibacterial Activities of Plant Essential Oils and Their Components against *Escherichia coli* O157:H7 and *Salmonella enterica* in Apple Juice. Journal of Agricultural and Food Chemistry. 2004; 52(19):6042-6048.
37. Kotan R, Kordali S, Cakir A. Screening of antibacterial activities of twenty-one oxygenated monoterpenes. Zeitschrift Für Naturforschung. 2007; 62c:507-513.
38. Bisht D, Padalia R, Singh L, Pande V, Lal P, Mathela CS. Constituents and antimicrobial activity of the essential oils of six Himalayan *Nepeta* species. Journal of the Serbian Chemical Society. 2010; 75(6):739-747.
39. Zenasni L, Bouidida H, Hancali A, Boudhane A, Amzal H, Idrissi A *et al*. The essentials oils and antimicrobial activity of four *Nepeta* species from Morocco. Journal of Medicinal Plants Research. 2008; 2(5):111-114.