SHORT REPORT

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Chemical Composition, Antimicrobial and Antioxidant Activities of the Essential Oil of *Nepeta deflersiana* Growing in Yemen

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Abstract: In the course of our phytochemical studies of essential oils, the oil obtained from the aerial part of *Nepeta deflersiana* (Lamiaceae) was analyzed by GC and GC/MS. In parallel to that, evaluation of the antimicrobial and antioxidant activities was also carried out. The investigation led to the identification of 51 components. The oil was rich in oxygenated monoterpenes (31.4%). The oil contained as well a high content of oxygenated sesquiterpenes (28.2%). Hexadecanoic acid (8.0%), caryophyllene oxide (6.4%), 2-methoxy-*p*-cresol (5.6%), camphor (4.7%) and eugenol (4.7%) were the most abundant constituents. In antimicrobial investigation, the essential oil has shown strong activity for Gram-positive bacteria with MIC-value of 0.4 mg/mL. Moreover, the DPPH-radical scavenging assay exhibited a moderate antioxidant activity (38%) at 1.0 mg/mL.

Keywords: Essential oil, *Nepeta deflersiana*, antimicrobial, antioxidant, GC, GC-MS

1. Plant Source

The genus *Nepeta* (Lamiaceae) is composed of only two species growing in Yemen [1]. *Nepeta deflersiana* Schweinf. ex Hedge is an erect and aromatic perennial herb [1]. Traditionally, it is used in the Yemeni folk medicine as antiseptic agent in the treatment of wounds, carminative as well as in the treatment of rheumatic disorders [2,3]. The plant was collected from the vicinity of Sana'a-Yemen in May 2007 and identified at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University. Voucher specimen (Mo-S12) was deposited at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University.

2. Previous Studies

In the course of our studies [4-6] on natural essential oils of plants growing in Yemen and their possible antimicrobial and antioxidant activities, the aim of this work was to investigate the chemical composition of the essential oil of the traditionally used *Nepeta deflersiana*. Reviewing the available current literature, nothing was found concerning the qualitative and quantitative analysis of the essential oil of *N. deflersiana*. In addition, a survey of the literature revealed that no studies on the

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potential antimicrobial and antioxidant activities of this oil had yet been undertaken; hence the antimicrobial and the radical scavenging activities of the essential oil were also investigated.

3. Present Study

The air-dried and powdered aerial parts of the plant were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus according to the European Pharmacopoeia. The oil was dried over anhydrous sodium sulfate and after filtration, stored at +4 °C until use. Oil yield was measured on the basis of volume of dried essential oil/primary dried plant material weight × 100 for each sample.

The analysis of the essential oils was performed using a Hewlett Packard GC (5890 Series II) equipped with a Flame Ionization Detector (FID) and a fused silica capillary CP-Sil 5 CB column (Varian) (30 m × 0.25 mm i.d., film thickness 0.26 μm). Nitrogen was used as a carrier gas (0.7 mL/min). Oven temperature was kept at 45 °C then gradually raised to 280 °C at 3 °C/min and finally held isothermally for 22 min. The injection volume was 1.0 μL (split ratio 1:16). Calculation of peak area percentage was performed on basis of the FID signal using the GC HP-Chemstation software (Agilent Technologies). The GC–MS analyses were performed using a Hewlett-Packard 5890 series II gas chromatograph coupled to a VG Analytical 70-250S mass spectrometer equipped with a fused silica capillary CP-Sil 5 CB column. Helium was used as carrier gas at flow rate of 1 mL/min. Injector temperature was 200° C. Oven temperature was programmed from 80 °C (2 min hold) at 10° C/min to 270° C and finally held isothermally for 20 min. The identification of the constituents was achieved by the comparison of their retention indices and mass spectra with data generated under identical experimental conditions. MassLib (V9.3-106) for processing and interpretation of mass spectra (MassLib, 1996-2008) was used with several commercially available libraries included Wiley Registry of Mass Spectral Data (4th ed.), NIST/EPA/NIH Mass spectral Library (2005), Library MPI Mühlheim (2006), Geochemicals (1st ed.) , MRC collection (1st ed.), and CC (4th ed.) – all from Chemical Concepts (Wiley). As additional library the electronic MS data base of Adams (2001) was used [7]. Moreover, the comparison was achieved with authentic reference compounds available in our laboratories. In order to determination of minimum inhibitory concentrations (MICs), a microbroth dilution method described by Mann and Markham (1998) [8] with modifications was applied. For the evaluation of the antioxidant activity, the DPPH free radical scavenging method was carried out [9].

The essential oil of *N. deflersiana* was colorless and possessed an aromatic odor. The yield was 0.35% (w/w). The chemical composition of the investigated oil, retention indices, percentages and identification methods are given in Table 1, where the identified components are listed in order of their elution on the CP-Sil 5 CB column. The GC-MS investigation led to the identification of 51 constituents representing 90.1% of the total oil. The most abundant compounds were hexadecanoic acid (8%), caryophyllene oxide (6.4%), 2-methoxy-*p*-cresol (5.6%), camphor (4.7%) and eugenol (4.7%). On the whole, the oil was constituted manily of oxygenated monoterpenes (31.4%) and oxygenated sesquiterpenes (28.2%) (Table 1).

To our knowledge this work represents the first GC-MS analysis of *N. deflersiana*. In previous studies [10-16], the chemical composition of the essential oils of different *Nepeta* species e.g. *N. menthoides*, *N. cataria*, *N. racemosa*, *N. crispa*, *N. kotschyi* , *N. nuda* and *N. meyeri*, was investigated. These studies revealed the predominance of nepetalactones e.g. 4aα,7α,7aβ-nepetalactone and 4aα,7α,7aα-Nepetalactone as major compounds. Moreover, the essential oil of *N. menthoides* at the prior flowering stage showed 1,8-cineol (57%) and β-pinene (8.8%) as the main constituents [10].

Conversely, the chemical composition of our investigated *N. deflersiana* differed quantitatively and qualitatively by completely absence of nepetalactones and 1,8-cineol and by higher content of hexadecanoic acid, caryophyllene oxide, 2-methoxy-*p*-cresol, camphor and eugenol. These results suggest that phenological stage of the plant as well as geographical environmental factors almost certainly contributed to create a spectacular chemical composition of *N. deflersiana*.

Table 1. Chemical composition of the essential oil of *N. deflersiana*.

No.	Compounds	RI	% Occurrence	Identification
1	(*E*)-2-Hexenal	824	0.4	1,2,3
2	β–Pinene	975	0.4	1,2,3
3	1-(1-cyclohexene-1-yl)-ethanone	1004	0.7	1,2
4	Limonene	1024	1.3	1,2,3
5	*trans*-Linalool oxide	1059	0.7	1,2
6	Fenchone	1070	1.3	1,2,3
7	Linalool	1085	2.6	1,2,3
8	α-Fenchol	1102	2.4	1,2,3
9	*exo*-Fenchol	1111	0.5	1,2
10	Camphor	1125	4.7	1,2,3
11	Borneol	1152	0.3	1,2,3
12	Terpinen-4-ol	1165	1.0	1,2,3
13	α-Terpineol	1175	1.0	1,2,3
14	Myrtenol	1183	0.3	1,2
15	2-Methoxy-*p*-cresol	1191	5.6	1,2
16	*trans*-Carveol	1201	1.6	1,2
17	*cis*-Carveol	1213	1.2	1,2
18	Carvone	1219	0.9	1,2
19	Geraniol	1236	0.4	1,2
20	Nonanoic acid	1257	0.5	1,2
21	Thymol	1272	0.9	1,2,3
22	Eugenol	1333	4.7	1,2,3
23	Decanoic acid	1353	0.5	1,2
24	*(E)*-β-Damascenone	1365	0.3	1,2
25	Methyleugenol	1373	1.0	1,2
26	β-Bourbonene	1388	0.8	1,2
27	(*E*)-β-Caryophyllene	1423	1.0	1,2,3
28	(*E*)- β-Farnesene	1445	0.7	1,2
29	α-Humulene	1456	0.3	1,2
30	β-Selinene	1488	0.6	1,2
31	α-Alaskene	1513	0.4	1,2
32	7-*epi*-α-Selinene	1520	0.9	1,2
33	α-Calacorene	1535	1.2	1,2
34	*n*-Dodecanoic acid	1551	1.9	1,2
35	Caryophyllene oxide	1580	6.4	1,2,3
36	α-Guiaol	1595	1.8	1,2
37	Humulene epoxide II	1604	2.8	1,2
38	γ-Eudesmol	1623	1.5	1,2
39	τ-Cadinol	1633	2.2	1,2
40	*epi*- α-Muurolol	1639	3.4	1,2
41	β-Eudesmol	1646	3.2	1,2
42	Eudesm-11-en-4 α-ol	1654	2.9	1,2
43	Bulnesol	1663	2.3	1,2
44	β-Bisabolol	1674	1.7	1,2
45	NI	1684	1.2	
46	*n*-Tetradecanoic acid	1745	1.4	1,2
47	NI	1816	2.2	
48	6,10,14-Trimethylpentadecane-2-one	1828	3.3	1,2
49	Hexadecanoic acid	1948	8.0	1,2
50	Cemberene A	1969	0.3	1,2
51	Manool	2060	2.1	1,2
52	Phytol	2102	3.5	1,2
53	*n*-Pentacosane	2496	0.3	1,2
	Monoterpene hydrocarbons		1.7	
	Oxygenated monoterpenes		31.4	
	Sesquiterpene hydrocarbons		5.9	
	Oxygenated sesquiterpenes		28.2	
	Diterpene hydrocarbons		0.3	
	Oxygenated diterpenes		5.6	
	Aliphatic acids		12.3	
	Other compounds		4.7	
	Total		90.1	

RI, retention indices relative to C8-C30 n-alkanes on the CP-Sil 5 CB column, 1: retention index, 2: mass spectrum, 3: spiking with authentic compound.

Table 2. Antimicrobial activity (MIC-values) and free radical scavenging activity of the investigated essential oils.

Plant species	Radical scavenging activity in %					MIC [a]				
	10 (µg/mL)	50 (µg/mL)	100 (µg/mL)	500 (µg/mL)	1000 (µg/mL)	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeuginosa*	*C. albicans*
N. deflersiana	5.7	6.0	9.6	22.8	37.9	0.40	0.40	3.25	3.25	-
Amoxicillin						3.5	3.5	nt	nt	nt
Gentamicin						nt	nt	3.5	7.0	nt
Nystatin						nt	nt	nt	nt	3.5
Ascorbic acid	48.2	89.5	95.8	96.1	96.0					

[a]: minimum inhibitory concentration values are given as mg/mL for essential oils and µg/mL for standard antibiotics, nt: not tested

The results of the antimicrobial activity are shown in Table 2. The Gram-positive strains showed more susceptibility to the tested essential oil than the Gram-negative ones. On the other hand, no activity was registered against *Candida albicans*. The essential oil of *N. deflersiana* demonstrated a great activity where the lowest MIC values (0.4 mg/mL) were obtained against *Staphylococcus aureus* and *Bacillus subtilis* (Table 2). Previous studies on other *Nepeta* species reported a strong antibacterial and antifungal activities [10,11,17,18]. It was suggested that the nepetalactones as well as 1,8-cineol as main components could be responsible for that activity. The results could partly concur with these findings. From the above observations, one can conclude that the antifungal activity is due to nepetalactones and 1,8-cineol which are lacking in this particular species (*N. deflersiana*). Oxygenated monoterpenes such as camphor, eugenol, borneol, linalool and α-terpineol, were reported to be responsible of the antimicrobial activity of several essential oils [19]. Consequently, the antimicrobial activity of the three investigated oils could be attributed to the high percentage of oxygenated monoterpenes. Moreover, the predominance of aliphatic acids such as hexadecanoic acid could probably contribute to the observed strong activity [20].

The potential antioxidant activity of the oils was determined on the basis of scavenging activity of the stable free radical DPPH. The oil was able to reduce DPPH and to demonstrate a moderate antioxidant activity (Table 2). This observed low antioxidant activity could be associated with low content of phenolic compounds such as thymol and carvacrol in the investigated oil [21].

In conclusion the GC/MS analysis of the essential oil of *N. deflersiana* revealed that the chemical compositions obtained differed from that of the other well-investigated *Nepeta* species. The results clearly showed that the oil of *N. deflersiana* present interesting antimicrobial but moderate antioxidant activity.

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