Health Biotechnology and Biopharma (2018), 1(4): 45-55

Original Research Article

Constituents and antimicrobial activity of the volatile oils from different parts of *Nepeta gloeocephala*

Elham Ezzatzadeh[1]*, Ehsan Pourghasem[2]

[1]Department of Chemistry, Ardabil Branch, Islamic Azad University, Ardabil, Iran

[2]University of Tehran, School of Chemical Engineering, Tehran, Iran

***Corresponding author:** Elham Ezzatzadeh, Department of Chemistry, Ardabil Branch, Islamic Azad University, Ardabil, Iran. Emails: dr.ezzatzadeh@yahoo.com*

DOI: 10.22034/HBB.2018.04

Received: March 4, 2018; **Accepted:** March 19, 2018

ABSTRACT

In this study, we identified the chemical composition and antimicrobial effects of different parts of essential oils of *Nepeta gloeocephala*. Totally 65 volatile compounds from the essential oils in *N. gloeocephala* were obtained by means of hydrodistillation and its components were analyzed using GC and GC-MS. These compounds are mainly oxygenated monoterpenes, monoterpene hydrocarbons and oxygenated sesquiterpenoids compound. The antimicrobial activity of leaf, flower, stem and root essential oils from *N. gloeocephala* were studied against seven gram-positive and gram-negative bacteria and two fungi by the disc diffusion method. The results of the bio assays showed an interesting antimicrobial activity, in which the gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, were the most sensitive to the oils, as well the oils exhibited a remarkable antifungal activity against all the tested fungi.

Keywords: *Nepeta gloeocephala*, essential oil, antimicrobial, chemical composition

INTRODUCTION

Essential oils and secondary metabolites of plants have many applications in medicine as well as food and cosmetics industry [1]. Essential oils have several health benefits including antioxidant and antimicrobial

properties. However, since essential oils are concentrated mixtures and may exhibit higher toxicity compared with the original plant or crude extracts, investigation of cytotoxic effects of essential oils is necessary prior to any therapeutic application. *Nepeta* is a large genus belonging to the Lamiaceae family. The majority of the known species (more than 280 spp.) of the genus *Nepeta* is spread out, principally, over the larger part of central and southern Europe, the Near East and central and southern Asia. Remarkably, about 67 of these species are found wild all over Iran [2], with the common Persian name of 'pune-sa'. Many *Nepeta* species are used in folk medicine because it was used in many countries as antispasmodic, diuretic, diaphoretic, antiseptic, antitussive, vulnerary, febrifuge, tonic, emmenagogue, sedative and antiasthmatic agent [3,4]. Also, many of them had the antifungal, antibacterial, and anti-viral activities that studied in previous literature [5-9]. Phytochemical analyses of *Nepeta* spp. have revealed the presence of several bioactive phytochemicals such as phenolics, terpenoids (sesquiterpenes) and flavonoids [3]. Part of the health benefits and biological activities of this genus may be attributed to its essential oil. Although the literature survey showed the essential oil composition of some other *Nepeta* species [10-19] have previously been studied, but there is no report on the antimicrobial activities of different parts of *N. gloeocephala* oils. In this research different parts including leaves, flowers, stems and roots of the *N. gloeocephala* were separated and essential oils obtained from these parts analysis via GC and GC-MS and then we evaluated the antimicrobial activity of the samples.

MATERIALS AND METHODS

Plant material

N. gloeocephala were collected from Tazerjan village, province of Yazd, Iran in June 2016, during the flowering stage. Voucher specimens have been deposited in the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran. The plants, dried in shade and different parts including leaves, flowers, stems and roots were separated.

Isolation of oils

Air-dried leaves (100 g), flowers (90 g), stems (100 g) and roots (95 g) of *N. gloeocephala* were separately subjected to 3.5 h of hydrodistillation in a clevenger-type apparatus. The resulting oil yield (leaves 1.1%, flowers 0.6%, stems 0.8 % and roots 0.5 % v/w) were dried over anhydrous

sodium sulfate and immediately placed into glass tubes and sealed. The samples were stored in the dark at 4 °C until analyzed.

GC analysis

Analysis of the oil was carried out using a Hewlett-Packard-6890 gas chromatograph equipped with a split/splitless (20:1) injector (250 °C) and a flame ionization detector (250 °C). Nitrogen gas was the carrier gas (1mL/min). A DB-5 capillary column was used (30 m × 0.25 mm, 0.32 µm film thicknesses). The oven temperature was held at 60 °C for 3 min, then heated to 220 °C with a 5 °C rate and kept constant at 220 °C for 5 min.

GC-MS analysis

This was performed using a Hewlett-Packard 6890/5973 GC-MS equipment with a 30 m × 0.25 mm, film thickness 0.32 µm HP-5MS column. Helium gas (99.999 %) was used as carrier gas (1.0 ml/min). The temperature program was the same as for GC. The GC-MS was equipped with chemstation software and a Wiley 275 library.

Identification of the constituents of the oil was made by comparison of their mass spectra and retention indices (RI) with those given in the literature and authentic samples [20].

Pharmacological Screening

Test microorganisms used in this study were the gram-positive bacteria *Bacillus cereus* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 15753 and *Staphylococcus epidermidis* ATCC 12228; the gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27852, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 27736; the fungi *Aspergillus niger* ATCC 9142 and *Candida albicans* ATCC 6258. The microorganisms were obtained from the research center of science and industry, Tehran, Iran. The antibacterial and antifungal activity of the essential oil was evaluated by disc diffusion method using Muller-Hinton and Sabouraud Dextrose agar, respectively [21]. A suspension of the tested microorganism (0.1 ml of a suspension of the tested microorganisms, containing 1.5×10^8 CFU/ml) was spread on the solid media plates. Mueller-Hinton and Sabouraud dextrose agar sterilized in a flask and cooled to 45 °C and distributed to sterilize petri dishes with a diameter of 9 cm. A serial dilution of the oils was prepared in Mueller-Hinton and Sabouraud dextrose broth for bacteria and fungi, respectively. The filter paper discs (6 mm in diameter) were individually impregnated

with 15 µl of the *N. gloeocephala* essential oil and then placed into the agar plates which had previously been inoculated with the tested microorganisms. The plates were inoculated with bacteria incubated at 37 °C for 24 h and at 28 °C for 48-72 h for the fungal strains. Standard reference antibiotics were used in order to control the sensitivity of the tested bacteria (ampicillin and tetracycline) and fungi (nystatine). All the experiments were carried out in triplicate and averages were calculated for the inhibition zone diameters.

RESULTS

Chemical composition of the essential oil

The use of essential oils for medical purposes has been the subject of increasing research in recent decades. This surge of interest in essential oil research is due to the diverse biological activities, simple oil extraction techniques, and natural origin of essential oils. This study evaluated the volatile composition and biological activities of the essential oils from *N. gloeocephala*. The results obtained in the analyses of the oils of *N. gloeocephala* leaf, flower, stem and root are listed in Table 1, in which the percentage and retention indices of the components are given.

As it is shown, about 98.91% (forty-eight compounds) of the leaf oil, 99.02% (fifty-one compounds) of the flower oil, 97.81% (fifty-three compounds) of the stem oil and 99.26% (forty-eight compounds) of the root oil were identified. The leaf oil consisted mainly of teen monoterpene hydrocarbons (14.46 %), thirty oxygenated monoterpenes (78.35%) and two oxygenated sesquiterpenes (1.39%). This oil was characterized by the presence of 1, 8-cineole (44.58%), myrtenol (7.28%), ß-pinene (5.62%), terpinene-4-ol (5.02%) and trans-pinocarveol (3.71%). The volatile oil of the flower contained nine monoterpene hydrocarbons (15.29%), thirty-one oxygenated monoterpenes (78.79%) and five oxygenated sesquiterpenes (2.3%). 1,8-Cineole (45.86%), and myrtenol (7.40%) were the major components of this oil, followed by ß-pinene (6.52%), terpinene-4-ol (4.59%) and pinocarvone (3.14%). The stem oil was characterized by a large amount of nine monoterpene hydrocarbons (20.83%), thirty-three oxygenated monoterpenes (70.92%) and seven oxygenated sesquiterpenes (5.03%). This oil was characterized by the presence of 1,8-cineole (35.87%), ß-pinene (7.77%), trans-pinocarveol (5.69%), terpinen-4-ol (4.27%), pinocarvone (3.81%), α-pinene (3.69%), γ-

terpinene (3.36%) and caryophylleneoxid (3.24%). The root oil contained teen monoterpene hydrocarbons (17.26 %) thirty-two oxygenated monoterpenes (77.81%) and two oxygenated sesquiterpenes (1.56%). 1,8-Cineole (38.02%) was the major component of this oil, followed by terpinen-4-ol (6.77%), myrtenol (6.41%), trans-pinocarveol (6.22%), ß-pinene (5.35%) ,α-pinene (3.31%) and γ-terpinene (3.17%).

Antimicrobial activity

The antimicrobial activities of leaf, flower, stem and root essential oils of *N. gloeocephala* were tested against seven gram-positive and gram-negative bacteria and two fungi. The results of the bioassay in Table 2 showed that the oils exhibited moderate to strong antibacterial activity against all the tested bacteria and strong activity against the fungi. The oils from leaf and flower showed inhibitory activity against all the tested bacteria, especially *Bacillus cereus*, and two fungi tested. The stem oil of *N. gloeocephala* was active against all tested microorganisms, except two gram-negative strains, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Table 2 also shows that the root oil had no antibacterial activity against *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Enterococcus faecalis*. This present study confirms that there is a positive correlation between the chemical content of the oil and their antimicrobial activities.

Table1: Comparative percentage compositions of the essential oils of *Nepeta gloeocephala*

No.	Compounds[a]	RI[b]	% Leaf	% Flower	% Stem	%Root
1	α- Thujene	927	0.33	0.46	0.73	0.55
2	α- Pinene	935	2.59	2.65	3.69	3.31
3	Camphene	949	0.41	0.55	0.77	0.49
4	Verbenen	954	0.18	0.2	0.3	0.19
5	Sabinene	974	0.39	-	-	0.6
6	β-Pinene	979	5.62	6.52	7.77	5.35
7	Dehydro-1,8-cineole	991	0.38	0.34	0.45	0.27
8	*p*-Mentha-1(7),8-diene	1005	0.22	0.29	0.47	0.35
9	α-Terpinene	1018	1.41	1.33	2.24	1.99
10	1,8-Cineole	1039	**44.58**	**45.86**	**35.87**	**38.02**
11	γ-Terpinene	1061	2.19	2.12	3.36	3.17

12	*cis*-Sabinene hydrate	1069	0.66	1.43	1.54	1.47
13	*trans*-Linalool oxide	1074	0.16	0.2	-	0.1
14	Camphenilone	1084	0.16	0.27	0.18	0.15
15	Terpinolene	1089	1.12	1.17	1.5	1.26
16	Linalool	1097	0.57	-	-	-
17	*trans*-Sabinene hydrate	1100	-	1.04	1.2	0.96
18	α-Fenchocamphorone	1107	0.2	0.29	0.14	0.17
19	*trans*-Thujone	1118	-	0.21	0.21	0.1
20	Dehydro-sabina ketone	1121	0.27	0.22	-	-
21	*cis*-*p*-Menth-2-en-1-ol	1123	0.64	0.78	0.81	1.18
22	α-Campholenal	1128	0.7	0.94	1.41	1.03
23	Nopinone	1140	2.11	-	-	-
24	*trans*-Pinocarveol	1143	3.71	-	5.69	6.22
25	*trans*-Verbenol	1149	1.9	2.20	2.39	2.29
26	*p*-Menth-3-en-8-ol	1152	0.24	0.17	-	0.25
27	Sabina ketone	1161	0.84	0.84	-	0.75
28	Pinocarvone	1166	2.23	3.14	3.81	2.40
29	δ-Terpineol	1169	-	1.08	-	-
30	*p*-Mentha-1,5-diene-8-ol	1172	1.27	-	0.76	0.91
31	Terpinene-4-ol	1183	5.02	4.59	4.27	6.77
32	Lavandulol	1187	0.23	0.2	0.15	0.21
33	Crypton	1190	1.13	0.91	0.58	1.56
34	neo-Dihydro carveol	1194	-	-	2.23	-
35	α-Terpineol	1197	1.67	1.97	-	1.62
36	Myrtenol	1202	7.28	7.40	-	6.41
37	*trans*-Piperitol	1211	-	0.19	0.26	0.23
38	Verbenone	1213	1.25	0.8	0.4	0.9
39	*trans*-Carveol	1221	0.58	0.54	0.53	0.66
40	*cis*-Sabinene hydrate acetate	1229	-	-	0.17	-
41	Ascaridole	1233	0.19	0.31	0.27	0.18
42	Cumin aldehyde	1243	1.12	1.34	1.87	1.51

43	Carvon	1246	0.43	0.53	0.52	0.6
44	*cis*-Chrysanthenyl acetate	1254	0.46	0.58	0.88	0.43
45	Perilla aldehyde	1276	0.16	0.18	0.22	0.17
46	*cis*-Verbenyl acetate	1282	0.25	0.17	0.24	0.2
47	neoiso-3-Thujyl acetate	1288	0.23	0.31	0.46	0.3
48	Thymol	1290	-	-	1.17	-
49	*p*-Cymen-7-ol	1292	0.58	0.53	-	0.58
50	Terpinen-4-ol acetate	1301	0.97	1.16	1.89	1.2
51	Myrtenyl acetate	1330	0.53	0.32	0.1	0.4
52	*trans*-Carvyl acetate	1342	-	-	0.51	-
53	α-Terpinyl acetate	1350	0.16	0.13	0.21	0.13
54	*cis*-Carvyl acetate	1360	0.2	0.15	0.2	0.11
55	Nepetalactone	1394	-	-	0.12	-
56	*p*-Cymen-7-ol acetate	1419	-	-	0.11	-
57	α-Cedrene epoxide	1556	-	0.17	0.28	-
58	1-nor-Bourbonanone	1564	-	0.11	0.13	-
59	Spathulenol	1582	1.24	1.56	3.24	1.44
60	Caryophylleneoxid	1587	0.15	0.32	0.58	0.12
61	Globulol	1611	-	0.1	0.17	-
62	*allo*-Alloaromadendrene	1640	-	-	0.11	-
63	α-Cadinol	1654	-	-	0.5	-
64	Widdrol	1658	-	0.15	-	-
65	β-Bisabolol	1693	-	-	0.15	-
	Total peak area (%)		**98.91**	**99.02**	**97.81**	**99.26**

[a]Compounds listed in order of elution. [b]RI, Kovats indices as determined on a DB-5 column using the homologous series of n-alkanes (C_8-C_{22}).

Table 2: Antimicrobial activity of the essential oils of *Nepeta gloeocephala*

Microorganism	Essential oils				Standards[b]		
	Leaf	Flower	Stem	Root	Tet	Amp	Nys
	DD[a]	DD	DD	DD	DD	DD	DD
Bacillus cereus	26.5	21.5	19	16.5	21	15	nt
Staphylococcus aureus	20	17	13	9.5	20	13	nt
Enterococcus faecalis	10	9.5	7	-	9	11	nt
Staphylococcus epidermidis	14	11	10.5	10	34	19	nt
Pseudomonas aeruginosa	9.5	7.5	-	-	-	10	nt
Escherichia coli	16	12.5	9.5	11	16	12	nt
Klebsiella pneumoniae	10	7	-	-	-	-	nt
Aspergillus niger	24	21	22	21	nt	nt	16
Candida albicans	23	20	20	23	nt	nt	18

[a]Diameter of inhibition zones (mm) including diameter of the sterile disk (6 mm) [b]Tet, Tetracycline (30 μg/disc); Amp, Ampicillin (10 μg/disc); Nys, Nystatine (30 μg/disc); (-) Inactive; (7–13) moderately active; (>14) highly active; nt: not tested.

DISCUSSION

According to our results, nepetalactone isomers were not detected in *N. gloeocephala* oil as they were in *N. makuensis* [22], *N. fissa* [23], *N. macrosiphon* [24], *N. denudate* [16], *N. glomerulosa* [25], and *N. isaphanica* [15]. The main components found in *Nepeta* oils of Iranian origin were 1,8-cineole [26-28], α-pinene [25], viridiflorol [29], spathulenol [30], ß-caryophyllene [23], linalool acetate [31] and α- terpinen-4-ol [32]. The comparison of the results with the literature showed significant differences for oils,

which can be attributed to either climatological factors or genetic differences of the plants.

CONCLUSION

In conclusion, findings of the present study indicated that the essential oil obtained from the different parts of *N. gloeocephala* is rich in oxygenated monoterpenes, mainly 1,8-cineole which constitutes (less 50%) of the total oil composition. Biological evaluations revealed that the oil possesses strong antibacterial and antifungal activities. Our results support the ethno-pharmacological uses of this plant in folk medicine and could provide useful data for the utilization of this essential oil in pharmaceutical, cosmetic and food industries.

ACKNOWLEDGMENT

The authors are grateful to Dr Mozaffarian, Institute of Forest and Rangelands for identifying the plant material.

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