Chemical composition, antioxidant and antimicrobial activity of *Nepeta persica* Boiss. essential oil

MOHADDESEH MAHBOUBI[1*], NASTARAN KAZEMPOUR[1], FATEMEH GHAZIAN[2], MOHSEN TAGHIZADEH[3]

[1]Department of Microbiology
Medicinal Plants Research Center of Jundi Shapour
87135-1178 Kashan, Iran

[2]Department of Phytochemisrty
Medicinal Plants Research Center of Jundi Shapour
87135-1178 Kashan, Iran

[3]Department of Biochemistry
Kashan University of Medical Sciences
Kashan, Iran

[*]corresponding author: phone: +98 866436 2112, fax: +98 866436 2187,
e-mail: mahboubi57@yahoo.com

S u m m a r y

The essential oil from aerial parts of *Nepeta persica* Boiss was obtained by steam distillation and was analyzed by GC and GC/MS. Fourteen components were identified as accounting for 97.3% of total oil composition. The 4aα,7α,7aα-nepetalactone (80%) and Spiro[5.6]dodecane (14.2%) were the main components of essential oil. Antimicrobial activity of essential oil against different kinds of microorganisms was determined by micro-broth dilution assay. The minimal inhibitory concentration and minimal lethal concentration values of oil were in the ranges from 1-8 to 1-16 μl/ml, respectively. Most of all, the oil was sensitive to *Candida albicans*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Antioxidant activity was evaluated by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging. The IC_{50} values of *N. persica* essential oil and BHT were nearly 0.03%, 2.98%, respectively and in β-carotene/linoleic acid system, the essential oil did not show effective antioxidant activity.

Key words: antioxidant, antimicrobial, *Nepeta persica*, neptalactone

INTRODUCTION

The genus of *Nepeta* (Lamiaceae) is annual plant, native to Asia, Europe and North America. *Nepeta* genus has 250 species. There are 67 species that found in Iran and 39 of them are exclusive to Iran [1]. *Nepeta persica* is native and grows in different part of Iran. Essential oil is one of the important ingredients of the plant. *N. persica* is a favorable feline plant; the feline used *Nepeta* for treatment. The chemical composition of *N. persica* essential oil was the subject of some studies [2-4]. 2,3,4,5-Tetramethyl-1,4-hexadiene and 4aβ, 7a, 7aα –nepetalactone were reported to be the main component of its essential oil by Javidnia et al [2], while 4αβ,7α,7aα-nepetalactone (26.5%), (E)-β-farnesene (4.4%) were the main components of oil from aerial parts of plant [3]. Main components of essential oil from the leaf, stem of *N. persica* were 4αβ,7α,7aβ-nepetalactone and 4α,7α,7αβ-nepetalactone, while the root oil contains α-pinene (40.4%) and 4αβ,7α,7αβ-nepetalactone (27.1%) as major components [4].

Anti-anxiety activity [5] and antibacterial activity of *N. persica* oil against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella thyphi* and *Enterococcus faecalis* [4] was confirmed but other biological activities such as antifungal and antioxidant activity were not studied yet. Thus, in this investigation, the antioxidant and antimicrobial activity of *N. persica* essential oil was evaluated and the chemical composition of essential oil responsible for this activity was analyzed.

MATERIALS AND METHODS

Plant material

The aerial parts of *Nepeta persica* Boiss. were collected at a flowering stage from Central Province (Novazen, Arak, Iran) in July 2009. The species were identified by Agricultural Department of Jundi shapour, Kashan, Iran, where a voucher specimen was deposited under the number 171-1.

Essential oil isolation and analysis

Air-dried aerial parts of *N. persica* were subjected to steam distillation using a Clevenger type apparatus for 6 h. The oil was dried on anhydrous sulfate. The oil analysis was carried out using GC and GC/MS. The GC apparatus was equipped with agilent technology (HP) 6890 system, capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 μm). The oven temperature program was initiated at 40°C, held for 1 min then raised up to 230 °C at a rate of 3°C/min held for 10

min. Helium was used as a carrier gas at a flow rate 1.0 ml/min. The detector and injector temperatures were 250 and 230°C, respectively. The GC/MS analysis was conducted on a HP 6890 GC system coupled with 5973 network mass selective detector with a capillary column the same as above, carrier gas helium with flow rate 1 ml/min with a split ratio equal to 1/50. The programmed injector and oven temperature was identical to GC. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those on the stored Wiley 7n.1 mass computer library, and NIST (National Institute of Standards and Technology) [6].

Microbial strains

Staphylococcus aureus ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* ATCC 1247, *Staphylococcus saprophyticus* ATCC 15305, *Klebsiella pneumoniae* ATCC 10031, *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, and *Candida albicans* ATCC 10231, 7 clinical isolates of *C. albicans*, *Aspergillus flavus*, 5 field isolates of *A. flavus*, *Aspergillus niger* ATCC 16404, *Aspergillus parasiticus* ATCC 15517 were used. Bacterial suspensions were made in Brain Heart Infusion (BHI) broth to a concentration of approximately 10^8 CFU/ml using standard routine spectrophometrical methods. Suspensions of fungi were made in Sabouraud dextrose broth. Subsequent dilutions were made from the above mentioned suspensions, then used in the tests.

Assay for antimicrobial potential

The minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) values of oil were determined by micro-broth dilution assay. The *N. persica* oil was dissolved in dimethyl sulfoxide and was twofold serially diluted with distilled water which each well contains 16–0.0125 μl/ml of oil. These dilutions were prepared in a 96-well microtitre plate. MOPS-buffered RPMI 1640 (fungi) [7], cation adjusted Muller Hinton broth (non fastidious bacteria) [8] and Todd Hewitt broth (fastidious bacteria) [9] were used as broth media. After shaking, 100 μl of oil solution was added to each well. The above mentioned microbial suspensions were diluted (1×10^6 CFU/ml for bacteria; 10^4 CFU/ml for fungi) and then 100 μl sample was added to each well and incubated at 35 °C. Antibiotics (32–0.5 μg/ml) were used as a positive control. MICs were defined as the lowest concentration of compound that inhibits bacteria or fungi after 24 and 48 h, respectively. The MLC values were the first well that showing no growth on solid media. All experiments were done in duplicates.

Antioxidant activity assays

The antioxidant activity was assessed by 2,2-diphenyl-2-picrylhydrazyl (DPPH) assay and β-carotene bleaching test. Tests were carried out in triplicate and final results were the averages of three determinations of three independent experiments. For the preparation of sample solution, essential oil and BHT separately were weighted accurately, and then dissolved in 96% ethanol in volumetric flask, then this solution was pipetted into 10 ml of 96% ethanol until the final volume reach to 10 ml. Furthermore, sample test solutions contained different concentrations of oil or BHT.

The β-carotene bleaching test

In this assay, the antioxidant activity was determined by measuring the inhibition of volatile organic compounds and the conjugated dienehydroperoxides arising from linoleic acid oxidation [10]. The β-carotene was dissolved in chloroform in the amount sufficient to reach 10 ml. The β-carotene-chloroform solution was pipetted into boiling flask containing 50 mg linoleic acid and 3000 mg Tween 80. The chloroform was removed using a laminar flow of nitrogen (50 ml/min, 30 min at 30˚C) and 50 ml of distillated water saturated with oxygen (30 min, 100 ml/min) was added to residue, mixed vigorously, to formed an clear solution, if this solution was not clear, the absorbance was not corrected (reactant solution). 5 ml of this solution (reactant solution) was added to 500 μl of various concentrations sample solutions separately and the absorbance was immediately measured at 470 nm against a blank, consisting of a solution without β-carotene [11].

The cap of test tube fitted and placed in a water bath at 50˚C, oxidation of the sample was monitored spectrophotometrically by measuring absorbance at 470 nm for 60 min. The antioxidant activity of sample was calculated using the following equation:

$AA\ \% = [(DRC - DR_S)/(DR_C)]^{*}100$,

where $DR_C = [\ln (a/b)/60]$, degradation rate of the control; $DR_S = [\ln (a/b)/60]$, degradation rate in presence of the sample; a = absorbance at time 0; b = absorbance after 60 min.

The same procedure was repeated with BHT test solution and antioxidant activities of sample solutions were compared with BHT.

Radical scavenging capacity of the oil by DPPH assay

The electron donation abilities of the corresponding extracts, pure compounds and oil were measured from the bleaching of the purple-colored ethanol solution of 2.2'-diphenyl picryl hydrazyl (DPPH) [12]. This used spectrophotometric assay, measured the stability of DPPH radical as a reagent. Test sample solutions in the volume of 500 μl were added to 2.5 ml of 6×10^{-5} molar DPPH solutions in ethanol.

After 70 min incubation period at a room temperature, the absorbance of each samples were read against a blank at 517 nm. Inhibition of free radical by DPPH in percent (I %) was calculated using the following equation:

$$I = [(A_{blank} - A_{sample})/A_{blank}],$$

where A_{blank} – absorbance of the blank (containing all reagents except the test compound); A_{sample} – absorbance of the test sample solution.

The sample concentration providing 50% inhibition (IC_{50}) was calculated from the linear program of inhibition percentage against sample concentration, and it is the sample concentration that has 50% inhibition.

RESULTS

Chemical composition of essential oil

The steam distillation of aerial parts of *N. persica* gave yellowish oil in 0.28% (w/w). The chemical composition of *N. persica* essential oil was analyzed by GC and GC/MS. Fourteen components representing 97.3% of the total oil were identified, the major components were 4α, 7α, 7α-nepetalactone (80%), Spiro[5.6]dodecane (14.2%) and β-bourbonene (1.5%) (tab. 1).

Table 1.

Chemical composition of *N. persica* essential oil

compound	RI*	percent
meparfynol	715	0.2
3-methyl cyclopentanone	858	0.1
3-octanone	991	0.2
1-cyclohexenylethanone	1039	0.2
α-terpinolene	1107	0.1
methyl salicylate	1178	0.1
2-methyl-3-octyne	1190	0.2
2-methoxy-4-methylphenol	1194	0.8
4aα, 7α, 7aα-nepetalactone	1344	80.0
5-ethylnona-2,3-dien-2-one	1261	0.2
β-bourbonene	1394	1.5
β-farnesene	1446	0.2
spiro[5.6]dodecane	1496	14.2
caryophyllene oxide	1587	0.7

*retention index

Antimicrobial activity

The antimicrobial activity of essential oil was tested against gram positive bacteria, gram negative bacteria, yeast and filamentous fungi. The antimicrobial assay showed that the oil exhibited strong activity against *C. albicans*, *S. aureus* and *K. pneumoniae*. The MIC and MLC values were 1-2 and 1-2 μl/ml for clinical isolates of *C. albicans* and 8 and 16 μl/ml for *E. faecalis*. *E. faecalis* is less sensitive to the oil. The oil showed inhibitory effect against filamentous fungi such as *A. flavus*, *A. parasiticus* and *A. niger*. The oil had cidal effect against some clinical isolates of *C. albicans* and *S. saprophyticus*. The MIC and MLC values for gram-negative bacteria, gram-positive bacteria, yeast and filamentous fungi were in the range of 2–8, 4–16; 2–8, 4–8; 1–2, 1–4; 1–2, 8–16 μl/ml, respectively. Thus, the most sensitive microorganism to essential oil was *C. albicans* with lower MIC and MLC values. The oil showed strong inhibitory effect against filamentous fungi but oil could not kill them (tab. 2).

Table 2.

Antimicrobial activity of *N. persica* essential oil by micro-broth dilution test

	N. persica oil (μl/ml)		Vancomycin (μg/ml)		Gentamycin (μg/ml)		AmphotericinB (μg/ml)	
	MIC*	MLC**	MIC	MLC	MIC	MLC	MIC	MLC
S. aureus	2	4	1	2	–	–	–	–
E. faecalis	8	16	4	4	–	–	–	–
B. cereus	2	4	0.5	0.5	–	–	–	–
S. saprophyticus	4	4	2	2	–	–	–	–
K. pneumoniae	2	4	–	–	2	2	–	–
E. coli	4	8	–	–	8	8	–	–
S. typhimorium	4	8	–	–	4	8	–	–
P. aeruginosa	8	8	–	–	4	8	–	–
C. albicans27	2	4	–	–	–	–	1	2
C. albicans 29	2	4	–	–	–	–	2	4
C. albicans 31	1	1	–	–	–	–	2	4
C. albicans green	1	2	–	–	–	–	1	2
C. albicans 25	2	4	–	–	–	–	0.5	0.5
C. albicans purple	2	2	–	–	–	–	1	2
C. albicans 21	1	2	–	–	–	–	1	2
C. albicans 10231	1	2	–	–	–	–	0.5	1
A. flavus	2	8	–	–	–	–	16	16
A. flavus K3	2	16	–	–	–	–	4	8
A. flavusS48	1	8	–	–	–	–	16	16

	N. persica oil (µl/ml)		Vancomycin (µg/ml)		Gentamycin (µg/ml)		AmphotericinB (µg/ml)	
	MIC*	MLC**	MIC	MLC	MIC	MLC	MIC	MLC
A. flavus Z16	2	8	–	–	–	–	8	8
A. flavus CZP3	2	8	–	–	–	–	16	16
A. flavus N1	2	4	–	–	–	–	4	4
A. niger	2	8	–	–	–	–	16	>32
A. parasiticus	2	16	–	–	–	–	16	16

*MIC – minimal inhibitory concentration; **MLC – minimal lethal concentration

Antioxidant activity

Evaluation of antioxidant activity by DPPH free radical scavenging showed that the IC_{50} values (the concentration required to inhibit radical formation by 50%) of *N. persica* essential oil were 2.98%. The IC_{50} values of BHT as a positive control were about 0.03%. Compared to BHT, the *N. persica* essential oil was lower than that of BHT and has inhibitory activity against the DPPH radicals (fig. 1). Antioxidant activity of *N. persica* essential oil as measured by the bleaching of β-caroetene, in β-caroetene/linoleic assay, oxidation of linoleic acid was not effectively inhibited by essential oil at a concentration of 1.79 g/ml.

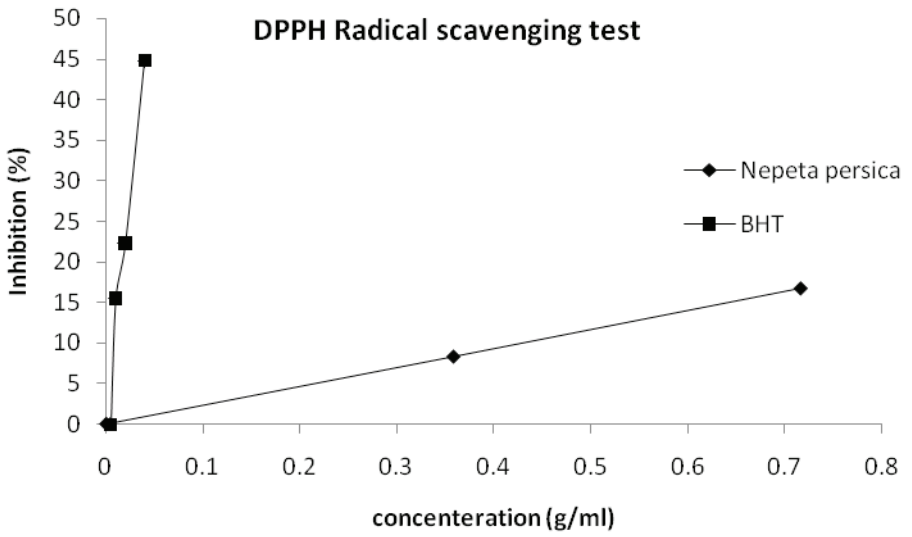

Figure 1.
Free radical scavenging of *N. persica* essential oil by DPPH assay

DISCUSSION

The yield of *N. persica* oil was 0.28% (w/w). The yield from other study was 0.08% [2]. Nepetalactone isomers were the main component aerial parts of *N. persica* oil [2-4]. Three main chemotypes were reported for the essential oil of *Nepeta* genus: Nepetalactone chemotype, caryophyllene oxide chemotype, 1,8-cineole/linalool chemotype [13]. *N. persica* assessed in this study belongs to nepetalactone chemotype.

Nepetalactone, a bicyclic terpenoid, was isolated for the first time from *N. cataria* [14]. It repels cockroaches and mosquitoes and is poisonous to some common flies and is also an aphid sex pheromone that is being investigated as a novel protection agent [15].

The antimicrobial activity observed for *N. persica* essential oil in this study may be attributed to the presence of main components in the essential oil: 4α, 7α, 7α-nepetalactone which account for 80% of the essential oil. Sonboli et al. suggested that other *Nepeta* species containing nepetalactones as main component possesses antibacterial and antifungal effect [16].

N. persica essential oil is concluded to have a slight antioxidant activity when compared to that of BHT. In the case of the linoleic acid system, essential oil did not show linoleic oxidation. It is concluded that the essential oil could not be used as natural antioxidant.

CONCLUSIONS

The essential oil from aerial parts of N. persica belongs to nepetalactone chemotype and this oil exhibited good antimicrobial activity against clinical isolates of C. albicans, S. aureus and K. pneumonia but the oil did not show strong antioxidant activity. The essential oil can be used as antimicrobial agent in pharmaceutical industries, but more research is needed for evaluation of its efficacy in clinical trials, also, the oil is a good source of neptalactone as insecticidal agent.

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SKŁAD CHEMICZNY ORAZ AKTYWNOŚĆ ANTYOKSYDACYJNA I PRZECIWBAKTERYJNA OLEJKU Z *NEPETA PERSICA*

MOHADDESEH MAHBOUBI[1*], NASTARAN KAZEMPOUR[1], FATEMEH GHAZIAN[2], MOHSEN TAGHIZADEH[3]

[1]Wydział Mikrobiologii
Centrum Biologii Jondi Shapour
87135-1178 Kashan, Iran

[2]Wydział Fitochemii
Centrum Fitochemii Jondi Shapour
87135-1178 Kashan, Iran

[3]Wydział Biochemii
Uniwersytet Medyczny Kashan,
Kashan, Iran

[*]autor, do którego należy kierować korespondencję: tel.: +98 866436 2112, faks: +98 866436 2187, e-mail: mahboubi57@yahoo.com

Streszczenie

Za pomocą chromatografii gazowej (GC) oraz chromatografii gazowej sprzężonej ze spektrometrią mas (GC-MS) przeprowadzono analizę składu chemicznego olejku eterycznego otrzymanego w procesie hydrodestylacji nadziemnych części *Nepeta persica* Boiss. Zidentyfikowano 14 związków, które stanowią 97,3% spośród wszystkich składników występujących w badanym olejku. W wyniku analizy chromatograficznej stwierdzono, że głównymi związkami olejku są 4aα,7α,7aα-nepetalakton oraz spiro[5.6]dodekan, które stanowią odpowiednio 80% i 14,2% wszystkich składników. Ustalono minimalne bakteriostatyczne stężenie (MIC) dla olejku, które mieści się w zakresie 1–8 μl/ml, natomiast bakteriobójcze (MBC) 1–16 μl/ml. Wśród mikroorganizmów najbardziej wrażliwe na działanie olejku są *Candida albicans, Staphylococcus aureus* oraz *Klebsiella pneumoniae*. Zbadano właściwości przeciwutleniające, wykorzystując 2, 2'-diphenyl-1-picrylhydrazyl (DPPH), BHT oraz system β-karoten/ kwas linolowy. Ustalono wartości $IC_{50.}$

Słowa kluczowe: *aktywność antyoksydacyjna (przeciwutleniająca), aktywność przeciwbakteryjna, nepetalakton*