Original Article

Comparative Analysis of Nepeta Crispa Essential Oil Composition in Flowering and Vegetative Stages

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ABSTRACT

Objective: growth stage is one of important factors in component of essential oil, both from quantity and quality aspects. *Nepeta crispa* (Lamiaceae) with "Mofarrah" as local name is one of endemic plants with several traditional uses in Iran. It's aerial parts are used to prepare infusions and beverages for use as sedative, relaxant, carminative, tonic for respiratory and nervous disorders. Evaluation of the essential oil pattern in two above mentioned growth stages was the aim of this study.

Methods: Aerial parts of plant in both stages of growth were dried, powdered and essential oils were isolated by Clevenger type apparatus. Obtained essential oils were injected to GC/MS.

Results: In vegetative stage 32 constituents were identified which the main components were: 1,8-cineol (43.8%), α-Terpineol(11.03%), 4aα,7α,7aα-Nepetalactone (10.36%), δ-Terpineol(4.95), α-Bisabolene (4.55), 4aα,7β,7aα-Nepetalactone (3.67) and 4-Terpineol (3.65) and in flowering stage, 31 compounds were identified while, 1,8-Cineole (44.25%), 4aα,7α,7aα-Nepetalactone (24.72%), α-Terpineol (6.3%), δ-Terpineol (2.99) had the major amounts.

Conclusion: these results present that the *N. crispa* essential oil in two growth stages were different both of content and presence of compounds.

Keywords: *Nepeta crispa*, Volatile oil, Lamiaceae, GC/MS, vegetative, flowering.

INTRODUCTION

Large therapeutic potential and uses of aromatic plants make them one of the widely studied subjects at present. Essential oils cause the aromatic property besides medicinal characteristics[1]. *Nepeta* genus belongs to Lamiaceae family, Nepetoideae subfamily and Mentheae tribe in plant kingdom. *Nepeta* genus comprising ca. 300

species, spread out in Africa, southern and central parts of Asia and Europe. *Nepeta* spp. has beautiful flowers and pleasant odor[2]. According to reports, 69 species of this genus grow in Iran to date from which 38, 31 are endemic and native, respectively[3-5]. The most of plant species have traditional uses. Up to now, from 88 studied species, 193 compounds with different C- skeleton and variety of bioactivities have been isolated[6]. Western Himalayas and Southwestern Asia have the greatest diversity and richness of species. The former and latter include Hindu Kush, Iran and Turkey, respectively. Punesa is the common name for the plant in Iran[7]. Several *Nepeta* species in folk medicine are used. They have different uses, such as: diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic. Some species have potent antiseptic and astringent properties which made them beneficial topical remedies in cutaneous eruptions of children and snake and scorpion bites[8]. *Nepeta crispa* with "Mofarrah" as local name, attracted great attention in traditional medicine of western regions of Iran[9]. It's aerial parts are utilized to prepare infusions and beverages for use as sedative, relaxant, carminative, tonic for respiratory and nervous disorders[10].

Essential oils are volatile compounds which can be occurred in different organs of plants. Most of the crude products from medicinal plants due to volatile oil are used directly in medicine. Volatile oils in addition, play a huge role in the cosmetic, food and pharmaceutical industries[10]. The composition of *N. crispa* essential oil in flowering stage was subject of several studies, although there are differences between studies[11-13]. Considering the differences of compounds in growth stages [14], and importance of *N. crispa* in western regions of Iran, the goal of present research was to evaluate volatile oil components of *N. crispa* in flowering and vegetative stages in June and May 2015, respectively.

MATERIAL AND METHODS

Plant material

The aerial plant material of *N. crispa* in flowering and vegetative stages were gathered from Alvand mountain (Hamadan, Iran). The plant material was authenticated and deposited a voucher specimen in Department of Pharmacognosy, School of Pharmacy, Hamadan University of Medical Sciences, Hamadan, Iran.

Essential oil isolation procedure

The plant material in both growth stages were air-dried, powdered and the essence was obtained by hydrodistillation using Clevenger-type apparatus. The oils were dried with sodium sulphate and kept in refrigerator. Considering the plant dry weight, the oil yields were 0.5 and 0.66% (v/w), respectively.

GC/MS analysis was performed on a GC/MS (Thermoquest- Finnigan Trace) with a DB- 5 column (30 m, 0.25 µm film thickness, 0.25 mm i.d.). The injection program was as follow: raising oven temperature from 60 °C to 250 °C at a rate of 5 °C/min, then held at 250 °C for 10 min. split ratio was 1/100. The carrier gas was Helium.

Constituents identification

By comparison of the mass spectrum of compounds with the mass library, the constituents of oils have been identified. Although the retention indices of components were compared with those published in the literature. In some cases the kovats indices have been calculated with n-alkanes[15].

RESULTS

N. crispa essential oils in both flowering and vegetative stages were analyzed by means of GC/MS. Thirty one and thirty two compounds were detected and identified that representing 98.86% and 99.85% of the oils in flowering and vegetative stages, respectively (Table I). The content of the two samples was different.

In vegetative stage, the major components were: 1,8-cineol(43.8%), α-Terpineol(11.03%), 4aα,7α,7aα-Nepetalactone (10.36%), δ-Terpineol(4.95), α-Bisabolene(4.55), 4aα,7β,7aα-Nepetalactone(3.67) and 4-Terpineol(3.65), and in flowering stage, the main constituents were: 1,8 Cineole(44.25%), 4aα,7α,7aα-Nepetalactone(24.72%), α-Terpineol(6.3%), δ-Terpineol (2.99). Oxygenated monoterpenoids were the dominant contributors in the oils accounting for 83.69% in stage of vegetation and 83.45% for flowering stage. The monoterpene hydrocarbons accounted for 8.62% and 6.97% of flowering and vegetative stages, respectively. Amount of Sesquiterpenoids identified in flowering and vegetative stages were 0.86% and 6.61%, respectively.

DISCUSSION

All reports on *N.crispa* showed that 1,8-cineol is the main constituent of its essential oil [11, 12, 13]. 1,8-cineol showed slight rise in flowering stage. Chemical analysis of *Nepeta crispa* volatile oils have shown 4aα, 7a, 7aα-Nepetalactone content increase during the flowering stage. But another isomer i.e. 4aα, 7β, 7aα-Nepetalactone was only identified in vegetative stage. But the amount of α-Terpineol in vegetative stage was more than flowering stage. Some important constituents was only found in vegetative stage such as Anethole (1.11%), Thymol (0.98%), Carvacrol (0.34%), trans-Caryophyllene (0.35%) and Germacrene D (0.17%). Constituents that were identified only in stage of flowering were α-Campholene aldehyde (0.04%), Nopinone (0.03%), trans-Pinocarveol(0.33%) and trans-Verbenol(0.1%). Compounds with the largest changes in both growth stages were 4aα, 7a, 7aα-Nepetalactone with 24.72% and 10.36% in flowering and vegetative stages, respectively. *Nepeta crispa* oil in stage of flowering have been previously studied by other researchers. the results of one study Showed that the oil main constituents were 1,8-cineol (47.9%) and 4aα,7a,7aß-nepetalactone(20.3%), ß-pinen (6.9%), α-terpineol (4.8%), 4-terpineol (2.8%), α-pinene (2.5%), d-terpineol (2.1%) and 4aß,7a,7aß-nepetalactone(1.9%) and overall twenty-three compounds had been identified [11]. In another report for *N.crispa* oil, twenty-eight contributors have been identified from which the mains were 1,8-cineole(62.8%), 4aα-7α-7aα-nepetalactone (10.3%), 4aβ-7α-7aβ-nepetalactone (9.2%), β -pinene (3.6%) and α-terpineole (3.3%) [12]. Although in other research, 1,8-cineole(71%), β-pinene (5%) and β-terpineol (4.1%) were found to be the major, there was no trace of nepetalactone[13]. Some studies showed that monoterpenes act as analgesics [16]. In different reports, 1,8-Cineol was the major compound of the plants, such as: *N. ispahanica* (65.2%) and *N. binaludensis* (42.3%)[17], *N. denudata* (48%) (18), *N. meyeri* (29.3%)[19], *N. heliotropifolia* (19%)[20]. Nepetalactone is a cyclopentanoid monoterpene which has two rings, one lactone and one cyclopentane. Nepetalactone has eight stereoisomers, four diastereoisomers and their corresponding enantiomers. The first methyl cyclopentane monoterpenoid fully characterized [25– 26] was the 4aa, 7a, 7aα-nepetalactone isolated from *N. cataria* [21-24].

The analgesic action may be attributable to 4aa, 7a, 7aα-nepetalactone which is an opioid agonist with a subtype-

specific action [27].4aα, 7α, 7aα-Nepetalactone, the active isomer in *Nepeta cataria* has a characteristic effect on cats [28] and also repels cockroaches and mosquitos [22].In another research same to present study, chemical analysis of *N.parnassica* was done in flowering and vegetative stages that results showed differences in the quantitative and qualitative pattern of the two samples[13].

CONCLUSIONS

These results present that the *N. crispa* essential oil in two growth stages were different both of content and presence of compounds.

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Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Almeida R.N, Agra M, Souto F.N, Maiorand D.P. Essential oils and their constituents: Anticonvulsant activity. *Molecules*. 2011; 16: 2726–2742.
2. Cantino P. D, Harley R. M, Wagstaff S. J. Genera of Labiatae: Status and classification, in Advances in Labiate Science. Harley R. M, Reynolds T, Eds. *Royal Botanic Gardens, Kew*. 1992; 511–522.
3. Jamzad, Z. A new species of Satureja (Lamiaceae) from Iran. *Iran. J. Bot*. 2010; 16 (2): 213-217.
4. Delghandi, M. Nepeta leucostegia (Labiatae), a new record for the flora of Iran. *Iranian J. Bot*. 1993; 6: 149-151.
5. Formisano C, Rigano D, Senatore F.Chemical Constituents and Biological Activities of *Nepeta* Species. *Chemistry & Biodiversity* .2011; 8: 1783-1818.
6. Jamzad Z, Chase M.W, Ingrouille M, Simmonds M. S. J, Jalili A. Phylogenetic relationships in *Nepeta* L. (Lamiaceae) and related genera based on ITS sequence data. *Taxon*; 2003: 52, 21.
7. Zargari A. Medicinal Plants. 4th ed. Tehran. *Tehran University Publications*, 1990.
8. Mozaffarian V. A Dictionary of Iranian Plant Names. Tehran.FarhangMoaser Iran, 1996.
9. Jamzad Z, Grayer R., Kite G.C, Simmonds M.S.J, Ingrouille M, Jalili A. Leaf surface flavonoids in Iranian species of *Nepeta* (Lamiaceae) and some related genera. *Bioch. Sys. Ecol*. 2003; 31: 587-600.
10. Rasoli I, Rezaei M.B, Kamrani A, Zarpak B. comparison antibacterial effect of thymus essential oil and ampicillin invitro. *Scientific Journal of Hamadan University of Medical Sciences*. 1381; 9(4).(Persian)
11. Sonboli A, Salehi P, Yousefzadi M. Antimicrobial activity and chemical composition of the essential oil of *Nepeta crispa*Willd. from Iran. *Zeitschriftfür Naturforschung*.2004; 59c: 653–656.
12. 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 Fragrance Journal*. 2006; 21: 764–767.
13. Mojab F, Nickavar B, Hooshdar H. Essential Oil Analysis of *Nepeta* crispa and *N. menthoides* from Iran. *Iranian Journal of Pharmaceutical Sciences*. 2009; 5(1): 43-46.
14. Gkinis G, Tzakou O, Iliopoulou D, Roussis V. Chemical Composition and Biological Activity of *Nepeta parnassica* Oils and Isolated Nepetalactones. *Zeitschriftfür Naturforschung*. 2003; 58c: 681- 686.
15. Shibamoto T. Retention indices in essential oil analysis in Capillary Gas Chromatography in Essential Oil Analysis. Sandra P, Bicchi C, Eds. *HuethigVerlag*, New York, 1987.
16. Adriana G. Guimarães, Jullyana S. S. Quintans, Lucindo J. Quintans-Júnior. Monoterpenes with Analgesic Activity—A

Systematic Review. *Phytotherapy research*. 2013; 27: 1–15.
17. Rustaiyan A, Nadji K. Composition of the essential oils of *Nepeta ispahanica* Boiss, and *Nepeta binaludensis* Jamzad from Iran. *FlavourFragr. J*. 1999; 14: 35-37.
18. Rustaiyan A, Komeilizadeh H, Monfared A, Nadji K,MasoudiSh, Yari M. Volatile constituents of *Nepeta denudate* Benth. and*N. Cephalotes* Boiss. from Iran. J. *Essent. Oil Res*. 2000; 11: 459-461.
19. Sefidkon F, Shaabani A. Essential oil composition of *Nepeta meyeri* Benth. from Iran. *FlavourFragr. J*. 2004; 19: 236-238.
20. Sajjadi S.E, Khatamsaz M. Volatile constituent of *Nepeta heliotropifolia* Lam. *J. Essent. Oil Res*.2001; 13: 204-205.
21. Regnier F. E, Waller G. R, Eisenbraun E. J. Studies on the composition of the essential oils of three *Nepeta* species. *Phytochemistry*.1967; 6: 1281-1289.
22. De Pooter H.L, Nicolai B, De Laet J, De Buyck L.F, Schamp N.M, Goetghebeur, P. The essential oils of five *Nepeta* species. A preliminary evaluation of their use in chemotaxonomy by cluster analysis. *FlavourFragr. J*. 1986; 3:155-159.
23. Heuskin S, Godin B, Leroy P, Capella Q, Wathelet JP, Verheggen F. Fast gas chromatography characterisation of purified semiochemicals from essential oils of *Matricaria chamomilla* L.(Asteraceae) and *Nepeta cataria* L.(Lamiaceae). *Journal of Chromatography*. 2009; 1216 (14): 2768-2775.
24. McElvain S. M, Eisenbraun E. J. The Constituents of the Volatile Oil of Catnip. III. The Structure of Nepetalic Acid and Related Compounds. *J. Am. Chem. Soc*. 1955; 77(6):1599–1605.
25. Bates R. B, Eisenbraun E. J, McElvain S. M.The Configurations of the Nepetalactones and Related Compounds. *J. Am. Chem. Soc*. 1958; 80 (13): 3420–3424.
26. McElvain SM, Eisenbraun EJ. The interconversion of nepetalic acid and isoiridomyrmecin (iridolactone). *Journal of Organic Chemistry*.1957; 22: 976-977.
27. Aydin S, Beis R, Ozturk Y,HusnuK , Baser C.Nepetalactone: A New Opioid Analgesic from *Nepeta caesarea* Boiss. *J. Pharm. Pharmacol*. 1998; 50: 813-817.
28. Turner R. How does catnip work its magic on cats? *Scientific American*. 2007; 14.

Table 1: Chemical composition of *Nepeta crispa* oils in vegetative and flowering stages

Compound	Rt	%compound (vegetative)	%compound (flowering)
α-Thujene	3.95	0.36	0.62
α-Pinene	4.09	1.06	1.66
Sabinene	4.75	0.85	1.4
β-Pinene	4.85	3.58	3.91
β-Myrcene	5.04	0.83	0.58
Isobutyl 2-methylbutyrate	5.24	0.15	0.81
α-Terpinene	5.61	0.36	0.07
1,8-Cineole	6.04	43.8	44.25
Butyl 2-methylbutyrate	6.09	-	0.08
γ-Terpinene	6.49	0.62	0.3
Sabinene hydrate<cis->	6.71	0.37	2.63
α-Terpinolene	7.17	0.18	0.08
Linalool	7.45	3.29	2.39
2-Methyl butyl-2-methyl butyrate	7.48	-	0.54
Amyl isovalerate	7.58	-	0.07
cis-para-Menth-2-en-1-ol	7.98	0.14	0.18
α-Campholene aldehyde	8.07	-	0.04
Nopinone	8.38	-	0.03
trans-Pinocarveol	8.43	-	0.33
trans-Verbenol	8.6	-	0.1
Pinocarvone	8.99	4.95	0.13
δ-Terpineol	9.15	4.95	2.99
4-Terpineol	9.4	3.65	2.62
α-Terpineol	9.79	11.03	6.3
Data MS	10.35	-	0.45
Data MS	13.87	-	0.41
4aα,7α,7aα-Nepetalactone	14.31	10.36	24.72
4aα,7β,7aα-Nepetalactone	14.97	3.67	-
Isobutyl phenylacetate	14.8	-	0.04
Data MS	14.9	-	0.27
trans-β-Farnesene	16.38	1.46	0.21
Phenethyl 2-methylbutyrate	17.16	0.2	0.43
2-Phenylethyl 3-methylbutirate	17.26	-	0.21
(Z)-α-Bisabolene	17.51	4.55	0.65
β-Bisabolene	17.66	0.08	-
(E)-2-Hexenal	2.97	0.08	-
2-Methylbutyl acetate	3.23	0.04	-
p-Cymene	5.77	0.75	-
n-Nonanal	7.47	0.3	-
Anethole<E>	12.16	1.11	-

Thymol	12.44	0.98	-
Carvacrol	12.7	0.34	-
Benzyl isovalerate	14.69	0.19	0.49
trans-Caryophyllene	15.52	0.35	-
Germacrene D	17.03	0.17	-
Total of identified		**99.85**	**98.86**

Table 2: Class composition of compounds in *N. crispa*

Class of compounds	*N. crispa* vegetative (%)	*N. crispa* flowering (%)
Oxygenated monoterpenoids	83.69	83.45
monoterpene hydrocarbons	6.97	8.62
Sesquiterpene hydrocarbons	6.61	0.86
Others	2.58	5.93
Total	99.85	98.86