Research Article

ANTIVIRAL, ANTIBACTERIAL ACTIVITIES AND CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *NEPETA DEFLERSIANA* SCHWEINF GROWING IN KSA

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ABSTRACT

The spread of respiratory tract infections, especially with resistant organisms, makes it urgent to search for new safe antimicrobial agents. Makkah city in Saudi Arabia is always crowded due to pilgrims & Muslim visitors for Hajj and Omra. This facilitates the rapid spread of infectious respiratory diseases. The essential oil of *Nepeta deflersiana*, grown in Saudi Arabia was tested against the highly pathogenic avian influenza (H5N1) virus and eight respiratory tract pathogenic bacteria, six of them are multi-drug resistant bacteria. The *Nepeta deflersiana* oil showed high antibacterial and moderate antiviral activity. These results make *N. deflersiana* oil an important, promising and economic agent for eradication of the multiple antibiotics-resistant bacterial strains. The chemical composition of the essential oil of *Nepeta deflersiana*, was determined using GC/MS. A total of 26 compounds were identified that constitutes 95.28% of the total volatiles. The GC profile dominated by oxygenated constituents that represented 80.77% while the identified hydrocarbons were 14.51%. A series of the oxygenated monoterpene neptalactone isomers representing 72.64 % of the oil composition were detected. Linalool which is another oxygenated monoterpene represented 4.86%. Caryophyllene oxide was the major oxygenated sesquiterpene representing 3.93 % of the oil composition.

KEY WORDS: *Nepeta deflersiana*, antiviral activity, antibacterial, essential oil, GC/MS

INTRODUCTION

The rapid spread of infectious diseases in crowded areas constitutes a major problem facing great number of pilgrims and visitors in Saudi Arabia especially in high seasons of Omra and Hajj. Improving the air quality by spraying safe antimicrobial agents presents a considerable solution for this problem. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics along with the spread of drug-resistant pathogens, which has become one of the most serious threats to successful treatment of microbial diseases^{1,2}.

This research aimed to discover active antimicrobial natural essential oils from plants of the Saudi flora. This can be used in spray form for eradication of respiratory infectious diseases that commonly spread in crowded areas.

Nepeta is a multiregional genus (280 species) belonging to the family Labiate. It has spread all over the world but widely grown in many regions of North America, Asia, Africa and central and southern parts of Europe³.

Nepeta deflersiana Schweinf was chosen for this study. It is an aromatic perennial herb. Traditionally, it was used in folk medicine as carminative, antiseptic for wounds and for the treatment of rheumatic disorders^{4,5}.

Herein we report the antiviral and antibacterial activities of the essential oil together with its chemical composition.

MATERIALS AND METHODS

Plant material

Aerial parts of *Nepeta deflersiana* were collected in January 2015 from Taeif region and was identified by Prof. Dr. Kadry Abdel Khalik, Professor of Plant taxonomy at Umm Al-Qura University. A voucher specimen no. 1543 was kept in the herbarium of Umm-Al-Qura University (UQU proposed abbreviation)

Microbial strains

The antibacterial activity of the essential oil was evaluated using eight isolated respiratory pathogenic strains: *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Acinetobacter nosocomialis*, *Acinetobacter baumannii*, *Proteus mirabilis*, *Enterobacter cloacae*, *Enterobacter aerogenes*, and *Serratia marcescens*.

For evaluation of the antiviral activity, Reasserted avian influenza A virus (H5N1) previously isolated from Egypt in 2006 (rgA/chicken/Egypt/1/2006), was used.

Extraction

Nepeta deflersiana essential oil was isolated by hydro-distillation in a Clevenger type apparatus. One hundred grams of the plant material was boiled with water for three hours in 1000 ml round bottom flask surmounted by a 25 ml column connected to the condenser. The essential oil sample (0.2 ml) is then isolated and stored in opaque sealed bottles under low temperature (4 to 5°C).

Gas chromatography-mass spectrometry analysis (GC/MS)

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1 mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used, Helium gas was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at 280° C. The oven temperature was programmed at an initial temperature 50° C (hold 2 min) to 150 °C at an increasing rate of 7° C /min. then to 270° C at an increasing rate 5° C /min (hold 2 min) then to 310° C as a final temperature at an increasing rate of 3.5 °C /min (hold 10 min).

The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system (Table 1 and Figure 1).

Antiviral activity

MTT cytotoxicity assay (TC50)

Samples were diluted with Dulbecco's Modified Eagle's Medium (DMEM). A stock solution of the test oil was prepared in 10 % DMSO in double distilled H₂O. The cytotoxic activity of the oil was tested in Madin Darby Canine kidney (MDCK) cells by using the 3-(4, 5-dimethylthiazol -2-yl)-2, 5-diphenyltetrazolium bromide (MTT) method⁶ with minor modification. Briefly, the cells were seeded in 96 well-plates (100 µl/well at a density of 3×10⁵ cells/ml) and incubated for 24 hours at 37°C in 5% CO₂. After 24 hours, cells were treated with various concentrations of the tested oil in triplicates. After further 24 hours, the supernatant was discarded and cell monolayers were washed with sterile phosphate buffer saline (PBS) 3 times and MTT solution (20 µl of 5 mg/ml stock solution) was added to each well and incubated at 37°C for 4 hours followed by medium aspiration. In each well, the formed formazan crystals were dissolved in 200 µl of acidified isopropanol (0.04 M HCl in absolute isopropanol = 0.073 ml HCl in 50 ml isopropanol). Absorbance of formazan solutions was measured at λ max 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation.

% Cytotoxicity = (Absorbance of cell without treatment — Absorbance of cell with treatment) X 100 / Absorbance of cell with treatment

The plot of % cytotoxicity versus sample concentration was used to calculate the concentration which exhibited 50% cytotoxicity (LD50).

Plaque reduction assay

Assay was carried out according to a standard method⁷ in a six well plate where MDCK cells (10⁵ cells / ml) were cultivated for 24 hours at 37°C. A/CHICKEN/M7217B/1/2013 (H5N1) virus was diluted to give 104 PFU/ well and mixed with the safe concentration of the tested oil and incubated for 1 hour at 37°C before being added to the cells. Growth medium was removed from the cell culture plates and virus-oil and Virus-Zanamivir mixtures were inoculated (100 µl / well). After 1 hour contact time for virus adsorption, 3 ml of DMEM supplemented with 2% agarose was added onto the cell monolayer. Plates were left to solidify and incubated at 37°C till formation of viral plaques (3 to 4 days). Formalin (10%) was added for two hours then plates were stained with 0.1 % crystal violet in distilled water. Control wells were included where untreated virus was incubated with MDCK cells and finally plaques were counted and percentage reduction in plaques formation in comparison to control wells was recorded as following:

% inhibition = viral count (untreated)-viral count (treated) x 100 / viral count (untreated)

Antibacterial activity

The antibacterial activity of the oil was assessed using agar well diffusion method⁸. Shortly, sterile test solutions were prepared in DMSO at concentration of 1 mg/ml. The medium was sterilized by autoclaving at 120 °C (15 lb/in²). About 30 ml of the Agar medium with the respective strains of bacteria was transferred aseptically in to each sterilized Petri plate. The plates were left at room temperature for solidification. A well of 6 mm diameter was made using a sterile cork borer. The oil was placed in the 6 mm diameter well. The plates were incubated at 37 ± 2°C for 24 hours. Experiments were run in duplicates. Both +ve (Imipenem) and -ve (DMSO) controls respectively were run simultaneously and results were recorded by measuring the diameter of inhibition zones in millimeter. The results are recorded in Table 3 and Figure 2.

RESULTS AND DISCUSSION

The average yield of the essential oil was calculated based on the dry plant material. *Nepeta deflersiana* yielded 0.2 % (v /w) dark yellow oil heavier than water. Chemical composition of the essential oil was determined using GC-MS analysis. Chromatogram is shown in figure1 and the identified compounds are represented in table 1. A total of 26 compounds were identified that constitutes 95.28% of the total volatiles. The GC profile dominated by oxygenated constituents that represented 80.77% while identified hydrocarbons were 14.51%. A series of oxygenated monoterpene neptalactone isomers representing 72.64 % of the oil composition were detected. Linalool which is another oxygenated monoterpene represented 4.86%. Caryophyllene oxide was the major oxygenated sesquiterpene representing 3.93 % of the oil composition. Percentage of the identified nonterpenes was 9.94%, the major was 11,14-eicosadienoic acid methyl ester which represented 3.04%.

Table 1: GC/MS of the essential oil of *Nepeta deflersiana*

No.	RT	RRT	Compound	Area %
1	11.37	0.358	1-Octen-3-ol	0.56
2	12.99	0.410	Ethanone, 1(1-cyclohexen-1-yl)	0.54
3	13.76	0.434	2- α -pinene	0.23
4	14.60	0.460	Cis Linalool Oxide	0.52
5	15.20	0.479	Trans Linalool Oxide	0.41
6	15.94	0.503	Llinalool	4.86
7	17.36	0.547	p-Ment-4(8)-ene	0.10
8	20.05	0.632	4-Acetyl-1-methylcyclo Hexane (Triplol)	4.29
9	20.18	0.636	Cyclopentene,1-hexyl	0.18
10	24.47	0.772	Carvacrol	0.12
11	25.06	0.791	Nepetalactone	0.21
12	25.25	0.797	Nepetalactone	1.37
13	25.35	0.800	Nepetalactone	0.09
14	25.58	0.807	Nepetalactone	15.03
15	25.84	0.815	Nepetalactone	34.12
16	25.91	0.817	Nepetalactone	21.82
17	26.09	0.823	1,1-Diethoxy-2-octyne	0.18
18	26.75	0.844	transCaryophyllene	0.59
19	28.60	0.902	Germacrene-D	1.34
20	29.79	0.940	Cadina-1(10),4diene	0.11
21	31.08	0.981	1,5 epoxysalvial 4(14)- ene	0.13
22	31.68	1.00	(-)- Caryophyllene oxide	3.93
23	32.34	1.02	Ethyl linoleate	1.15
24	32.75	1.03	11,14-Eicosadienoic acid, methyl ester	3.04
25	33.31	1.05	Epiglobulol	0.19
26	34.58	1.09	Trans-Z- α -Bisabolene epoxide	0.17
Total percentage of:				
Identified components				
Oxygenated compounds				
Non-oxygenated compounds				
Monoterpenes				
Sesquiterpenes				
Nonterpene				
95.29				
78.55				
2.04				
0.18				
0.33				
4.42				
9.76				

*RRT: Retention time relative to Caryophyllene oxide.

Table 2: Antiviral activity of *Nepeta deflersiana*

Sample	TC50 (μ g/ml)	Initial viral count	Conc. μ l/ml (V/V)	Viral count (PFU/ml)	% of Inhibition
<i>Nepeta deflersiana</i> oil	0.31	33 X 10 ⁵	0.078	20 X 10 ⁵	39
Zanamivir		33 X 10 ⁵	0.156	15 X 10 ⁵	54.5
			10 μ g	0	100

TC50: It is the half maximal (50%) toxic concentration of the sample on cell line under examination.

PFU: Plaque forming unit: refers to one infectious virion that is capable of initiation of infection in one cell rises into a plaque after incubation.

Table 3: Antibacterial activity of *Nepeta deflersiana*, inhibition zones (IZ, mm)

Pathogens	IZ (mm)	Negative control (DMSO)	Positive control Imipenem (mm)
<i>Klebsiella pneumonia</i>	14	0	0
<i>Pseudomonas aeruginosa</i>	12	0	30
<i>Acinetobacter nosocomialis</i>	19	0	8
<i>Acinetobacter baumannii</i>	16	0	0
<i>Proteus mirabilis</i>	12	0	0
<i>Enterobacter cloacae</i>	13	0	10
<i>Enterobacter aerogenes</i>	15	0	9
<i>Serratia marcescens</i>	12	0	22

R: Resistant, S: Sensitive

Figure 1: Gas chromatogram of *Nepeta deflersiana* essential oil. Figure 2: Antibacterial activity of *Nepeta deflersiana* essential oil.

Avian influenza (AI) is a highly contagious disease of poultry caused by influenza viruses type-A of the family Orthomyxoviridae⁹. Due to the rapid spread and the high possibility for genetic alterations the spectra of wide spread human infection has been raised. Highly pathogenic avian influenza (HPAI) of H5 and H7 subtypes in poultry, has become devastating human health challenge. Searching for alternatives for antivirals that can effectively inhibit H5N1 or other influenza A viruses, and/or act in synergy with available antivirals, is an urgent need of the hour¹⁰.

In the present study the essential oils of *Nepeta deflersiana* was tested for the first time for its antiviral activity against avian influenza (H5N1) virus. MTT cytotoxicity assay was done to determine the minimum save dose of the tested oil on the host cells, the LD50 was found to be 0.31 µl/ml. Plaque reduction assay showed that the tested oil has moderate activity (54.5%) against strain 17B of the H5N1 influenza virus at concentration 0.156 µl/ml (Table 2)

Nepta oil was tested against eight respiratory tract pathogenic bacteria, six of them are multi-drug resistant bacteria namely, *Klebsiella pneumonia*, *Acinetobacter nosocomialis*, *Acinetobacter baumannii*, *Proteus mirabilis*, *Enterobacter cloacae* and *Enterobacter aerogenes*. The +ve control (Imipenem) showed weak or no activity against these bacteria, while the *Nepta* oil showed good activity against them. These results are very important and promising to discover new antibacterial agents able to eradicate the resistant strains to multiple antibiotics. Moreover, this is of highly economic value to benefit from the local flora for the discovery of new drugs.

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