Polish Journal of Microbiology
2009, Vol. 58, No 1, 69–76

ORIGINAL PAPER

Antimicrobial and Antioxidant Activity of the Essential Oil and Methanol Extract of *Nepeta cataria*

AHMET ADIGUZEL[1], HAKAN OZER[2*], MUNEVVER SOKMEN[3], MEDINE GULLUCE[4], ATALAY SOKMEN[5], HAMDULLAH KILIC[6], FIKRETTIN SAHIN[7], OZLEM BARIS[4]

[1] Health Services Vocational Training School, Ataturk University, Yenisehir-Erzurum, 25070, Turkey
[2] Department of Field Crops, Faculty of Agriculture, Ataturk University, Erzurum 25240, Turkey
[3] Department of Chemistry, Faculty of Science, Karadeniz Technical University, Trabzon 61080, Turkey
[4] Department of Biology, Faculty of Science, Ataturk University, Erzurum, 25240, Turkey
[5] Department of Biology, Faculty of Science, Karadeniz Technical University, Trabzon 61080, Turkey
[6] Department of Chemistry, Faculty of Science, Ataturk University, Erzurum 25240, Turkey
[7] Department of Genetics and Bioengineering, Faculty of Engineering and Architectur, Yeditepe University, Istanbul 34755, Turkey

Received 24 September 2008, revised 5 December 2008, accepted 9 December 2008

Abstract

Catnip (*Nepeta cataria*) is an important medicinal herb belonging to the mint family, Lamiaceae. In this study, the *in vitro* antimicrobial and antioxidant activities of the essential oil and methanol extract from *Nepeta cataria,* and its essential oil composition were investigated. The essential oil, which has 4aα,7α,7aβ-nepetalactone (70.4%), 4aα,7α,7aβ-nepetalactone (6.0%), thymol (2.3%), and 4aα,7α, 7aβ-nepetalactone (2.5%), as main components, exhibited activity against eleven bacteria, and twelve fungi and a yeast, *C. albicans*; with Minimum Inhibitory Concentrations (MIC) values ranging from 12.50 to 250 µl/ml; the methanol extract showed weaker activity. The samples were also subjected to a screening for their possible antioxidant activities by using 2.2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene/linoleic acid assays. In DPPH assay, the extract showed slight antioxidant activity whereas the essential oil remained inactive. In the latter case, both the extract and the essential oil exerted weak activity having inhibiton ratios of linoleic acid oxidation at 16.4% and 27.0%, respectively. The weak antioxidative nature of the extract could be attributed to the low phenolic content, estimated as gallic acid equivalent at 22.6 ± 2.07 µg/ml or 2.26%. In both systems, antioxidant capacity of BHT was determined in parallel experiments.

Key words: *Nepeta cataria*, antimicrobial activity, antioxidant activity, essential oil, gas chromatography/mass spectrometry (GC-MS), methanol extract

Introduction

Catnip (*Nepeta cataria*), a typical aromatic plant of the Lamiaceae, is an erect perennial herb, which produces small whitish or pinkish flowers. It is distrubuted from the eastern Mediterranean region to the western Himalayas, central Asia, southern Siberia, and China (Small, 1997).

The principal culinary use of catnip is as a tea, which is reputedly sedative and soporific. Fresh or dried leaves and young shoots are sometimes used for flavoring sauces, soups, and cooked foods. *Nepeta cataria* has been long used medicinally, as a juice, tea, tincture, infusion, and poultice. Its leaves and flowering tops are strongly antispasmodic, antitussive, astringent, carminative, diaphoretic, slightly emmenagogue, refrigerant, sedative, slightly stimulant, stomachic and tonic (Duke, 1987).

The medicinal properties of *Nepeta* species are usually attributed to their essential oils and flavonoids (Jamzad *et al*., 2003). Previous studies (Bourrel *et al*., 1993; Handjieva *et al*., 1996; Malizia *et al*., 1996; Ganzera *et al*., 2001; Hethelyi *et al*., 2001; Peterson and Ems-Wilson, 2003; Morteza-Semnai and Saeedi, 2004; Wang *et al*., 2007) on the essential oils of *Nepeta cataria* reported nepetalactone and its isomers as major components. In literature there is only one report (Baser *et al*., 2000) on the essential oil of *Nepeta cataria* from Turkey, but the research did not gave a detailed knowledge on the essential oil

* Corresponding author: H. Ozer, Department of Field Crops, Faculty of Agriculture, Ataturk University, Erzurum 25240, Turkey; phone: (+90) 442 231 2493; fax: (+90) 442 218 3647; **e-mail: haozer@atauni.edu.tr.**

composition of *N. cataria* and it only reported nepatalactone as main constituent.

Much attention has been recently devoted to the essential oil and antimicrobial activities of the plants since they have been used to extend the self life of foods and in folk medicine. Many studies suggest that the antibacterial and antifungal activities of these plants are particularly related to the main components of their essential oils. Surprisingly, less is known about antimicrobial activity of the essential oil of catnip (Bourrel *et al*., 1993; Angelini *et al*., 2006; Suschke *et al*., 2006), and in these studies the antimicrobial activity of catnip essential oil was tested on the limited number of bacteria or fungi.

During the past two decades, great emphasis has been placed on the antioxidant action of the essential oils and/or extracts of medicinal and aromatic plants. For this reason they can be used as safe and effective alternatives to synthetic preservatives. Natural antioxidants are extensively studied also for their capacity to protect organisms and cells from damage induced by oxidative stress, the latter being considered a cause of ageing, degenerative diseases and cancer (Cozzi *et al*., 1997). It has long been known that some plant extracts and/or essential oils have antioxidant activity (Lee and Shibamoto 2001; Kordali *et al*., 2005; Gulluce *et al*., 2007; Ozer *et al*., 2007). However, less information is available on the antioxidant activity (Dapkevicius *et al*., 1998) of the essential oil or extract of *N. cataria*

As part of our work on the characterization of aromatic and medicinal plants from Turkey, we report here the antimicrobial and antioxidant activity of the essential oil and methanol extract of *Nepeta cataria,* as well as its essential oil composition.

Experimental

Materials and Methods

Plant material. *Nepeta cataria* plants at flowering stage in August 2004 were collected at a height of 800 m in Olur, Erzurum, Turkey. The taxonomic identification of plant materials was confirmed by a senior plant taxonomist, Meryem Sengul, in Department of Biology, Atatürk University, Erzurum, Turkey. Collected plant materials were dried in shadow and ground in a grinder with a 2 mm in diameter mesh. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Ataturk University, Erzurum-Turkey (ATA HERB No 9791).

Chemicals and standarts. Methanol used in preparing the methanol extract was obtained from Sigma (St. Louis, MO.). Sodium sulphate needed for isolation of the essential oil was obtained from Sigma (St. Louis, MO.). Nutrient agar (NA), sabouraud dextrose agar, potato dextrose agar (PDA) used for Disk-diffusion assay were obtained from Fluka. Ofloxacin, sulbactam cefoperazone, netilmicin were obtained from Oxoid. Dimethylsulfoxide for micro-well dilution assay was obtained from Sigma (St. Louis, MO., USA). Nutrient broth was obtained from Fluka. Amphotericin and Tween 20 used for MIC agar dilution assay were obtained from Sigma (St. Louis, MO., USA). Chemicals used in antioxidant activity and total phenolics assays were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). All solvents were of analytical grade and obtained from Merck (Darmstadt, Germany). Pure chemicals used for GC/MS were obtained by Sigma, Fluka, Aldrich, and Merck.

Preparation of the methanol extract. The dried and powdered leaves (500 g) were extracted with 1 l of methanol using a Soxhlet extractor (ISOPAD, Heidelberg, Germany) for 72 h at a temperature not exceeding the boiling point of the solvent. The extract was filtered using Whatman filter paper (No 1) and then concentrated in vacuo at 40°C using a Rotary Evaporator (BÜCHI Labortechnic AG, Flawil, Switzerland). The residues obtained were stored in a freezer (Nauire, Plymouth, USA) at –80°C until further tests.

Isolation of the essential oil. Air-dried plant material was subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus (Ildam Ltd., Ankara, Turkey). The essential oil yield was calculated as 0.74 % v/w. The obtained essential oil was dried over anhydrous sodium sulphate and, after filtration, stored at +4°C until tested and analysed.

GC-MS analysis conditions. The analysis of the essential oil was performed using a Thermofinnigan Trace GC/Trace DSQ/A1300, (E.I Quadrapole) (Thermo Finnigan, CA, USA) equipped with a SGE-BPX5 MS capillary column (Scientific Instrument Services Inc., NJ. USA) (30 m × 0.25 mm i.d., 0.25 μm). For GC-MS detection an electron ionization system with ionisation energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220°C and 290°C, respectively. The programme used was 50–150°C at a rate of 3°C/min, held isothermal for 10 minutes and finally raised to 250°C at 10°C/min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μl were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, Wiley7N, TRLIB library data of the GC-MS system and literature data (Adams, 2007). The quantitative data were expressed as area %. The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature (Adams, 2007).

Antimicrobial activity

Microbial strains. The methanolic extracts and the essential oil were tested individually against a range of 40 microorganisms, including 24 bacteria, 15 fungi, and a yeast species. The microorganisms used are listed in Tables II and III. Microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine, and Plant Diagnostic Laboratory, Faculty of Agriculture at Ataturk University, Erzurum, Turkey. The identity of the microorganisms used in this study was confirmed by the Microbial Identification System (Sherlock Microbial Identification System Version 4.0, MIDI Inc., Newyork, DE, USA) in the Biotechnology Application and Research Center at Ataturk University.

Disk-diffusion assay. The dried plant extracts were dissolved in methanol to a final concentration of 30 mg/ml and sterilized by filtration through 0.45 µm Millipore filters (Schleicher & Schuell, Microscience, Dassel, Germany). Antimicrobial tests were then carried out by the disk diffusion method (Murray *et al*., 1995) using 100 µl of suspension containing 10^8 CFU (Colony Forming Units)/ml of bacteria, 10^6 CFU/ml of yeast and 10^4 spore/ml of fungi spread on nutrient agar (NA), sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. The disks (6 mm in diameter) impregnated with 10 µl of essential or 10 µl of the methanol solution of the dried plant extracts (300 µg /disk) were placed on the inoculated agar. Negative controls were prepared with the same solvents used to dissolve the plant extracts. Ofloxacin (10 µg/disk), sulbactam (30 µg) + cefoperazone (75 µg) (105 µg/disk) and/or netilmicin (30 µg/disk) were used as positive reference standards to determine the sensitivity of one strain/isolate in each bacterial species tested. The inoculated plates were incubated at 37°C for 24 h for clinical bacterial strains, 48 h for the yeast and 72 h for fungi isolates. Plant-associated microorganisms were incubated at 27°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay was repeated twice.

Micro-well dilution assay. The minimal inhibition concentration (MIC) values were determined for the bacterial strains which were sensitive to the essential oil in the disk diffusion assay. The inocula of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils and extracts of *N. cataria* dissolved in 10% dimethylsulfoxide (DMSO), were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial two-fold dilutions were made in order to obtain concentration range from 7.8 to 500 µg/ml in 10 ml sterile test tubes containing nutrient broth. The MIC values of the *N. cataria* extracts against bacterial strains and *Candida albicans* isolates were determined based on a micro-well dilution method (Gulluce *et al*., 2004) with some modifications.

The 96-well plates were prepared by dispensing 95 µl of nutrient broth and 5 µl of the inoculum into each well. A 100 µl from the stock solutions of *N. cataria* essential oil prepared at the 500 µg/ml concentration was added into the first wells. Then, 100 µl from the serial dilutions was transferred into the six consecutive wells. The last well containing 195 µl of nutrient broth without compound and 5 µl of the inoculum on each strip was used as a negative control. The final volume in each well was 200 µl. Maxipime (Bristol-Myers Squibb, Princeton, NJ., USA) at a concentration range of 500–7.8 µg/ml was prepared in nutrient broth and used as a standard drug for positive control. The plate was covered with a sterile plate sealer. Contents of each well were mixed on a plate shaker (MS2-Minishaker, IKA, Labortechnik, Staufen, Germany) at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth in each medium was determined by reading the respective absorbance (Abs) at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc, Highland Park, VT, USA) and confirmed by plating 5 µl samples from clear wells on nutrient agar medium. The oil tested in this study was screened twice against each organism.

MIC agar dilution assay. The agar dilution method, as described previously by Gul *et al*. (2002) was used to determine the MIC values of the fungi isolates. The essential oils of *Nepeta cataria* were added aseptically to sterile molten PDA medium, containing Tween 20 (Sigma 0.5%, v/v), at the appropriate volume to produce the concentration range of 7.8–500 µg/ml. The resulting PDA solutions were immediately poured into Petri plates after vortexing. The plates were spot inoculated with 5 µl (10^4 spore/ml) of each fungal isolate. Amphotericin B (Sigma A 4888) was used as a reference antifungal drug. The inoculated plates were incubated at 27°C and 37°C for 72 h for plant and clinical fungi isolates, respectively. At the end of the incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the essential oil where absence of growth was recorded. Each test was repeated at least twice.

Antioxidant activity

DPPH assay. The hydrogen atoms or electron donating ability of the corresponding extracts and butylated hydoxytoluene (BHT) was determined from the bleaching of purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical 2,2'-diphenylpicrylhydrazyl (DPPH) as a reagent (Cuendet *et al*., 1997; Dapkevicius *et al*., 1998;

Burits and Bucar, 2000). Fifty microliters of various concentrations of the extracts in methanol were added to 5 ml of a 0.004% methanol solution of DPPH in a 10 ml test tube. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I%) was calculated in the following way:

$I\ \% = (A_{blank} - A_{sample} / A_{blank}) \times 100$

Where A_{blank} is the absorbance of the control reaction (containing all the reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted as inhibition percentage against extract concentration. Tests were carried out in triplicate.

β-Carotene-linoleic acid assay. In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide formation from linoleic acid oxidation (Dapkevicius *et al*., 1998). A stock solution of β-carotene-linoleic acid mixture was prepared as follows: 0.5 mg β-carotene was dissolved in 1 ml of chloroform (HPLC grade), 25 ml linoleic acid and 200 mg Tween 40 were added as emulsifier since β-carotene is not water soluble. Chloroform was completely evaporated using a vacuum evaporator. Then, 100 ml of distilled water saturated with oxygen were added with vigorous shaking at a rate of 100 ml/min for 30 min. 2500 ml of this reaction mixture was dispersed to test tubes and 350 ml portions of the extracts, prepared in 2 g/l concentrations, were added and the emulsion system was incubated up to 48 hours at room temperature. The same procedure was repeated with a positive control BHT and a blank. After this incubation time, the absorbance of the mixtures was measured at 490 nm. Antioxidative capacities of the extracts were compared with those at the BHT and the blank. Tests were carried out in triplicate.

Assay for total phenolics. The total phenolic constituents of the N. cataria extracts were determined using the literature methods involving Folin-Ciocalteu reagent and gallic acid as standard (r^2=0.998) (Slinkard and Singleton, 1977; Chandler and Dodds, 1983). The extract solution (0.1 ml) containing 1000 µg extract was put in a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent was added and the flask was shaken thoroughly. After 3 min, 3 ml of a 2% Na_2CO_3 were added and the mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm. The same procedure was repeated for all the standard gallic acid solutions (0–1000 µg 0.1 ml^{-1}) and the standard curve was determined using the equation given below:

Absorbance: 0.0012 × Gallic acid (mg) + 0.0033

Table I
Chemical composition of the essential oil of *Nepeta cataria* L

RIa	Components	Composition (%)	Identification
921	β-pinene	0.4	GC-MS, RI, CoI
994	Myrcene	0.1	GC-MS, RI, CoI
1039	Limonene	0.3	GC-MS, RI, CoI
1044	1,8-Cineole	0.7	GC-MS, RI, CoI
1151	Camphor	0.4	GC-MS, RI, CoI
1160	Pinocamphone	0.4	GC-MS, RI
1162	Pinocarvone	1.2	GC-MS, RI
1171	trans-pinocamphone	0.1	GC-MS, RI
1172	terpinen-4-ol	0.1	GC-MS, RI, CoI
1184	α-Terpineol	0.2	GC-MS, RI, CoI
1211	Pulegone	1.8	GC-MS, RI
1221	Piperitone	0.8	GC-MS, RI
1240	Thymol	2.3	GC-MS, RI, CoI
1245	Carvacrol	0.5	GC-MS, RI, CoI
1266	Piperitenone	1.7	GC-MS, RI
3276	Piperitenone oxide	1.7	GC-MS, RI
1278	4aα,7α,7aα-nepetalactone nepetalactone	6.0	GC-MS, RI
1293	4aα,7a,7aβ-nepetalactone nepetaiactone	70.4	GC-MS, RI
1294	4aα,7β,7aα-nepetalactone nepetalactone	2.5	GC-MS, RI
1298	trans-Caryophyllene	0.9	GC-MS, RI, CoI
1362	Spathulenol	0.1	GC-MS, RI
1364	Caryophyllene oxide	0.4	GC-MS, RI, CoI
Total		93.0	

RI, retention index ; CoI, co-injection SGE-BPX5 capillary column GC; Compounds listed in order of elution from a BPX5 MS column

Statistical analysis. SPSS for Windows version 11.0 was used for statistical analysis. Results were expressed as average ± standard deviation (SD).

Results and Discussion

Chemical composition of the essential oil. The genus *Nepeta* is represented in Turkey by 33 species and altogether 38 taxa, 17 of these being endemic in Turkey (Davis, 1982). *N. cataria* is one of the best known species of the genus around the world. In this study, as can be seen in Table I, GC/MS analysis of *N. cataria* oil resulted in the identification of 22 compounds, representing 93.0% of the oil. 4aα, 7α, 7aβ-nepetalactone (70.4%), 4aα,7α,7aα-nepetalactone (6.0%), thymol (2.3%), and 4aβ,7α,7aβ-nepetalactone (2.5%) were the main compounds. The results of our study were in accordance with those reported by other authors (Bourrel *et al*., 1993; Handjieva *et al*., 1996; Malizia *et al*., 1996; Ganzera *et al*., 2001; Hethelyi *et al*., 2001; Morteza-Semnai and Saeedi, 2004; Peterson and Ems-Wilson, 2003; Wang *et al*., 2007). These authors determined that the essential oil composition of *N. cataria* is characterized nepetalactones and its isomers as main components.

Antimicrobial activity. The antimicrobial activities of *N. cataria* oil and extracts assayed against the microorganisms in the current study were qualitatively and quantitatively assessed by evaluating the presence of inhibition zones, zone diameter, and MIC values. The results were given in Tables II and III. The results showed that the essential oil of *N. cataria* had antimicrobial activity against 11 bacteria, and 12 fungi and 1 yeast species. On the other hand, the methanol extract from aerial parts of *N. cataria* plants showed antimicrobial activity on only 5 bacteria and 7 fungi (Tables II and III), suggesting that methanol

Table II
Antimicrobial activities of *N. cataria* extract and essential oil against the bacterial strains tested

Test bacterium	Plant extract (MeOH)		Essential oil		Antibiotics	
	DD[a]	MIC[b]	DD[c]	MIC[b]	DD[d]	MIC[e](max)
Plant Origin						
Bacillus megaterium A59	ND	ND	ND	ND	9 (SCF)	15.62
Burkholdria cepacia A225	ND	ND	10	125	22 (SCF)	125
Clavibacter michiganense A227	10	62.50	ND	ND	25 (SCF)	16.62
Enterobacter cloacae A135	ND	ND	ND	ND	20 (NET	31.25
Klebsiella pneumoniae A137	ND	ND	32	15.62	12 (OFX)	125
Pseudomonas syringae pv. tomato A35	ND	ND	ND	ND	24 (OFX)125	
Xanthomonas campestris A235	ND	ND	ND	ND	20 (SCF)	31.25
Clinic Origin						
Acinetobacter baumanii A8	ND	ND	ND	ND	18 (OFX)	31.25
Bacillus subtilis ATCC-6633	11	125	12	62.50	28 (OFX)	62.50
Bacillus subtilis A57	ND	ND	12	125	28 (OFX)	125
Brucella abortus A77	ND	ND	10	125	12 (SCF)	62.50
Bacillus macerans A199	16	31.25	18	15.62	19 (OFX)	15.62
Enterococcus faecalis ATCC-29122	ND	ND	ND	ND	18 (SCF)	31.25
Escherichia coli A1	16	31.25	12	125	20 (OFX)	62.50
Proteus vulgaris A161	ND	ND	ND	ND	12 (OFX)	125
Proteus vulgaris KUKEM1329	ND	ND	10	250	13 (OFX)	125
Pseudomonas aeruginosa ATCC-9027	ND	ND	ND	ND	22 (NET)	31.25
Pseudomonas aeruginosa ATCC-27859	ND	ND	ND	ND	22 (NET)	15.62
Salmonella enteritidis ATCC-13076	ND	ND	ND	ND	27 (SCF)	62.50
Staphylococcus aureus A215	12	62.50	29	15.62	22 (SCF)	31.25
Staphylococcus aureus ATCC-29213	ND	ND	ND	ND	22 (SCF)	62.50
Staphylococcus epidermis A233	ND	ND	10	62.50	12 (SCF)	15.62
Streptococcus pyogenes ATCC-176	ND	ND	14	62.50	10 (OFX)	62.50
Streptococcus pyogenes KUKEM-676	ND	ND	ND	–	13 (OFX)	31.25

[a] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 300 µg/disk of methanol extract.
[b] Minimal inhibitory concentration as µ*glmL.*
[c] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 10 µL. of essential oil.
[d] DD, diameter of disk diffusion (mm); OFX, ofloxacin (10 µg/disk); SCF, sulbactam (30 µg) + cefoperazone (75 µg) (105 µg/disk); and NET, netilmicin (30 µg/disk) were used as positive reference standards antibiotic disks (Oxoid).
[e] Maxipine (µg/mL) was used as reference antibiotic in micro well dilution assay (Sigma).
–: Not Detected (ND)

Table III
Anticandidal and antifungal activities of the extract and essential oil of *N. cataria* against the yeast and fungus isolates tested

Test microorganism	Plant extract (MeOH)		Essential oil		Antibiotics
	DD[a]	MIC[b]	DD[c]	MIC[b]	MIC Amp B[d]
Yeast					
Candida albicans A117	ND	ND	10	125	31.25
Fungi					
Alternaria alternate	ND	ND	24	62.50	31.25
Aspergillus flavus	16	62.50	39	15.62	15.62
Aspergillus variecolor	12	125	29	31.25	15.62
Fusarium acuminatum	ND	ND	ND	ND	62.50
Fusarium oxysporum	ND	ND	17	125	62.50
Fusarium solani	14	125	35	15.62	62.50
Fusarium tabacinum	15	62.50	35	15.62	62.50
Moniliania fructicola	ND	ND	ND	ND	15.62
Penicillum spp.	ND	ND	27	62.50	31.25
Rhizopus spp.	14	125	34	31.25	125
Rhizoctonia solani	15	125	35	15.62	31.25
Sclorotinia minor	ND	ND	ND	ND	125
Sclorotinia sclerotiorum	ND	ND	16	125	62.50
Trichophyton rubrum	ND	ND	18	125	31.25
Trichophyton mentagrophytes	16	62.50	35	15.62	15.62

[a] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 300 µg/disk of methanol extract.
[b] Minimal inhibitory concentration as (µ*glml*).
[c] DD, inhibition zone in diameter (mm) around the disks (6 mm) impregnated with 10 µL. of essential oil.
[d] Amphotericin B (µg/mL) was used as reference antibiotic in MIC agar dilution (Sigma).
–: Not Detected (ND)

extract of *N. cataria* exhibited weaker activity comparing to essential oil. The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the essential oil of *N. cataria*, were in the range of 10–32 mm, and 15.62 – 250 µl/ml (Tables II and III). The maximal inhibition zones and MIC values of the yeast and fungi were also 10–39 mm and 15.62–125 µl/ml, respectively (Tables II and III). These results indicate that the essential oil showed good antimicrobial spectrum of action (Tables II and III).

The antimicrobial activity observed for *Nepeta cataria* in this study may be attributed to the presence of main components in the essential oil, *i.e.*, nepetalactone and its isomers, which account for 78.9% of the essential oil (Table I). Earlier studies also suggest that other Nepeta species containing nepetalactons as main components possess antibacterial and antifungal activity (Sonboli *et al*., 2004; Sojanovic *et al*., 2005). Limited evidence exists for the antimicrobial activity of *N. cataria* oils and extracts (Bourrel *et al*., 1993; Nostro *et al*., 2000; Suschke *et al*., 2006). In an earlier study, Bourrel *et al*. (1993) determined that the essential oil of *N. cataria* exhibited bacteriostatic and fungistatic activities against a variety of test organisms, and some of the antifungal activity of the oil was due to the presence of lactones. However, it should also be noted that at least a part of this antimicrobial activity observed in the present study is likely to be due to the presence of thymol, pulegone, piperitenone, and piperitenone oxide in the essential oil although they account for a low percentage in the essential oil composition (Flamini *et al*., 1999; Gulluce *et al*., 2004; Tepe *et al*., 2005; Gulluce *et al*., 2007). Indeed, to better analyze the contribution of the essential oil to antimicrobial activity, more insight is needed on the antibacterial and antifungal activities of individual pure components.

In the literature, some iridoid compounds including non-volatile nepetalactones have been reported in the methanol extract of some *Nepeta* species (Galati *et al*., 2004; Kökdil *et al*., 1999; Takeda *et al*., 1999). Therefore, the difference in antimicrobial activities of essential oil and methanol extract of *Nepeta cataria* could be attributed to have their different compounds.

Antioxidant activity. As shown in Table IV, the methanol extract was able to reduce the stable radical, DPPH the yellow-coloured diphenylpicrylhydrazine with an IC_{50} value of 171.98 ± 3.6 µg/ml, which is concluded as a slight activity when compared to that of positive control, BHT with 19.80 ± 0.52 µg/ml whereas the essential oil remained inactive.

Table IV
Antioxidative potentials of the essential oil and methanolic extracts of *Nepeta cataria* and positive control (BHT) in DPPH and β-carotene/linoleic acid assays

Sample	DPPH assay (μg/ml)	Inhibition ratio of linoleic acid oxidation (% ± 1)	Amounts of total phenolics*
MeOH	171.98 ± 3.6	16.4 ± 1.4	22.6 ± 2.07
Essential oil	NA	27.0 ± 2.1	–
BHT (positive control)	19.80 ± 0.52	96.0 ± 0.05	–

–: Not Active (NA); * as gallic acid equivalent (mg/mL)

In the case of the linoleic acid system, both the MeOH extract and the essential oil seemed to possess weak linoleic acid oxidation at 16.4% and 27.0%, respectively. It is concluded that none of the samples tested could be used as natural antioxidant in food preservation. The antioxidative nature of *Nepeta* species is scarce in the literature. Only two comprehensive studies point out the notable antioxidative capacity of *Nepeta* species (Dapkevicius *et al*., 1998; Tepe *et al*., 2007). In the latter case, antioxidative capacitities of the essential oil were attributed to 1.8-cineole and linalool, both presented in the oil at relatively high amounts, 38.9% and 25.1%, respectively. Also, activity in polar extract was positively correlated with the high phenolic content. However, neither the essential oil proved to contain 1,8-cineole and linalool nor the extract contained high phenolic content in our study carried out with *N. cataria*, as presented here. As aforesaid, antioxidative nature of the members of *Nepeta* is scarce in the literature and therefore a comprehensive evaluation is needed for this genus.

Previous studies on the antimicrobial activity of *N. cataria* oils and/or extracts have limitations for the number and diversity of microorganisms tested compared to the current study. In this context, our study is the first comprehensive report that assesses antimicrobial activity of catnip oil against a wider range of microorganisms. Our findings provide a better understanding of antimicrobial and antioxidant activity of *N. cataria* oils and extracts, suggesting that the study can be useful as a starting point for further applications of catnip essential oil and their constituents, particularly nepetalactones, in food and pharmaceutical preparations.

Acknowledgements

We thank Dr. Ahmet CAKIR for his valuable comments in GC/MS analysis.

This study was supported in part by a grant from the Research Funds appropriated to Ataturk University.

Literature

Adams R.P. 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 4th Edition. Allured Publishing Corp., Carol Stream, IL, USA.

Angelini P., R. Pagiotti, A. Menghini and B. Vianello. 2006. Antimicrobial activities of various essential oils against foodborne pathogenic or spoilage moulds. *Ann. Microbiol.* 56: 65–69.

Baser K.H.C., N. Kirimer, M. Kurkcuoglu and B. Demirci. 2000. Essential oils of *Nepeta* species growing in Turkey. *Chem. Nat. Compd.* 36: 356–359.

Bourrel C., F. Perineau, G. Michel and J.M. Bessiere. 1993. Catnip (*Nepeta cataria* L.) essential oil: analysis of chemical constituents, bacteriostatic and fungistatic properties. *J. Essent. Oil Res.* 5: 159–167.

Burits M. and F. Bucar. 2000. Antioxidant activity of *Nigella sativa* essential oil. *Phytother. Res.* 14: 323–328.

Chandler S.F. and J.H. Dodds. 1983. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum lacinitum*. *Plant Cell Rep.* 2: 105–108.

Cozzi R., R. Ricordy, T. Aglitti, V. Gatta, P. Petricone and R. De Salvia. 1997. Ascorbic acid and beta-carotene as modulators of oxidative damage. *Carcinogenesis* 18: 223–228.

Cuendet M., K. Hostettmann, O. Potterat and W. Dyatmyko. 1997. Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helv. Chim. Acta* 80: 1144–1152.

Dapkevicius A., R. Venskutonis, T.A. Van Beek and J.P.H. Linssen. 1998. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agr.* 77: 140–146.

Davis P.H. 1982. Flora of Turkey and the East Aegean Islands. pp. 270–271, vol.7, Edinburg University Press, Edinburg.

Duke J.A. 1987. CRC Handbook of Medicinal Herbs, 5th ed., CRC Press, Boca Raton, FL.

Flamini G., P.L. Cioni, R. Puleio, I. Morelli and L. Panizzi. 1999. Antimicrobial activity of the essential oil of *Calamintha nepeta* and its constituent pulegone against bacteria and fungi. *Phytother. Res.* 13: 349–351.

Galati E.M., N. Miceli, M. Galluzzo, M.F Taviano and O. Tzakou. 2004. Neuropharmacological effects of epinepetalactone from *Nepeta sibthorpii* behavioral and anticonvulsant activity. *Pharm. Biology.* 42: 391–395.

Ganzera M., S. Crockett, M.R. Tellez and I.A. Khan. 2001. Determination of nepetalactone in *Nepeta cataria* by reversed phase high performance liquid chromatography. *Pharmazie* 56: 896–897.

Gul H.I., T. Ojanen and O. Hanninen. 2002. Antifungal evaluation of bis Mannich bases derived from acetophenones and their corresponding piperidinols and stability studies. *Biol. Pharm. Bull.* 25: 1307–1310.

Gulluce M., F. Sahin, M. Sokmen, H. Ozer, D. Daferera, A. Sokmen, M. Polissiou, A. Adiguzel and H. Ozkan. 2007. Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp *longifolia*. *Food Chem.* 103: 1449–1456

Gulluce M., M. Sokmen, F. Sahin, A. Sokmen, A. Adiguzel and H. Ozer. 2004. Biological activities of the essential oil and methanolic extract of *Micromeria fruticosa* (L) Druce ssp *serpyllifolia* (Bieb) PH Davis plants from the eastern Anatolia region of Turkey. *J. Sci. Food Agr.* 84: 735–741.

Handjieva N.V., S.S. Popov and L.N. Evstatieva. 1996. Constituents of essential oils from *Nepeta cataria* L., *N. grandiflora* M.B. and *N. nuda* L. *J. Essent. Oil Res.* 8: 639–643.

Hethelyi E.B., L.G. Szabo and K. Korany. 2001. Volatile constituents of *Nepeta cataria* L., *N. glechoma* Benth. and *N. parviflora* M. Bieb. from Hungary. *Int. J. Hortic. Sci.* 7: 47–50.

Jamzad Z., R.J. Grayer, G.C. Kite, M.S.J. Simmonds, M. Ingrouille and A. Jalili. 2003. Leaf surface flavonoids in Iranian species of *Nepeta* (Lamiaceae) and some related genera. *Biochem. Syst. Ecol.* 31: 587–600.

Kokdil G., S.M. Yalcin and G. Topcu. 1999. Nepetalactones and other constituents of *Nepeta nuda* ssp. *albiflora*. *Turkish J. of Chem.* 23: 99–104.

Kordali S., R. Kotan, A. Mavi, A. Cakir, A. Ala and A. Yildirim. 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium*, *A. dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* essential oils. *J. Agric. Food Chem.* 53: 9452–9458.

Lee K.G. and T. Shibamoto. 2001. Antioxidant activities of volatile components isolated from Eucalyptus species. *J. Sci. Food Agric.* 81: 1573–1579.

Malizia R.A., J.S. MolliJ, D.A. Cardell and J.A. Retamar. 1996. Volatile constituents of the essential oil of *Nepeta cataria* grown in Cordoba Province (Argentinia). *J. Essent. Oil Res.* 8: 565–567.

Morteza-Semnani K. and M. Saeedi. 2004. Essential oils composition of *Nepeta cataria* L. and *Nepeta crassifolia* Boiss. and Buhse from Iran. *J. Essent. Oil-Bear. Plants* 7: 120–124.

Murray P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolke. 1995. Manual of Clinical Microbiology, Vol. 6. ASM, Washington, DC.

Nostro A., M.P. Germano, V. D'angelo, A. Marino and M.A. Cannatelli. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett. Appl. Microbiol.* 30: 379–384.

Ozer H., M. Sokmen, M. Gulluce, A. Adiguzel, F. Sahin, A. Sokmen, H. Kilic and O. Baris. 2007. Chemical composition and antimicrobial and antioxidant activities of the essential oil and methanol extract of *Hippomarathrum microcarpum* (Bieb.) from Turkey. *J. Agric. Food Chem.* 55: 937–942.

Peterson C.J. and J. Ems-Wilson. 2003. Catnip essential oil as a barrier to subterranean termites (Isoptera: Rhinotermitidae) in the laboratory. *J. Econ. Entomol.* 96: 1275–1282.

Slinkard K. and V.L. Singleton. 1977. Total phenol analyses: automation and comparison with manual methods. *Am. J. Enol. Vitic.* 28: 49–55.

Small E. 1997. Culinary Herbs. pp. 441–449. In: National Research Council Press (eds), Ottawa, Ontario, Canada.

Sonboli A., P. Salehi and M. Yousefzadi. M. 2004. Antimicrobial activity and chemical composition of the essential oil of *Nepeta crispa* Willd. from Iran. *Z. Naturforsch. C J. Biosci.* 59: 653–656.

Stojanovic G., N. Radulovic, J. Lazarevic, D. Miladinovic and D. Dokovic. 2005. Antimicrobial activity of *Nepeta rtanjensis* essential oil. *J. Essent. Oil Res.* 17: 587–589.

Suschke U., H.K. Geiss and J. Reichling. 2006. Antibacterial activity of the essential oils of catnip (*Nepeta cataria* L.) and lemon balm (*Melissa officinalis* L.) against clinical isolates from the respiratory tract. *Planta Med.* 72: 992.

Takeda Y., Y. Kiba, T. Masuda, H. Otsuka, G. Honda, M. Tagawa, E. Sezik and E. Yesilada. 1999. Nepetaracemosides A and B, iridoid glucosides from *Nepeta racemosa*. *Chem. Pharm. Bull.* 47: 1433–1435.

Tepe B., M. Sokmen, A. Sokmen, D. Daferera and M. Polissiou. 2005. Antimicrobial and antioxidative activity of the essential oil and various extracts of *Cyclotrichium origanifolium* (Labill.) Manden & Scheng. *J. Food Eng.* 69: 335–342.

Tepe B., D. Daferera, A. Sihoglu-Tepe, M. Polissiou and A. Sokmen. 2007. Antioxidant activity of the essential oil and various extracts of *Nepeta ?avida* Hub.-Mor. from Turkey. *Food Chem.* 103: 1358–1364.

Wang M.F., K.W. Cheng, Q.L. Wu and J.E. Simon. 2007. Quantification of nepetalactones in catnip (*Nepeta cataria* L.) by HPLC coupled with ultraviolet and mass spectrometric detection. *Phytochem. Anal.* 18: 157–160