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In vitro antioxidant and antimicrobial assays of acetone extracts from *Nepeta meyeri* Bentham

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Abstract. – *Background and Objectives:* *Nepeta* species are used as diuretic, diaphoretic, antitussive, antispasmodic, antiasthmatic, febrifuge, emmenagogue, sedative agents, insecticidal, acaricidal, antiviral, anti-inflammatory and antioxidant. Acetone extract of *Nepeta meyeri* (Labiatae) was screened for antioxidant and antimicrobial activities.

Materials and Methods: **The antioxidant properties of the extract were investigated by using various methods established *in vitro* systems such as 1,1-diphenyl-2-picrylhydrazyl (DPPH)/nitric oxide (NO) radical scavenging activity. Reducing power and total phenolic substance analysis and also antimicrobial activity of acetone extract of Nepeta meyeri were tested against six gram negative, seven gram positive bacteria and the yeast strain using the disc diffusion method.**

Results and Discussion: **Acetone extract of the plant examined exhibited a significant concentration-dependent inhibition of DPPH and $NO^{\bullet}$ radical. Furthermore, Nepeta meyeri showed very high reducing power. In DPPH radical and $NO^{\bullet}$ scavenging assays the IC_{50} value of extract was 672.2 µg/ml and 165.32 µg/ml, respectively. The amounts of total phenolic compounds were also determined and 12.86 µg pyrocatechol equivalents of phenols were detected in the extract. The data obtained from these *in vitro* models clearly demonstrated antioxidant potential of acetone extract of *Nepeta meyeri*. The extract revealed antibacterial activity against all gram positive bacteria but not was active against gram negative bacteria.**

Key Words:

Nepeta meyeri, Lamiaceae, Antimicrobial activity, DPPH, NO, Antioxidant activity.

Introduction

Lamiaceae (Labiatae), a well known family, is represented by approximately 250 genera and 3000 species within the flowering plants. Multiregional genus *Nepeta* L. belonging to the family Labiatae contains approximately 250 species distributed mainly in South-West and Central Asia, Europe, Africa and North America and is one of the largest genera in the family[1,2]. The 40 *Nepeta taxa* growing in Turkey can be divided into 2 groups; Mediterranean (13 taxa) and Irano-Turanian (21 taxa). The Irano-Turanian taxa are found in the Central, South-east and East Anatolia, whereas, the Mediterranean taxa grow mainly in the Mediterranean, Marmara and Aegean regions. The other taxa are widely distributed throughout Turkey[1]. *Nepeta* species are used as diuretic, diaphoretic, antitussive, antispasmodic, antiasthmatic, febrifuge, emmenagogue and sedative agents[3]. In addition, insecticidal, acaricidal, antiviral, anti-inflammatory and antioxidant properties have been also reported[4-6]. *Nepeta* species contain monoterpenes, sesquiterpenes, cyclopentanoid iridoids derivatives and nepetalactones[2,3]. Nepetalactones which had been a peculiar physiological activity to cats has been known as a constituent of plants belonging to the genus *Nepeta*[7]. In previous studies, antibacterial, antifungal properties of several *Nepeta* species were reported. Stojanovic et al[8] found that essential oil of *Nepeta rtanjensis* was active against bacteria but not against a fungus species examined. Sonboli et al[9] found that essential oil of *Nepeta crispa* Willd. was mostly active against gram positive two bacteria, namely, *B*acillus *subtilis* and *Staphylococcus aureus*. Also, the oil exhibited a remarkable antifungal activity against all the tested fungi. Saxena and Mathela[10] isolated the compounds such as iridodial beta-monoenol acetate, isolated from the essential oil of *Nepeta leucophylla Benth*, and actinidine from *Nepeta clarkei Benth*. These compounds were

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screened for antifungal activities against *Aspergillus flavus*, *Aspergillus ochraceus*, *Penicillium citrinum*, and *Penicillium viridicatum*, all known mycotoxin-producing taxa, and *Sclerotium rolfsii* and *Macrophomina phaseolina*. Iridodial beta-monoenol acetate was most effective against *Sclerotium rolfsii*, while actinidine was highly active against *Macrophomina phaseolina*. Chemical constituents, nepetalactone, cineole and camphor, which are the main constituents found in the essential oil of *Nepeta meyeri* were reported by Sefidkon and Shaabani[11] and Esmaeili et al[12]. Although reports on the chemical composition as well as antimicrobial properties of some species of *Nepeta* have been documented. There has been no reports on antimicrobial properties and antioxidant capacity of *Nepeta meyeri*. Studies on free radicals showed that foods that are rich in antioxidants could protect from various diseases including cardiovascular disorders, cancer as well as neurodegenerative diseases like Parkinson's and Alzheimer disease[13]. Harmful effects of free radicals can be scavenged by antioxidants that are capable of scavenging these radicals. Recent studies indicated that foods that are rich in antioxidants are very effective in the prevention of many diseases.

The aim of the present work is to evaluate the possible antioxidant and antimicrobial properties from the acetone extracts from the aerial parts of *Nepeta meyeri*.

Materials and Methods

Plant Material and the Preparation of the Extract

Aerial parts of *Nepeta meyeri* were collected from Kars, Turkey. The plant was identified in the herbarium of the Biology Department at Kahramanmaras Sutcu Imam University (Turkey), where a voucher specimen is deposited as A. Ilçim 732 in the herbarium of Dr. A. Ilcim KSUH. Powdered plant was Soxhlet-extracted with acetone (8 to 10 h). The solvent was used with further purification before extraction step. The crude solvent-based extract initially subjected to filtration. Afterwards, the solvent removed in a rotary vacuum evaporator at 40°C (Büchi, Rotavapor R-210, Labortechnik AG, Flavil, Switzerland). Acetone extracts was resolved in purified methanol, then, sterilized through membrane filter (0.22 µm filter unit, Millex-GS Millipore). The extract was then stored at refrigerated conditions until further use.

In vitro Antioxidant Assays

Chemicals

1,1-Diphenly-2-picrylhydrazyl (DPPH), buthylated hydroxytoluene (BHT), Rutin were purchased from Sigma (Sigma, Aldrich GmbH, Sternheim, Germany). Pyrocatechole, Folin-Ciocalteu's phenol reagent, sodium carbonate, ethanol, chloroform and the other chemicals and reagents were purchased from Merck (Darmstat, Germany). All other chemicals and reagents were of analytical grade.

Reducing Power

The reducing power of acetone extract was measured using the method of Oyaizu[14]. Two hundred and fifty µl of the extract or standard solution were initially dissolved in methanol at different concentrations ranging from 0 to 600 µg/ml. Each concentration of the test compound or standard solution was sequentially mixed with 2.25 ml of 0.2 mol/L phosphate buffer (pH 6.6), 2.5 ml of potassium ferricyanide solution (1%) and incubated in a water bath (Memmert, Schwabach, Germany) (20 min/50°C). After incubation, a 2.5 ml of trichloroacetic acid (10%) was transferred into the mixture and centrifuged at 1000 × g for 10 min. The resulting supernatant (2.5 ml) was mixed with an equal volume of distilled water and 0.5 ml of ferric chloride (0.1%). The absorbance of the reaction mixture was read at 700 nm at spectrophotometer (Shimadzu, Kyoto, Japan). BHT was used as positive control.

Determination of Total Phenolics Compounds

Total phenolic compounds were determined with Folin-Ciocalteu's reagent according to the method of Slinkard and Singleton using pyrocatechol as a standard phenolic compound[15]. Briefly, the basic extract solution was mixed with distilled water and Folin-Ciocalteu's reagent (1:45:1). After 3 min, the reaction mixture was treated with 3 ml of Na_2CO_3 (2%) and left to stand for 2 h. The absorbance in test tubes was measured at 760 nm in a spectrophotometer. The concentration of total phenolic compounds of sample was calculated according to the formula: Absorbance = [0.00237 × µg pyrocatechol equivalent] – 0.00947 (R^2=0.996).

DPPH Radical Scavenging Activity

DPPH· free radical scavenging activity was measured using the method of Mokbel and Hashinaga[16]. A 100 µl of extract or standard solution was dissolved in methanol at different concentrations from 0 to 550 µg/ml. The solution was sequentially mixed with 2 ml of 0.05 M acetate buffer (pH 5.5) and 1.9 ml of methanol and 1 ml of 0.3 mM DPPH· in methanol. As soon as the addition of DPPH·, the tubes were mixed immediately and left to stand at room temperature in a dark. The decrease in absorbance at 517 nm was measured (Shimadzu, Kyoto, Japan) after 30 min until the reaction reached a plateau. In this assay, blank included a 2 ml of 0.05 M acetate buffer (pH 5.5) + 1.9 ml methanol +100 µl extract or standard. Control group contained 2 ml of 0.05 M acetate buffer (pH 5.5) + 1 ml DPPH· + 2 ml methanol. The inhibitory percentage of DPPH· was calculated according to the formula:

Scavenging Effect (%)=$[(A_0-(A-A_b))/A_0] \times 100\%$, where: A_0: A_{517} of DPPH without sample (control); A: A_{517} of sample or standard and DPPH; A_b: A_{517} of sample or standard without DPPH[17]. Rutin was used as positive control. The IC_{50} value is the concentration of sample required to inhibit 50% of DPPH· radical. The inhibition 50% of DPPH· radical value for *Nepeta* acetone extract was calculated from the following calibration curve, determined by linear regression: Inhibition=[0.075 × amount of sample (µg)] –0.415 (R^2: 0.970).

Assay of Nitric Oxide ($NO^·$) Scavenging Activity

The assay of nitric oxide scavenging activity was employed by the method of Kumaran and Karanukaran[18]. A 3 ml of reaction mixture containing 2 ml of sodium nitroprusside (10 mM) and 0.5 ml of PBS (0.1 M, pH 7.4) was mixed with different concentration of acetone extract of *Nepeta* (10, 25, 50, 75, 100 and 150 µg) or standard solution dissolved in the methanol were incubated at 25 °C for 150 min. After incubation, 0.5 ml of the reaction mixture was treated with 0.5 ml of Greiss reagent (1% sulfanilamide, 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 5% H_3PO_4) and kept for 30 min at room temperature. The absorbance of chromophore resulting from the reaction was read at 548 nm in a spectrophotometer. Rutin was used as positive control. The IC_{50} value is the concentration of sample required to inhibit 50% of nitric oxide radical. The inhibition 50% of NO· radical value for Nepeta acetone extract was calculated from the following calibration curve, determined by linear regression: Inhibition=[0.28 × amount of sample (µg)] + 3.71 (R^2: 0.942).

Antimicrobial Assay

Bioassay of crude acetone extracts of *Nepeta meyeri* was evaluated by the disc diffusion method. Tested microorganisms were: *Staphylococcus aureus* (ATCC 95045), *Listeria monocytogenes* (NCTC 96040), *Bacillus subtilis* (ATCC 6633), *Bacillus cereus* (soil isolate), *Bacillus polymyxa* (soil isolate), *Bacillus megaterium* (soil isolate), *Bacillus thurungiensis* (soil isolate), *Shigella dysenteriae* (NCTC 4837), *Salmonella typhi* (NCTC 9394), *Escherichia coli* O157:H7 (EDL 931), *Yersinia enterocolitica* O3, *Pseudomonas aeruginosa* (clinic isolate), *Enterobacter cloaca, and the yeast Candida albicans* (ATCC 10231). All bacterial species were inoculated into 10 ml of Mueller Hinton Broth II (LabM, IDG) and incubated for 24 at 37ºC. The yeast was cultured into 10 ml of Sabouroud Dextrose Broth (30 ºC/48 h). Microbial suspensions prepared in sterile 0.85% saline corresponding in an optical density of 0.5 McFarland standards corresponding to 10^8 cfu/ml. A 100 µl from each culture was added on to the Mueller Hinton Agar II (Lab M™, IDG plc, UK) for bacteria and Sabouraud Dextrose Agar (Lab M, IDG) for the yeast studied. Antibiotic susceptibility blank discs (6 mm in diameter, Oxoid) impregnated with 62.5-4000 µg of the test materials placed onto appropriate agar media. Each plate also received a control disc, prepared by applying acetone each to disc and drying at room temperature. The plates were inverted and pre-incubated at 4°C for 2 h to allow uniform diffusion into the agar medium. The incubation temperature for bacteria, yeast was 37°C (24-48 h), 30°C (24-48 h), respectively. The antimicrobial activity was evaluated by measuring the inhibition zone diameter formed around the disc. The antimicrobial assay was carried in duplicate and the readings were expressed as average values of inhibition.

Statistical Analysis

All assays were conducted in duplicate and the data were reported as the mean ± SD. Different concentration of *Nepeta* acetone extract were analyzed and then half-maximal inhibitory concentration (IC_{50}) values for all the experiments were calculated by linear regression analysis.

Results

Figure 1 shows reducing capacity of acetone extract of *Nepeta* compared with BHT. The reducing capacity of a compound shows the potential antioxidant activity of that compound. In this research, the reducing power of *Nepeta* acetone extract increased with increasing amount of sample (R^2: 0.996).

Nepeta acetone extract added into 0.3 mM $DPPH^{\cdot}$ containing medium at different amounts was compared with Rutin standard for $DPPH^{\cdot}$ free radical scavenging activity (Figure 2). Results analyzed by linear regression showed that IC_{50} of *Nepeta* extract that scavenged 50% of the $DPPH^{\cdot}$ radical in the medium was found to be 672.2 µg/ml (R^2=0.970).

$NO^{\cdot}$ radical was produced by using 10 mM sodium nitroprusside in phosphate buffered saline (PBS) buffer at pH 7.4, and *Nepeta* acetone extract which was added to the medium in different amounts was compared with Rutin standard for $NO^{\cdot}$ radical scavenging activity (Figure 3). $NO^{\cdot}$ radical plays an important role in many inflammatory processes. Following the linear regression analysis of data, it was found that IC_{50} of *Nepeta* extract which scavenged 50% of the $NO^{\cdot}$ radical in the medium was 165.32 µg/ml (R^2=0.942).

In the *Nepeta* acetone extract (1 mg) 12.86 µg pyrocatechol equivalents of phenols were detected. This result indicates a strong correlation between phenolic compounds and antioxidant capacity (R^2=0.996).

The results of the antimicrobial screening of the extract against microorganisms presented in Table I. Our results indicate that the extracts from *Nepeta meyeri* inhibit the growth of some of the tested microorganisms to various degrees. The acetone extract was found to be effective antimicrobial agent against gram positive bacteria. It was found that the acetone extract of the aerial parts of *Nepeta meyeri* were devoid of antibacterial and antiyeast activity against gram negative bacteria and the yeast examined. It showed activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus polymyxa*, *Bacillus megaterium*, and *Bacillus thurungiensis*. The maximum activity was found against *Staphylococcus aureus* and *Listeria monocytogenes*. No activity shown by the acetone extract against the Gram negative bacteria and the yeast.

Discussion

Many plants in Labiatae family are known to have antioxidant capacity. In a study, for example, ethanol extract of 16 plants belonging to *Ballota* species of Labiatae family which shows a distribution in Turkey were studied for the antioxidant activity on the lipid peroxidation and formation of superoxide anion. The extracts of all *Ballota* species were shown to have antioxidant activity and also inhibitory effect on lipid peroxidation by scavenging super oxide anion

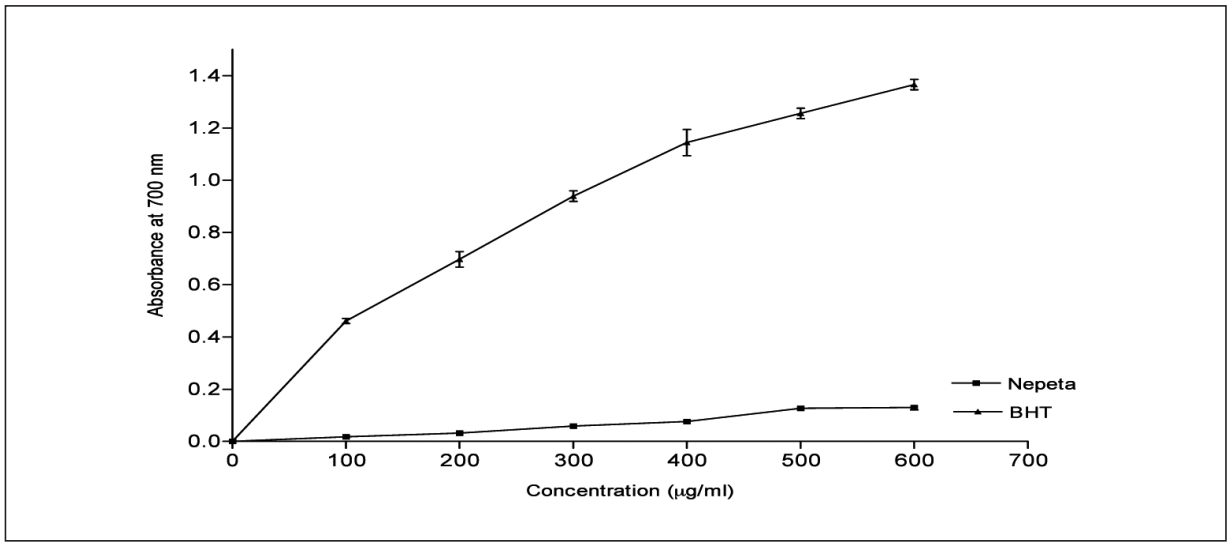

Figure 1. Reducing power of acetone extract of Nepeta and butylated hydroxy toluene (BHT).

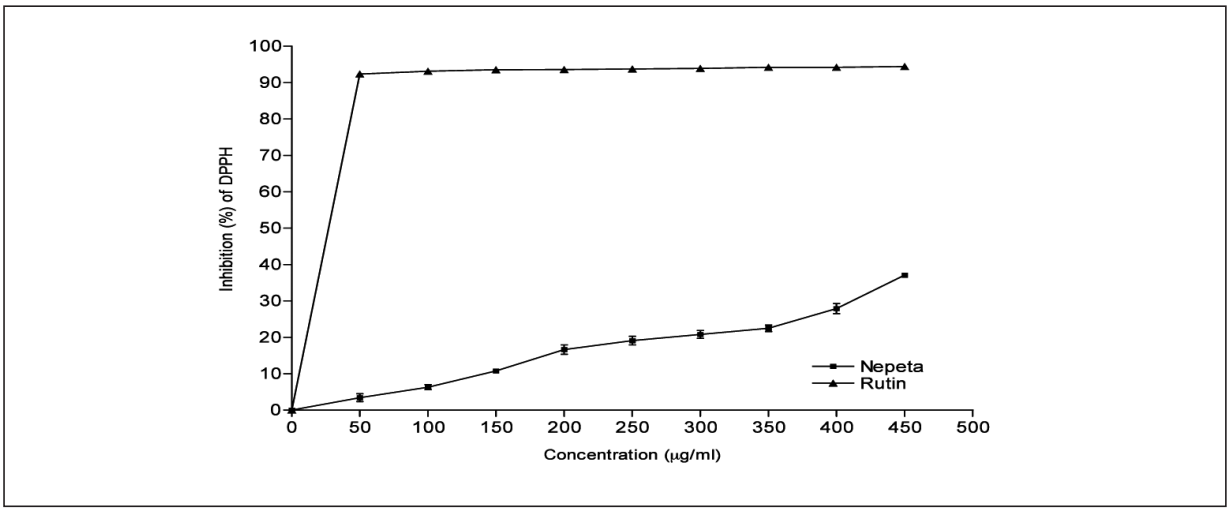

Figure 2. Radical scavenging activities of the Nepeta acetone extracts and Rutin against DPPH.

radicals[19]. In a separate study, the methanol extract of *Teucricum montbretii sp.* belonging to Labiatae family was studied for the antioxidant and antimicrobial activity. It was reported that the extract has a natural compound which prevents the auto-oxidation of lipids, and it was also suggested that the plant could be used as a natural food protectant due to its antibacterial and antioxidant activities[20]. Triantaphyllou et al[21] studied the antioxidant activity of water extract of 6 different aromatic plants which belong to Labiatae family in Greece. It was reported that the obtained extracts had protective effects against lipid peroxidation. Miceli et al[5] measured the radical scavenging activity of methanol extract of *Nepeta sibthorpii Bentham* plant by the DPPH· method. The IC_{50} value for the methanol extract of *Nepeta sibthorpii Bentham* was determined to be 97.18 µg/ml. In the same study, ursolic acid and polyphenols were separated from the *Nepeta* plants to find out the source of biological activity in the *Nepeta* plant. The IC_{50} for the

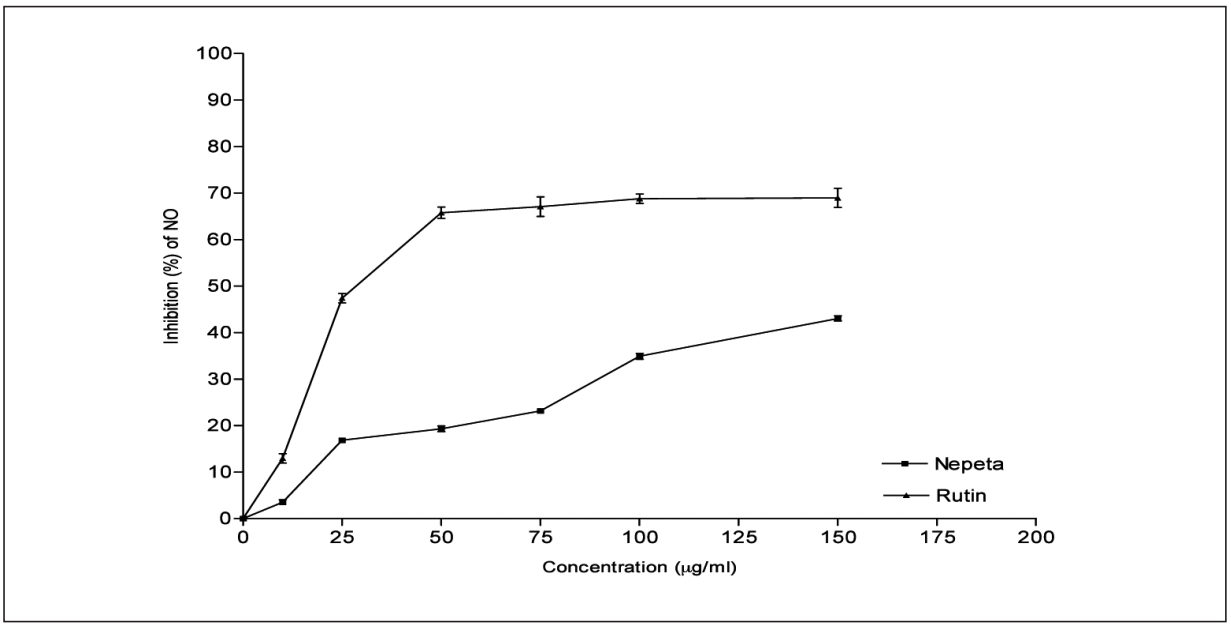

Figure 3. Inhibition of nitric oxide radicals by Nepeta and Rutin.

Table I. Antimicrobial activities of *Nepeta meyeri*.

Microorganism	Zone of inhibition (mm) Acetone extract (µg/disc)*						
	4000	2000	1000	500	250	125	62.5
Bacillus subtilis	10	10	10	9	9	8	–
Bacillus thurungiensis	10	9	9	8	–	–	–
Bacillus megaterium	10	10	9	8	8	8	8
Bacillus polymyxa	9	9	9	9	8	8	8
Bacillus cereus	10	9	9	8	–	–	–
Staphylococcus aureus	12	12	12	10	10	–	–
Listeria monocytog.	12	12	10	–	–	–	–
Salmonella typhi	–	–	–	–	–	–	–
Shigella dysenteriae	–	–	–	–	–	–	–
Yersinia enterocolitica	–	–	–	–	–	–	–
Enterobacter cloaca	–	–	–	–	–	–	–
Candida albicans	–	–	–	–	–	–	–

*Values are the mean of duplicates. Diameter of zone of inhibition (mm) included the diameter of disc. – = No activity against the tested microorganisms.

DPPH· scavenging activity of polyphenol fraction was 23.95 µg/ml. Similarly, Tepe et al[22] showed that various extracts of *Nepeta flavida* and their essential oils had antioxidant capacity.

Presence of radical scavenging activity of *Nepeta* determined in the present study supports the previous reports with respect to the antioxidant activity of *Nepeta*. It was reported that flavonoids, phenolic acids, essential oils and terpens found in plants of Labiatae family are the compounds leading to antioxidant effects[23]. Polyphenolic compounds existing in the structure of *Nepeta* could give the antioxidant effect. The most common herbal phenolic antioxidants are flavonoids, cinnamic acid derivatives, coumarins, tocopherols and phenolic acids. It was reported that phenolic compounds could directly contribute to the antioxidant activity of the plants[24]. The results of the antimicrobial screening of the extract against microorganisms presented in Table I. Our results indicate that the extracts from *Nepeta meyeri* inhibit the growth of some of the tested microorganisms to various degrees. The acetone extract was found to be effective antimicrobial agent against gram positive bacteria. It was found that the acetone extract of the aerial parts of *Nepeta meyeri* was devoid of antibacterial and antiyeast activity against gram negative bacteria and the yeast examined. It showed activity against *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus polymyxa*, *Bacillus megaterium*, and *Bacillus thurungiensis*. The maximum activity was found against *Staphylococcus aureus* and *Listeria monocytogenes*. No activity shown by the acetone extract against the Gram negative bacteria, could be due to the presence of compounds in the extract possessing lipophilic characteristic[25]. The present findings are in line with those from previous screenings of antimicrobial activities of some terrestrial plants, which showed activity against gram positive strains[25-32]. These differences may be attributed to the fact that the cell wall in gram positive bacteria consists of a single layer, whereas, the gram negative cell wall is a multi layered structure and quite complex[33].

In conclusion our results revealed that *Nepeta meyeri* has antioxidant capacity and potentially a rich source of antibacterial agents against most gram positive bacteria.

References

1) Dirmenci T, Yildiz B, Tumen G. Threatened categories of four Nepeta L. (Lamiaceae) species endemic to the East Anatolia. Turk J Bot 2004; 28: 221-226.

2) Topcu G, Ulubelen A. Structure elucidation of organic compounds from natural sources using 1D and 2D NMR techniques. J Mol Struct 2007; 834: 57-73.

3) Kaya A, Demirci B, Baser KHC. Micromorphology of glandular trichomes of Nepeta congesta Fisch & Mey. var. congesta (Lamiaceae) and chemical analysis of the essential oils. S Afr J Bot 2007; 73: 29-34.

4) Bourrel C, Perineau F, Michel G, Bessiere JM. Catnip (Nepeta cataria L.) essential oil: Analysis of chemical constituents, bacteriostatic and fungistatic properties. JEOR 1993; 5: 159-167.

5) Miceli N, Taviano MF, Giuffrida D, Trovato A, Tzakou O, Galati EM. Anti-inflammatory activity of extract and fractions from Nepeta sibthorpii Bentham. J Ethnopharmacol 2005; 97: 261-266.

6) Sattar AA, Bankova V, Kujumgiev A, Galabov A, Ignatova A, Todorova C, Popov S. Chemical-composition and biological-activity of leaf exudates from some lamiaceae plants. Pharmazie 1995; 50: 62-65.

7) Takeda Y, Ooiso Y, Masuda T, Honda G, Otsuka H, Sezik E, Yesilada E. Iridoid and eugenol glycosides from Nepeta cadmea. Phytochemistry 1998; 49: 787-791.

8) Stojanovic G, Radulovic N, Lazarevic J, Miladinovic D, Dokovic D. Antimicrobial activity of Nepeta rtanjensis essential oil. JEOR 2005; 17: 587-589.

9) Sonboli A, Salehi P, Yousefzadi M. Antimicrobial activity and chemical composition of the essential oil of Nepeta crispa Willd. from Iran. Z Naturforsch C 2004; 59: 653-656.

10) Saxena J, Mathela CS. Antifungal activity of new compounds from Nepeta leucoyhylla and Nepeta clarkei. Appl Environ Microb 1996; 62: 702-704.

11) Sefidkon F, Shaabani A. Essential oil composition of Nepeta meyeri Benth. from Iran. Flavour Frag J 2004; 19: 236-238.

12) Esmaeili A, Rustaiyan A, Masoudi S, Nadji K. Composition of the essential oils of Mentha aquatica L. and Nepeta meyeri Benth. from Iran. JEOR 2006; 18: 263-265.

13) Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE, Etherton TD. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. Am J Med 2002; 113(Suppl 9B): 71S-88S.

14) Oyaizu M. Studies of products browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. Japn J Nutr 1986; 44: 307-315.

15) Slinkard K, Singleton V. Total phenol analysis: automation and comparison with manual methods. Am J Enol Viticult 1977; 28: 49-55.

16) Mokbel MS, Hashinaga F. Antibacterial and antioxidant activities of banana (Musa, AAA cv. Cavendish) fruits peel. Am J Biochem Biotechnol 2005; 1: 125-131.

17) Shyu YS, Hwang LS. Antioxidative activity of the crude extract of lignan glycosides from unroasted Burma black sesame meal. Food Res Int 2002; 35: 357-365.

18) Kumaran A, Karunakaran RJ. Nitric oxide radical scavenging active components from Phyllanthus emblica L. Plant Food Hum Nutr 2006; 61: 1-5.

19) Citoglu GS, Coban T, Sever B, Iscan M. Antioxidant properties of Ballota species growing in Turkey. J Ethnopharmacol 2004; 92: 275-280.

20) Ozkan G, Kuleasan H, Celik S, Gokturk RS, Unal O. Screening of Turkish endemic Teucrium montbretii subsp pamphylicum extracts for antioxidant and antibacterial activities. Food Control 2007; 18: 509-512.

21) Triantaphyllou K, Blekas G, Boskou D. Antioxidative properties of water extracts obtained from herbs of the species Lamiaceae. Int J Food Sci Nutr 2001; 52: 313-317.

22) Tepe B, Daferera D, Tepe AS, Polissiou M, Sokmen A. Antioxidant activity of the essential oil and various extracts of Nepeta flavida Hub.-Mor. from Turkey. Food Chem 2007; 103: 1358-1364.

23) Erdemoglu N, Turan NN, Cakici I, Sener B, Aydin A. Antioxidant activities of some Lamiaceae plant extracts. Phytother Res 2006; 20: 9-13.

24) Duh P, Tu YY, Yen GC. Antioxidant Activity of Water Extract of Harng Jyur (Chrysanthemum morifolium Ramat). LWT. Food Sci Technol 1999; 32: 269-277.

25) Lall N, Meyer JJM. Antibacterial activity of water and acetone extracts of the roots of Euclea natalensis. J Ethnopharmacol 2000; 72: 313-316.

26) Mccutcheon AR, Ellis SM, Hancock REW, Towers GHN. Antibiotic screening of medicinal-plants of the British-Columbian Native Peoples. J Ethnopharmacol 1992; 37: 213-223.

27) Vlietinck AJ, Vanhoof L, Totte J, Lasure A, Vandenberghe D, Rwangabo PC, Mvukiyumwami J. Screening of 100 Rwandese medicinal-plants for antimicrobial and antiviral properties. J Ethnopharmacol 1995; 46: 31-47.

28) Rabe T, vanStaden J. Antibacterial activity of South African plants used for medicinal purposes. J Ethnopharmacol 1997; 56: 81-87.

29) Samy RP, Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J Ethnopharmacol 2000; 69: 63-71.

30) Ali NAA, Julich WD, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibac-

terial and cytotoxic activities. J Ethnopharmacol 2001; 74: 173-179.

31) Bhosale SH, Nagle VL, Jagtap TG. Antifouling potential of some marine organisms from India against species of Bacillus and Pseudomonas. Mar Biotechnol 2002; 4: 111-118.

32) Kelmanson JE, Jager AK, van Staden J. Zulu medicinal plants with antibacterial activity. J Ethnopharmacol 2000; 69: 241-246.

33) Essawi T, Srour M. Screening of some Palestinian medicinal plants for antibacterial activity. J Ethnopharmacol 2000; 70: 343-349.