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Chemical composition of the essential oil from *Calamintha nepeta* (L.) Savi plants growing in the flora of Azerbaijan

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Abstract: In this work, we investigated the chemical constituents of the essential oil extracted from the aerial parts of *Calamintha nepeta* (L.) Savi (Laminaceae Lindl.). The aerial parts of *C. nepeta* collected from in flowering time from the village Tengalti and the near of Velvelechay river of Quba region. The essential oil was obtained ant by hydrodistillation. The amounts of the essential oil extracted from the above-ground portion of were 0.8-1.2%. The color of oil was yellow with pleasant mint-like smell. Essential oil was analyzed for their chemical composition by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). In all, 78 compounds were identified, the major components were: Thymol (19.81%), Cyclopropane, 1,1-diethyl- (19.77%), Cyclohexanone, 3-vinyl-3-methyl- (18.66%), D-Limonene (7.45%), Caryophyllene (6.16%), the minor components were: beta.-Pinene (1.46%), 3-Octanol (1.85%), Terpinen-4-ol (1.43%), alpha.-Terpineol (1.6%), 1,4,7,-Cycloundecatriene, 1,5,9,9- tetramethyl-, Z,Z,Z- (3.10%), Germacrene D (1.18%), Caryophyllene oxide (2.41%), 12-Oxabicyclo [9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R*,3E,7E,11R*)] (1.32%). The components which the percentage of content less than one -alpha-Pinene (0.75%), Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis- (0.85%), beta.-Ocimene (0.74%), O-Cymen-5-OL (0.76%), 2-Dimethylamino-3-methylpyridine (0.77%), 3,4-Dimethoxytoluene (0.54%), 10,10-Dimethyl-2,6-Dimethylenebicyclo[7.2.0]undecan-5.beta.-ol (0.49%), Alloaromadendrene (0.35%), trans-Z-. alpha.-Bisabolene epoxide (0.38%) et al. This is the first report on the chemical compounds of the essential oil of this species growing in the territory of Azerbaijan.

Key Words: *Calamintha nepeta, Lamiaceae, essential oil, GC and GC-MS, compounds.*

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INTRODUCTION

Calamintha nepeta (L.) Savi (*Calamintha officinalis* (Moenc) [Czerepanov, 1995] is a plant species belonging to the family Lamiaceae Lindl. The genus *Calamintha* Mill. is distributed in Europe, Eastern Mediterranean region, Central Asia, North Africa and America. There are more than 7 species in the world flora, of which 4 species are found in the Caucasus and up 3 species in Azerbaidjan [Askerov, 2008, 2011; Flora Azerbaydjana, 1957].

There are a lot of literature information relating to research biological activities and the essential oil extracted from the *Calamintha* species [Kitic et al., 2005; Alan et al., 2010; Septi et al., 2011; Regina et al., 2012]. Aforementioned species contain bioactive materials like essential oils, flavonoids, vitamin C, saponins, triterpenoide,. tanins, and more [Rastitelnie resursi, 1991, 1996, 2011]. Because of their pleasant mint-like smell, many *Calamintha* species are used as spices in various culinary recipes. They are used in folk medicine like mints, mainly as stimulant, digestive, tonic, antiseptic etc. [Rastitelnie resursi, 1991; Chevallier, 2001]. These plants are used as antispasmodic, diaphoretic, diuretics, carminatives expectorant and for strengthening central nervous system [Grieve, 1982; Baytop, 1999; Bown, 2001; Nostro et al., 2002; Nostro et al., 2004; Burzo et al., 2006; Formisano et al., 2007; Alan et al, 2011; Mancini et al., 2013; Araniti, Grana et al., 2013; Colombo et al., 2013; Araniti, Grana et al., 2013]. Investigations showed that leaves and flowers of *Calamintha* species are effective as an antiseptic, antispasmodic and tonic [Small, 2006; Branković, 2009; Demirci et al., 2011; Ćavar, et al., 2013; Rastitelnie resursi, 1991, 1996, 2011], as well as antimicrobial and antispasmodic activities of their essential oils [Branković, 2009; Sarac, Ugur, 2009; Demirci et al., 2011]. The oils of some species exert significant sedating and antipyretic activities [De Ortiz Urbina et al., 1989; Rastitelnie resursi, 1991; Formisano, 2007]. *Calamintha* essential oils are also used for stomach, throat aches and kidney disorders [Rastitelnie resursi, 1991; Viney, 1994; Alan, et al., 2011; Ćavar, et al., 2013]. *Calamintha* species also have horticultural uses [Rastitelnie resursi, 1991; Baytop, 1999].

In order to investigate essential oils from local wild plants, one of the *Calamintha* species, *C. nepeta* (L.) Savi, was screened. Based on results, it was revealed

that *C. nepeta* is widely used in traditional medicine and as a spice in cousin.

The species contains such bioactive compounds as essential oil, flavonoids, vitamin C, saponins, triterpenoids, iridoides and etc. *C. nepeta* essential oil is used in cooking as an aromatic herb and also to improve the flavor and fragrance of several pharmaceutical products. Lately, the essential oil of *C. nepeta* used for its diaphoretic, expectorant, and flavouring properties has been reported for its preservative properties in culture medium and in cetomacrogol cream [Moattar et al., 2018; Morteza-Semani et al., 2007*;* Mancini et al., 2013; Marongiu et al., 2010; Eftekharinasab et al., 2012; Nostro et al., 2002; Nostro et al., 2004; Riela et al., 2008; Rastitelnie resursi, 1991, 1996, 2011]. The microbial activity of the Essential oil of *Calamintha nepeta* was screened against *Aspergillus niger, Escherichia coli, Staphylococcus aureus, Salmonella enteritidis, Bacillus subtilis* and *Pseudomonas aeruginosa*. It was found that all mentioned microorganisms were sensitive to the oil [Kitic et al., 2005; Rastitelnie resursi, 1991].

The aim of this study was to determine the quantity content of component of essential oil of *C. nepeta.*

MATERIAL AND METHODS

During our study, aerial parts of *Calamintha grandiflora* (L.) Moench were collected in 10th of July 2017 from the village Tengalti and the near of Velvelechay river of Quba region.

The essential oil of *C. nepeta* has been obtained by hydrodistillation [Qinzberg, 1932]. The composition of the essential oil obtained from the dried parts *C.nepeta* was analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS).

GC and GC-MS conditions. The oils were analyzed by capillary GC and GC/MS using a Agilent GC-MSD system (Agilent Technologies Inc., Santa Clara,CA).

Gas Chromatography (GC) analysis was conducted using a Shimadzu GC-17A with a capillary CP-SIL 8 CB column (15 m x 0.25-0.39 nm). Nitrogen was used as the carrier gas at the constant flow rate of 5 ml/min. The oven temperature was kept at 60^{o}C for 2 min, then programmed to 250^{o}C at a rate of 6^{o}C/min for 5 min, and then kept to 280^{o}C of 15^{o}C/min for 6 min. The injector and detector (FID) temperatures were kept at 280^{o}C and 300^{o}C, respectively. Gas Chromatography-Mass Spectroscopy (GC-MS) analysis was carried out using an Agilent 6890N Network CG system combined with an Agilent 5975 inert Mass Selective Detector. The GC conditions were: capillary column Agilent 19091S-433 HP-5MS 5% Phenyl Methyl Siloxane; nominal length 30.0 m, nominal diameter 250.00 um, nominal film thickness 0.25 um; oven temperature was kept at 70^{o}C for 2 min, then programmed to 280^{o}C at a rate of 5^{o}C/min, and then held for 6 min. Helium was used as a carrier gas; nominal unit pressure 7.64 psi; average velocity 36 cm/sec; initial flow 1.0 mL/min; split ratio 40:1; injected volume, 0.50 μL. Identification of the components was done by comparison of the mass spectral data using the Wiley and NIST 11.L electronic libraries. The percentages of the components were calculated from the GC peak areas, using the normalization method.

RESULTS AND DISCUSSION

C. nepeta refers to perennial plant which grows up 20-50 sm. Leaves on petioles, broadly ovate, pubescent on both sides. Inflorescence - the whorls 3-7-flowered. Corolla is pink. Fruit - nut spherical. Flowering time in July-November, fruiting – from August to November. It is xeromezophyte. Grows as groups in lighted forests and part of the riverine forest; found from the lowlands to the middle mountain belts.

The amounts of the essential oil extracted from the above-ground portion of *C. nepeta* were 0.8-1.2%. The color of oil was yellow of with pleasant mint-like smell. The composition of the essential oil obtained from the dried parts of *C. nepeta* was analyzed by GC and GC-MS. In all, 78 compounds were identified. Constituents of the essential oils of *C. nepeta* are shown in Table. As can be seen from the table, Thymol (19.81%), Cyclopropane, 1,1-diethyl- (19.77%), Cyclohexanone, 3-vinyl-3-methyl- (18.66%), D-Limonene (7.45%), Caryophyllene (6.16%) are the major components and 1,4,7,-Cycloundecatriene, 1,5,9,9- tetramethyl-, Z,Z,Z- (3.10%), Caryophyllene oxide (2.41%), 3-Octanol (1.85%), alpha.-Terpineol (1.60%), beta.-Pinene (1.46%), Terpinen-4-ol (1.43%) are minor components of the essential oil of Calamintha nepeta. It is followed by the components which the percentage of content < 1: alpha.-Pinene (0.75%), Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis- (0.85%), beta.-Ocimene (0.74%), O-Cymen-5-OL (0.76%), 2-Dimethylamino-3-methylpyridine (0.77%), 3,4-Dimethoxytoluene (0.54%), 10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol (0.49%), Alloaromadendrene (0.35%), trans-Z-.alpha.-Bisabolene epoxide (0.38%) etc.

Table. The composition of the essential oil of *Calamintha nepeta.*

Peak No.	Retention time	Compounds	Area (%)
1	2	3	4
1	6.404	alpha.-Pinene	0.75
2	7.040	beta.-Pinene	1.46
3	7.269	3-Octanol	1.85
4	7.534	(+)-4-Carene	0.20
5	7.710	D-Limonene	7.45
6	8.057	gamma.-Terpinene	0.461
7	8.234	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis-	0.85
8	8.381	2-Carene	0.27
9	8.528	1,6-Octadien-3-ol, 3,7-dimethyl-	0.41
10	8.598	beta.-Ocimene	0.74
11	8.840	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	0.24
12	8.957	1,4-Benzenediol, 2,5-dimethyl-	0.35
13	9.216	l-Menthone	0.15
14	9.310	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, trans-	0.13
15	9.492	Terpinen-4-ol	1.43
16	9.645	alpha.-Terpineol	1.60
17	9.869	L-.alpha.-Terpineol	0.89
18	10.522	Cyclopropane, 1,1-diethyl-	19.77
19	10.857	Thymol	19.81
20	11.075	O-Cymen-5-OL	0.76
21	11.169	2-Methoxy-4-vinylphenol	0.22
22	11.239	Phenol, m-tert-butyl-	0.14
23	11.628	Cyclohexanone, 3-vinyl-3-methyl-	18.66
24	11.739	(+)-3-Carene, 2-(acetylmethyl)-	0.54
25	11.798	2-Dimethylamino-3-methylpyridine	0.77
26	11.986	Caryophyllene	6.16
27	12.133	1,6-Cyclodecadiene, 1-methyl-5-met hylene-8-(1-methylethyl)-, [S-(E,E)]	0.15
28	12.257	1,4,7,-Cycloundecatriene, 1,5,9,9- tetramethyl-, Z,Z,Z-	3.10
29	12.439	Germacrene D	1.18
30	12.533	gamma.-Muurolene	0.06
31	12.598	beta.-copaene	0.10
32	12.651	Benzene, 1-methyl-3-[(2-methylpropyl)thio]-	0.22
33	12.739	2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)-	0.10
34	12.810	Benzene, 1,2,3-trimethoxy-5-(2-propenyl)-	0.06
35	12.939	3,4-Dimethoxytoluene	0.54
36	13.016	Caparratriene	0.12
37	13.122	6-Cyano-5-methoxyquinoline	0.21
38	13.210	1-Methyl-6-(3-methylbuta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptane	0.21
39	13.316	Caryophyllene oxide	2.41
40	13.428	Humulene	0.17
41	13.539	12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R* ,3E,7E,11R*)]-	1.32
42	13.751	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol	0.49
43	13.898	Alloaromadendrene	0.35
44	14.039	trans-Z-.alpha.-Bisabolene epoxide	0.38
45	14.263	Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-	0.16
46	14.439	Cyclohexane, 1,5-diethenyl-3-methy l-2-methylene-, (1.alpha.,3.alpha. ,5.alpha.)-	0.10

1	2	3	4
47	14.774	Cedren-13-ol, 8-	0.04
48	15.269	6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl-,	0.06
49	15.669	Isoaromadendrene epoxide	0.03
50	15.839	2-Pentadecanone, 6,10,14-trimethyl	0.06
51	15.157	Phthalic acid, isobutyl octyleste	0.08
52	16.492	9-Isopropyl-1-methyl-2-methylene-5-oxatricyclo[5.4.0.0(3,8)]undecane	0.11
53	16.921	trans-Geranylgeraniol	0.03
54	17.092	Hexadecanoic acid, methyl ester	0.05
55	17.604	Dibutyl phthalate	0.05
56	18.015	Aromadendrene oxide-(2)	0.02
57	19.862	Heneicosane	0.03
58	20.004	Phytol	0.19
59	20.392	Furazano[3,4-b]pyrazine-5,6-diamine, N-isopropyl-N'-(3,4-dimethylphenyl)-	0.06
60	21.192	Imidazole, 4-amino-5-ethoxycarbonyl-	0.06
61	21.474	Biphenyl, 4,4'-bis(trimethylsilyl)	0.09
62	21.674	Imidazole, 4-amino-5-ethoxycarbonyl-	0.06
63	22.050	2(1H)-Pyridinethione, 1-ethyl-6-methyl-	0.03
64	22.797	3,5-Dihydroxybenzamide	0.04
65	22.909	Heptadecane	0.03
66	23.456	Cyclodisilazane, 2,2,4,4-tetramethyl-1,3-diphenyl-	0.10
67	23.539	2H-3,7-Methanoazacycloundecino[5, 4-b]indole, 7-ethyl-1,4,5,6,7,8,9,10-octahydro-11-methoxy-, (-)-	0.03
68	24.003	1-Methyl-4-ethylaminocytosine	0.03
69	24.339	Tetracosane	0.03
70	25.468	4,4-Bis(1,1-dimethylethyl)-3'-methoxy-1.1'-biphenyl-2-ol	0.03
71	25.586	Benzene, 2-methoxy-1,3,4-trimethyl	0.06
72	25.715	3-(3-Hydroxy-4-methyl-phenyl)-3,4,4-trimethyl-cyclopentanone	0.07
73	26.091	Phthalic acid, di(2-propylpentyl) ester	0.06
74	27.027	Heneicosane	0.03
75	28.291	Eicosane	0.04
76	29.444	Decanedioic acid, bis(2-ethylhexyl) ester	0.02
77	30.044	2-[[3-(3,5-dimethyl-pyrazol-1-yl)- [1,2,4]triazol-4-ylimino]-methyl]-phenol	0.03
78	30.673	Eicosane	0.03

CONCLUSIONS

In the course of this study, it was revealed that during the flowering phase in the aerial part of *C. nepeta* (L.) Savi, collected from the village Tengalti and the near of Velvelechay river of Quba region the content of essential oil varies from 0.8% to 1.2%.

According to our results, essential oil components of *Calamintha* were conditionally divided into three groups (A, B, C). A groupincludes the leading components Thymol (19.81%), Cyclopropane, 1,1-diethyl- (19.77%), Cyclohexanone, 3-vinyl-3-methyl- (18.66%), D-Limonene (7,45%), Caryophyllene (6.16%). B components which percentage of >1: beta.-Pinene (1.46%), 3-Octanol (1.85%), Terpinen-4-ol (1.43%), alpha.-Terpineol (1.6%), 1,4,7,-Cycloundecatriene, 1,5,9,9- tetramethyl-, Z,Z,Z- (3.10%), Germacrene D (1.18%), Caryophyllene oxide (2.41%), 12-Oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-, [1R-(1R* ,3E,7E,11R*)]- (1.32%) and C - < 1: alpha.-Pinene (0.75%), Cyclohexanol, 1-methyl-4-(1-methylethenyl)-, cis- (0.85%), beta.-

Ocimene (0.74%), O-Cymen-5-OL (0.76%), 2-Dimethylamino-3-methylpyridine (0.77%), 3,4-Dimethoxytoluene (0.54%), 10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5.beta.-ol (0.49%), Alloaromadendrene (0.35%), trans-Z-.alpha.-Bisabolene epoxide (0.38%) et al.

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Azərbaycan florasında yayılan *Calamintha nepeta* (L.) Savi bitkisindən alınan efir yağının kimyəvi tərkibi

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Təqdim olunan məqalədə *Calamintha nepeta* (L.) Savi (fəs. Laminaceae Lindl.) növünün yerüstü hissəsindən alınmış efir yağının kimyəvi tərkibi tədqiq edilmişdur. Bitki çiçəkləmə fazasında Quba rayonunun Təngaltı kəndinin ətrafında Vəlvələçay çayının yaxınlığında yığılmışdır. Efir yağı hidrodistillyasiya metodu ilə alınmışdır. Efir yağının çıxımı 0.8-1.2% aralığında dəyişir. Alınmış efir yağı şəffaf sarı rənglı xoş nanə ətirli mayedir. Efir yağının kimyəvi tərkibi qaz xromatoqrafı (QX) və xromato-mass-spektrometriya metodları (XMS) ilə tədqiq olunmuş və 78 komponent müəyyən edilmişdur. Calamintha nepeta növünün efir yağının major komponentləri timol (19.81%), siklopropan, 1,1-dietil - (19.77%), sikloheksanon, 3-vinil-3-metil -

(18.66%), D-limonen (7.45%), kariofillen (6.16%) və minor komponentləri beta-pinen (1.46%), 3-oktanol (1.85%), terpinen-4-ol (1.43%), alfa.-terpineol (1.6%), 1,4,7,-siklondekatrien, 1,5,9,9-tetrametil-,Z,Z,Z- (3.10%), hermakren D (1.18%), kariofillen oksid (2.41%), 12-oksabisiklo [9,1,0]dodeka-3,7-dien, 1,5,5,8-tetrametil-[1R- (1R*,3E,7E,11R*)] - (1.32%) komponentləridir. Efir yağının tərkibində, həmçinin faizi vahiddən az olan komponentlər də müəyyən edilmişdir (<1): alfa-pinen (0.75%), sikloheksanol, 1-metil-4-(metiletenil)-sis-(0.85%), beta-oksimen (0.74%), O-simen-5-ol (0.76%), 2-dimetilamino-3-3-metilpiridin (0,77%), 3,4-dimetoksitoluol (0.54%), 10,10-dimetil -2,6-dimetilenbisiklo [7.2.0] undekan-5β-ol (0.49%), alloapomadendren (0.35%), epoksid-Z-alfa-bisabolen (0.38%) və s. Azərbaycanda yayılmış *Calamintha nepeta* növünün efir yağının komponent tərkibi haqqında ilk dəfə olaraq məlumat verilir.

Açar sözlər: *Lamiaceae, Calamintha nepeta, efir yağı, QX və QX-MS, komponentlər*

Состав эфирного масла *Calamintha nepeta* (L.) Savi из природной флоры Азербайджана

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В представленной работе мы исследовали химический состав эфирного масла, полученного из надземных частей *Calamintha nepeta* (L.) Savi (Laminaceae Lindl.). Растения были собранные в период цветения в окрестностях сел. Тенгальты вблизи реки Вельвелечай Губинского района. Эфирное масло было получено гидродистилляцией. Количество масла, колеблется в пределах 0,8-1,2%. Полученное эфирное масло представляет собой прозрачную жидкость желтого цвета с приятным мятным ароматом. Химический состав масла анализировали с помощью газовой хроматографии (ГХ) и газовой хромато-масс-спектрометрии (ГХ-МС). Было выявлено 78 соединений, из них основными компонентами эфирного масла *Calamintha nepeta* являлись-тимол (19.81%), циклопропан, 1,1-диэтил- (19.77%), циклогексанон, 3-винил-3-метил- (18.66%), D-лимонен (7.45%), кариофиллен (6.16%), и минорные компоненты: бета-пинен (1.46%), 3-октанол (1.85%), терпинен-4-ол (1.43) %), альфа.-терпинеол (1.6%), 1,4,7, -циклондекатриен, 1,5,9,9-тетраметил-, Z, Z, Z- (3.10%), гермакрен D (1.18%), оксида кариофиллена (2.41%), 12-оксабицикло [9,1,0]додека-3,7-диена, 1,5,5,8-тетраметил-,[1R-(1R*,3E,7E,11R*)] - (1.32%). Далее следуют компоненты, процентное содержание которых было меньше единицы (<1), это - альфа-пинен (0.75%), циклогексанол, 1-метил-4- (1-метилэтенил)-, цис- (0.85%), бета-оксимен (0.74%), O-Cymen-5-OL (0.76%), 2-диметиламино-3-метилпиридин (0.77%), 3,4-диметокситолуол (0.54%), 10,10-диметил -2,6-диметиленбицикло [7.2.0] ундекан-5β-ол (0.49%), аллоаромадендрен (0.35%), эпоксид транс-Z-альфа-бисаболена (0.38%) и другие. В данной работе впервые приводится компонентный состав эфирного масла растений *Calamintha nepeta*, произрастающих в Азербайджане.

Ключевые слова: *Lamiaceae, Calamintha nepeta, эфирное масло, ГХ и ГХ-МС, компоненты*