

PHYTOCHEMICAL AND BIOLOGICAL INVESTIGATION OF THE VOLATILE CONSTITUENTS OF *NEPETA SEPTEMCRENATA* EHRENB., GROWING IN EGYPT

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في هذا البحث ولأول مرة تمكن الباحثون من فصل المواد الطيارة من نبات مصيص النامي في سانت كاترين - جمهورية مصر العربية ، وتم ذلك بالتقطير المائي لأجزاء النبات الظاهرية الطازجة ووجد أن لون الزيت الناتج أصفر ضعيف وكانت نسبته % من وزن أجزاء النبات. كما تم التعرف على مكونات الزيت الناتج باستخدام كروماتوجرافيا الغاز المدمج بمطياف الكتلة ووجد أنها تحتوي على مركب، أمكن التعرف عليهم جميعا وكانت تمثل نسبة % من اجمالي محتوى المواد الطيارة.

The volatile constituents of *Nepeta septemcrenata* Ehrenb. herb growing in Egypt were prepared by hydrodistillation of the fresh herb and analysed by GC/MS. Sixteen major compounds (represent 94.96% of total) were detected and identified. They contain ten oxygenated compounds (70.67%) and six hydrocarbons (24.29%). The major oxygenated compounds were 1,2 benzene-dicarboxylic acid dibutyl ester (33.16%) and 4-methyl-2,6-diterbutyl phenol (23.30% w/w), while the major hydrocarbon is 4-nonyne C_9H_{16} , (9.00%). The volatile constituents had promising antimicrobial activities against some tested micro-organisms.

INTRODUCTION

Genus *Nepeta*, family Lamiaceae, includes 250 species of perennial plants with small crenate leaves and blue corolla with narrow long exerted tubes.¹ They are of medicinal and aromatic plants widely grown in Mediterranean countries.² *Nepeta septemcrenata* Ehrenb (Figure 1) is an erect slender plant with branches at base. Leaves are oppositely alternated, ovate with crenate or slightly dentate margins.¹ This plant was found in Sant Cathrine (Sinai, Egypt).¹ It was reported that *Nepeta* plants were prepared as tea and used in traditional medicine as anthelmintics, febrifuges, expectorants, to treat bronchitis, bites, stings of and scorpions.³ They have antispasmodic, astringent properties,³ cardiotoxic (*N. ciliaris*), diaphoretic, skin diseases (*N. hindostana*) and anticatarrhal (*N. hederacea*).⁴ Several *Nepeta* species are characterized by the presence of essential oil.⁵

¹¹. An isopimarane type diterpene and 7-O-methylapigenin were isolated from the ethanol extracts of *Nepeta septemcrenata* Ehrenb herb.¹² Nothing was found in literature, so far, concerning the chemical composition of the volatile constituents of *Nepeta septemcrenata* Ehrenb herb.

EXPERIMENTAL

Plant material

Nepeta septemcrenata Ehrenb (aerial parts) were collected on August, 2002 from wadi gebal in Sant cathrin (Sinai) and kindly identified by the late Prof. Dr. Nabil Al-Hadidi, Prof. of Plant Taxonomy, Department of Botany, Faculty of Science, Cairo University. A voucher herbarium specimen had been deposited in the Museum of Medicinal Plants, Department of Pharmacognosy, Faculty of Pharmacy Al-Azhar University, Cairo (Egypt).



Fig. 1: *Nepeta septemcrenata* Ehrenb. Herb.

Preparation of the volatile constituents

The volatile constituents were prepared from the fresh aerial parts of *Nepeta septemcrenata* Ehrenb. by hydrodistillation according to E.P. (1984).¹³ The volatile constituents were extracted with diethylether and dried over anhydrous sodium sulfate. The yielded oil of *Nepeta septemcrenata* Ehrenb was 0.14% w/w, yellowish green in colour and has an aromatic odour.

Identification of the volatile constituents

The prepared volatile constituents were analysed by GC using the apparatus Shimadzu GC/MS - QP 5050 A. Software Class 5000. Column: DBI, 30 m x 0.53 mm i.d, 1.5 μ m film thickness. Carrier gas: Helium (flow rate 1ml / min). Ionization mode: E.I. (70 eV). Temperature program: 30° (static for 2 min) then gradually increasing (at a rate of 2° / min) up to 250° (static for 5 min). detector (FID) temperature 280°. Injector temperature 280°. Qualitative identification of the volatile constituents was achieved by library searched data base Willey 229LIB. and by comparing their mass fragmentation patterns with those of the available published data.¹⁴⁻¹⁵

Quantitative estimation of the the volatile constituents was determined by computerized peak area measurements using internal normalization method..

Antimicrobial activities

The disc agar diffusion technique¹⁶ was used for testing antimicrobial activity of the volatile constituents against certain Gram +ve, Gram -ve bacteria and fungi. Pure strains of *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, as well as *Aspergillus niger*, *Penicillium marneffeii*, *Syncephalastrum racemosum* and *Candida albicans* were kindly supplied from Staff members of the Microbiology Department, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt. Four mm Discs (4 mm each) of filter paper (Whatmann No. 3) were impregnated in volatile oil solution (0.1 g% in diethyl ether) and placed on the surface of nutrient agar, and sabaroud's dextrose agar. seeded with tested micro-organisms. Discs impregnated in diethyl ether was used as a negative control in each plate. The plates were incubated at 37° for 24 hours and at 25° for 72 hours to investigate the antibacterial and antifungal activities, respectively. Chloramphenicol (5 μ g/discs) and Terbinafin (4.5 μ g/disc) were used as positive standards

RESULTS AND DISCUSSION

Phytochemical screening of *Nepeta septemcrenata* Ehrenb herb revealed the presence of phenolic and terpenoid compounds.⁽¹⁷⁾ The volatile constituents of *Nepeta septemcrenata* Ehrenb herb (50 g) was prepared by hydrodistillation using E.P 1984 apparatus. The obtained oil is yellowish green in colour, heavier than water and with characteristic odour. It yielded 0.14% w/w of volatile oil, soluble in pet.-ether, ether, chloroform, ethyl acetate and alcohol. The components of this oil were detected by silica gel TLC developed in benzene and showed several spots, some of them acquired blue colour with FeCl₃ indicating their phenolic nature⁽¹⁷⁾. Further investigation by TLC was impossible due to lack of authentic samples. GC/MS of the volatile components revealed the

presence of 16 peaks (represent 94.96% of total, Table 1) were identified. They could be classified to oxygenated (10 compounds) and non oxygenated (6 compounds) representing 70.67 and 24.29%, respectively. The most abundant one of the oxygenated derivatives is 1,2-benzenedicarboxylic acid dibutyl ester (R_t 83.02 min⁻¹) and 4-methyl-2,6-ditertbutylphenol (R_t 57.76), representing 33.16 and 23.30% w/w, respectively

Identification of the the volatile constituents were done on the bases of matching data with Wiley 229 LIB. library search system and comparison of mass spectra fragmentation patterns with those reported in the available literature.^{14,15,18-21} The

antimicrobial screening of the volatile constituents of the aerial parts of *Nepeta septemcrenata* Ehrenb. growing in Egypt (Table 2) revealed that they have a significant antimicrobial effect against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, but they are not active against *E. coli*. Compounds responsible for antimicrobial activities of *Nepeta septemcrenata* in the volatile constituents will be studied in detail in future, on availability of plant samples. These volatile constituents could be used for treatment of skin infections caused by *Staphylococcus aureus* or *Pseudomonas aeruginosa*.

Table 1: GC/MS data of the volatile constituents of *Nepeta Septemcrenata* Ehrenb.

R_t	%	M^+	B.p.	Other fragments*	Name of Compound
A. Oxygenated Volatile constituents					
28.17	0.53	170	59	43,55,41,67,94 and 95	$C_{10}H_{18}O_2$, Linalol oxide
36.03	T	172	60	59,43,73,71 and 94	$C_{10}H_{22}O_2$, Decanoic acid
47.11	5.45	152	53	82,54,110,81,41,67,109 and 124	$C_{10}H_{16}O$, Thujone
51.79	3.25	154	41	67,81,113 and 153	$C_{10}H_{18}O$, 2-Decyn-1-ol
57.76	23.3	220	205	41,57,220,91,77,105,115,145,155 and 177	$C_{15}H_{24}O$, 4-Me-2,6-Ditertbutylphenol
65.65	1.34	222	41	151,93,95,81,107 and 111	$C_{15}H_{26}O$, Widdrol
83.02	33.16	278	149	41,76,104,205 and 223	$C_{16}H_{22}O_4$, 1,2-benzene-Dicarboxylic acid dibutyl ester
86.76	3.64	256	41	56,60,73,83 and 129	$C_{16}H_{32}O_2$, Hexadecanoic acid
95.38	T	282	41	55,69,98,83,111,147 and 127	$C_{18}H_{34}O_2$, Oleic acid
110	T	390	149	167,57,43,71,279	$C_{24}H_{38}O_4$, 1,2-benzene-Dicarboxylic acid, bis(2-Ethylhexyl) ester
Total	70.67				
B. Volatile Hydrocarbon Constituents					
36.66	0.27	138	41	81,65,95 and 123	$C_{10}H_{18}$, 3,5-Octadiene-2,7-dimethyl
41.13	5.14	110	67	8,1,41 and 95	C_8H_{14} , 3-Octyne
43.22	9.00	124	67	81,41 and 95	C_9H_{16} , 4-Nonyne
99.6	0.83	226	43	57 and 71	$C_{16}H_{34}$, Hexadecane
103.9	2.36	254	43	57,71 and 85	$C_{18}H_{38}$, 2-methyl-Pentadecane
108.2	6.69	269	43	57,71 and 85	$C_{21}H_{44}$, n-Heneicosane
Total	24.29				

* MS fragments were arranged in a decreasing order of their intensities

Table 2: Antimicrobial activity of the volatile constituents of *Nepeta septemcrenata* Ehrenb.

Micro-organsims	DEE	1	A	B
<i>Staphylococcus aureus.</i>	00	+	++	00
<i>Bacillus subtilis</i>	00	+	++	00
<i>E.coli.</i>	00	00	++	00
<i>Pseudomonas aeruginosa</i>	00	+	++	00
<i>Candida albicans</i>	00	00	00	++
<i>Penicillium marneffeii</i>	00	00	00	++
<i>Syncephalastrum racemosum</i>	00	00	00	++
<i>Aspergillusniger</i>	00	00	00	++

1, volatile constituents; A, Antibacterial (+ve control, chloramphenicol); B, Antifungal (Terbinafin, +ve control); DEE, diethylether(-ve control); +, Inhibition values of 1: 5 mm beyond control; ++, Inhibition values of 6:10 mm beyond control and 00 = inhibition zones were not detected.

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