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In vitro Antibacterial Activities of *Nepeta transcaucasica*

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The present results showed that the methanol extract of the *Nepeta transcaucasica* had antibacterial activity against 14 out of 50 bacteria with inhibition zone between 8-13 mm. The plant extract found to be more active against *Corynebacterium ammoniagenes*. Some of the extracts had the lowest MIC values (31.25 µg/mL) compared the standard drug, maxipime indicating the plant had an important natural antimicrobial source. These results also support the traditional use of this plant in folk medicine by local peoples living in rural areas in Turkey.

Key Words: Antibacterial activity, Bioassay, Methanol extract, *Nepeta transcaucasica*.

INTRODUCTION

Turkey is situated at the junction of Mediterranean, Irano-Turanian and Euro-Siberian regions where three different climates are occur. Therefore its flora, which is highly used with medicinal purposes, is rich and diverse with over 10000 vascular plant taxa including *Nepeta* and a total of 32 % of endemism[1].

The genus *Nepeta* L. is one of the largest genera in Lamiaceae and has approximately 250 species distributed mainly in south-west and central Asia, Europe, North Africa and North America[2,3]. The widest variation of types and the greatest abundance of species within the genus *Nepeta* is found in two regions: south-west Asia (especially Iran) and the western Himalayas including the adjacent Hindu-Kush Mountains[3].

In Turkish flora, *Nepeta* has a special importance because Turkish *Nepeta* represented by 44 taxa of which 22 are endemic to Turkey. In other word endemism ratio is very high (50 %)[4,5]. Endemic and non-endemic *Nepeta* species widely distributed in both East Anatolia and the Taurus Mountains located Mediterranean region.

In Turkey, many of *Nepeta* species has been collecting mostly by local peoples and are used folk medicine as fortifier, disinfectant, bacteriostatic as well as against eczema-type disorders[6].

As well known, many infectious diseases treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries[7]. In addition, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases[8]. Therefore, researchers are increasingly turning their attention to folk medicine looking for new leads to develop better drugs against microbial infections[9,10].

As a part of our continuing research study to evaluate the therapeutic potentials of Turkish aromatic and medicinal plants in East Anatolia Region of Turkey, in this paper the antimicrobial properties of methanol extract of aerial parts of *Nepeta transcaucasica* against 50 food-associated bacteria are reported.

EXPERIMENTAL

The plant material (aerial parts) of *Nepeta transcaucasica* was collected in Eastern Anatolia region of Turkey. Plant materials were further identified by senior taxonomists, Avni Ozturk, from Department of Botany, Canakkale Onsekiz Mart University, Canakkale, Turkey.

Methanol extraction: The air-dried and powdered plant materials (400 g) were extracted in a Soxhlet apparatus with methanol (MeOH) for 72 h[11]. The extracts were filtered using Whatman filter paper (No. 1) and then concentrated *in vacuo* at 40 ºC using a Rotary evaporator. The residues obtained were stored in a freezer at -80 ºC until further tests.

Bacterial strains: A total 50 bacteria species which are listed in Table-1 were used in this study. The bacteria, maintained on Nutrient Agar (Merck, Darmstadt, Germany) were supplied by Microbiology Laboratory of Agricultural Faculty of Ataturk University, Erzurum, Turkey. The food-associated bacteria were selected because they are frequently reported in foods. Identity of the bacteria used in this study was confirmed by Microbial Identification System in Biotechnology Application and Research Center at Ataturk University.

Antibacterial activity test: The methanol extract of plant was tested against a panel of microorganisms. The antibacterial activity of the extracts was carried out by disc diffusion test[12] using 100 µL of suspension containing 10^8 CFU/mL of bacteria spread on nutrient agar (NA) medium. Sterile 6 mm diameter filter paper discs were impregnated with 10 µg of essential oil or 300 µg of extract/disc and placed onto nutrient agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ofloxacin (5 µg/disc), sulbactam (30 µg) + cefoperazona (75 µg) (105 µg/disc) and/or netilmicin (30 µg/disc) were used as positive reference standards to determine the sensitivity of one strain in each bacterial species tested. The inoculated plates with food-associated bacteria were incubated at 27 °C for 24 h. The diameter of the clear zone around the disc was measured and expressed in millimeters as its antibacterial activity. Five discs per plate used and each test was run in triplicate[13].

Microdilution assays: The inocula of bacteria were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The minimal inhibitory concentration (MIC) of *Nepeta transcaucasica* extract against bacterial strains was determined based on a micro-well dilution method[14]. The 96-well plates were prepared by dispensing into each well 95 µL of nutrient broth and 5 µL of the inoculum. A 100 µL from *Nepeta transcaucasica* extracts initially prepared at the concentration of 500 µg/mL was added into the first wells. Then, 100 µL

from their serial dilutions was transferred into 6 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 negative control. The final volume in each well was 200 µL. Maxipime (Bristol-Myers Squibb) at the concentration range of 500-7.8 µg/mL was prepared in nutrient broth and used as standard drug for positive control. Contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by absorbance at 600 nm using the ELx 800 universal microplate reader (Biotek Instrument Inc., Highland Park, Vermont, USA) and confirmed by plating 5 µL samples from clear wells on nutrient agar medium. The extract tested in this study was screened two times against each organism. The MIC of each extracts was taken as the lowest concentration that showed no growth[15].

RESULTS AND DISCUSSION

The present study describes antibacterial activity of aerial parts of *Nepeta transcaucasica* against a number of food-borne bacteria. The microbial growth inhibition of aerial parts of the plants is summarized in Table-1.

As indicated in Table-1, methanol extract of *Nepeta transcaucasica* was found active against 14 out of 50 bacteria species tested.

The methanol extract of aerial parts of *Nepeta transcaucasica* was active against Gram-positive and Gram-negative bacteria with inhibition zone between 8-13 mm. (*Bacillus psychrosaccharolyticus*, *Bacillus* spp., *Bacillus subtilis*, *Corynebacterium ammoniagenes, Corynebacterium flavescens, Enterobacter hormaechei, Erwinia chrysanthemi, Flavimonas oryzihabitans, Kocuria kristinae, Kocuria rosea Micrococcus luteus, Paenibacillus apiarius, Paenibacillus macerans* and *Pseudomonas syringae syringae* (Table-1). The plant extract found to be more active against *Corynebacterium ammoniagenes* (13 mm inhibition zone). On the other hand the inhibition zone of all methanol extract had lower value than standard antimicrobial disc. For example as mentioned before the plant extract was more active against to *Corynebacterium ammoniagenes* with 13 mm inhibition zone. However, inhibition zone of standard antimicrobial disc against this microorganism was 20 mm (Table-1).

The minimal inhibitory concentration (MIC) values showed by the methanol extract were in the range of 31.25-500 µg/mL (Table-1). It was interesting that in most cases the plant extracts had lower MIC values than standard drug, maxipime indicating the plant can be used as antimicrobial agent and may possess antimicrobial compounds. Many reports on *Nepeta* species previously showed that the main constituents of the plant extract are diastereomeric nepetalactones and 1,8-cineole. These compounds are responsible for their feline attractant or insect repellant properties[16].

Some of the extracts had the same MIC values compared the standard drug, maxipime. According to these results, it is possible to conclude that *Nepeta transcaucasicus* possess a broad spectrum of antibacterial activity. To the best of our knowledge, this is the first study to provide data that the methanol extracts of *Nepeta transcaucasicus* evaluated against a wide range of bacteria.

TABLE-1
ANTIBACTERIAL ACTIVITY OF *Nepeta transcaucasica* METHANOL EXTRACTS AGAINST THE BACTERIA STRAINS BASED ON DISC DIFFUSION AND MICRODILUTION ASSAY

Bacterial species	Inhibition zone in diameter (mm)		MIC values (µg/mL)	
	Nepeta transcaucisica methanol extract	Positive control standard antibiotic disc	Maxipime	*Nepeta transcaucisica* methanol extract
Acidovorax facilis	-	28 (OFX)	250	-
Arthrobacter agilis	-	31(SCF)	7.81	-
Arthrobacter atrocyaneus	-	15 (OFX)	62.50	-
Arthobacter ilicis	-	20 (OFX)	7.81	-
Arthrobacter protophormiae	-	21(NET)	-	-
Bacillus cereus	-	21 (OFX)	7.81	-
Bacillus dipsauri	-	26 (OFX)	250	-
Bacillus lentimorbus	-	33 (OFX)	7.81	-
Bacillus lichemiformis	-	29 (OFX)	-	-
Bacillus megaterium	-	26 (OFX)	-	-
Bacillus marinus	-	14 (OFX)	-	-
Bacillus psychrosaccharolyticus	8	15 (OFX)	7.81	31.25
Bacillus pumilus	-	24(SCF)	-	-
Bacillus sphaericus	-	21 (OFX)	31.25	-
Bacillus spp	10	20(SCF)	7.81	31.25
Bacillus-subtilis	12	29 (OFX)	7.81	62.50
Brevibacillus agri	-	27 (OFX)	500	-
Brevibacillus brevis	-	32(NET)	7.81	-
Brevibacterium linens	-	22(SCF)		-
Chryseomonas luteola	-	30 (OFX)	31.25	-
Citrobacter amalonaticus	-	23(NET)	7.81	-
Corynebacterium ammoniagenes	13	20 (OFX)	7.81	62.50
Corynebacterium cystitidis	-	18 (OFX)	7.81	-
Corynebacterium flavescens	10	24 (OFX)	7.81	250
Enterococcus faecalis	-	10(SCF)	-	-
Enterobacter hormaechei	8	22 (OFX)	7.81	250
Enterobacter intermedius	-	16(SCF)	31.25	-
Enterobacter sakazakii	-	21(NET)	7.81	-
Erwinia corotovora	-	20(NET)	31.25	-
Erwinia chrysanthemi	9	17(SCF)	15.60	500
Exiguobacterium acetylicum	-	20 (OFX)	7.81	-
Flavimonas oryzihabitans	9	30 (OFX)	15.60	125
Kocuria kristinae	10	24(NET)	15.60	250
Kocuria rosea	12	15 (OFX)	31.25	250
Micrococcus luteus	8	28 (OFX)	62.50	250
Micrococcus lylae	-	30 (OFX)	7.81	-
Moraxella catarrhalis	-	18 (OFX)	7.81	-
Neisseria subflava	-	24 (OFX)	7.81	-

Paenibacillus apiarius	10	30 (OFX)	62.50	250
Paenibacillus macerans	-	30 (OFX)	250	-
Paenibacillus polymyxa	-	10 (OFX)	-	-
Pantoea agglomerans	-	30 (OFX)	7.81	-
Proteus vulgaris	9	20 (OFX)	62.50	62.50
Pseudomonas syringae syringae	9	15 (OFX)	250	250
Psychrobacter immobilis	-	20 (OFX)	15.60	-
Salmonella typhimuirum	-	28 (OFX)	-	-
Serratia liquefaciens	-	30 (OFX)	62.50	-
Shigella dysenteriae	-	21(NET)	7.81	-
Staphylococcus cohnii cohnii	-	12 (OFX)	7.81	-
Xanthomonas arboricola corylina	-	22 (OFX)	250	-

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REFERENCES

1. K.H.C. Baser, *Pure Appl. Chem.*, **4**, 527 (2002).
2. I.C. Hedge, Proceedings of The Royal Society of Edinburgh, Vol. 89B, pp. 23-25 (1986).
3. T. Dirmenci, *Bot. J. Linnean Soc.*, **147**, 229 (2005).
4. Z. Aytaç and G. Yildiz, *Turk. J. Bot.*, **20**, 385 (1996).
5. T. Dirmenci, Taxonomic Studies on *Nepeta* species (Lamiaceae) in Turkey, Ph.D. Thesis, Balikesir University (2003).
6. T. Baytop, Türkiye'de Bitkiler ile Tedavi. I.U. Yayinlari No. 3255, Eczacilik Fak. No. 40, p. 444 (1984) (in Turkish).
7. M. Zakaria, *Asia Pacific J. Pharmacol.*, **6**, 15 (1991).
8. R. Rojas, B. Bustamante, J. Bauer, I. Fernandez, J. Alban and O. Lock, *J. Ethnopharmacol.*, **88**, 199 (2003).
9. D. Srinivasan, N. Sangeetha, T. Suresh and P.L. Perumalsamy, *J. Ethnopharmacol.*, **74**, 217 (2001).
10. N. Benkeblia, *Lebensm.-Wiss. Technol.*, **37**, 263 (2004).
11. J. Lin, A.R. Opoku, M. Geheeb-Keller, A.D. Hutchings, S.E. Terblanche, A.K. Jager and J. van Staden, *J. Ethnopharmacol.*, **68**, 267 (1999).
12. J. Kim, M.R. Marshall and C. Wie, *J. Agric. Food Chem.*, **43**, 2839 (1995).
13. C.D. Djipa, M. Delmee and J. Quetin-Leclercq, *J. Ethnopharmacol.*, **71**, 307 (2000).
14. K.M.S. Swanson, F.F. Busta, E.H. Peterson and M.G. Johanson, in eds.: C. Vanderzant, D.F. Splittstoesser, Colony Count Methods, Compendium of Methods for Microbiological Examination of Food, American Public Health Association, Washington, edn. 3, pp. 75-95 (1992).
15. F. Sahin, I. Karaman, M. Gulluce, H. Ogutcu, M. Sengul, A. Adiguzel, S. Ozturk and R. Kotan, *J. Ethnopharmacol.*, **87**, 61 (2003).
16. K.H.C. Baser, B. Demircakmak, A. Altinas and H. Duman, *J. Essen. Oil Res.*, **10**, 299 (1998).

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