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Full Length Research Paper

Chemical composition and *in vitro* antimicrobial and antioxidant activities of the essential oil of *Nepeta nuda* L. subsp. *Albiflora* (Boiss.) gams

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The chemical composition of *Nepata nuda* essential oil was hydrodistilled and characterized by gas chromatography-mass spectrometry (GC-MS) and altogether 24 components were detected. The main constituents were trans-caryophyllene (23.9%), isopulegone (12.6%), cis-sabinol (10.1%), and β-pinene (10.0%). Antimicrobial activity of the oil against Gram-positive and -negative bacteria and fungus, was determined by the disc diffusion assay. The oil showed antibacterial activity against *Klebsiella pneumoniae* and *Salmonella typhi.* The antioxidant activity of the essential oil was investigated using 2,2-diphenylpicryl-hydrazyl (DPPH) radical scavenging assay and the β-carotene-linoleic acid test. butylated hydroxytoluene (BHT) was employed as a positive control. The essential oil of *N. nuda* showed weak antioxidant activities. The results suggest that *N. nuda* essential oil could be a natural antibacterial agent.

Key Words*: Nepeta nuda*, essential oil, antimicrobial and antioxidant activity, chemical composition.

INTRODUCTION

Nepeta (Lamiaceae) is a genus of perennial or annual herbs which is found in Asia, Europe and North Africa. There are more than 250 species of the genus *Nepeta (Lamiaceae)* throughout the world (Evans, 1996) and 33 species are present in Turkey, half of them being endemic (Davis, 1982).

Nepeta species are widely used in folk medicine because of their antispasmodic, expectorant, diuretic, antiseptic, antitussive, antiasthmatic and febrifuge activities (Baser et al., 2000; Newall et al., 1996; Zargari, 1990). The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents (Bhattacharjee et al., 2005). Essential oils and extracts of many plants have potential in medical procedures and applications in the pharmaceutical, cosmetic and food industry. Numerous researchers have shown interest to biologically active components, isolated from plants, and their influence on the elimination of pathogenic microorganisms (Tepe et al., 2005). The resistance developed by certain microorganisms against antibiotics initiated antimicrobial investigations and different applications of essentials oils (Jimenez-Arellanes et al., 2007; Hammer et al., 1999 and 1998; Nelson, 1997).

In the last few years, there has been a growing interest to providing natural antioxidants (Halliwell et al., 1999; Gutteride et al., 1994). The antioxidant properties of many herbs and spices have been reported effective in this respect (Kanner et al., 1994; Schuler, 1990). Many terpenoids have been identified as potential antioxidants (Choi et al., 2000). Free radicals, e.g., superoxide ions (O_2^-), hydroxyl radicals (OH·) and non-free radical compounds, can be responsible for lipid peroxidation (deterioration) in foods (Squadriato et al., 1998; Robinson et al., 1997; Halliwell, 1995) and for various diseases such as malaria, acquired immunodeficiency syndrome, been

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reported that some synthetic antioxidant compounds such as butylated hydroxytoluene and butylated hydroxyanisole, commonly used in processed foods, have side effects (Rice-Evans et al., 1997; Ito et al., 1983). Therefore, investigations on identifying the natural antioxidants have become very important issue (Gulcin et al., 2003; Vutto et al., 2000; Demo et al., 1998).

The essential oil composition of *Nepeta nuda* has been reported previously (Kokdil et al., 1998), but we have found no information on antimicrobial and antioxidant activities of *N. nuda*. Therefore the objective of this study was to evaluate the *in vitro* antimicrobial and antioxidant activities of the essential oil of *N. nuda*.

MATERIALS AND METHODS

Plant material

N. nuda plants were collected from the village of Cayozu, Cimen district (1850 - 2050 m), Divrigi-Sivas, Turkey when flowering (late February, 2006). The taxonomic identification was made during flowering season (Jully, 2006). The voucher specimen was identified by Dr. Erol Dönmez at the Department of Biology, Cumhuriyet Universty, Sivas-Turkey and has been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher No: ED 8802).

Isolation of the essential oil

The air-dried and finely ground aerial parts of *N. nuda* were subjected for 3 h to water distillation using a Clevenger- type apparatus (yield 2.1% v/w). The essential oil obtained was dried over anhydrous sodium sulphate and after filtration, stored at +4°C until tested and analyzed. The amount of distilled material obtained is 10.3 g.

Gas chromatography mass spectrometry (GC/MS) analysis

The chemical composition of *N. nuda* essential oil was analyzed using a Shimadzu QP5000 gas chromatograph/mass spectrometer (Kyoto, Japan) equipped with a GL Science capillary column TC-5 (30 m × 0.25 mm i.d., 0.25 mm) and a 70 eV EI quadrupole detector. For GC - MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1.9 ml/min. Injector and MS transfer line temperatures were set at 250 and 280°C, respectively. Column temperature was initially set at 40°C and held for 2 min, then gradually increased to 125°C at the rate of 2°C/min, held for 2 min, and finally increased to 250°C at 5°C/min held for 2 min. Diluted samples (1:100 v/v, in acetone) of 1.0 µl amounts were injected manually and in splitless manner for 10 s. The components were identified by comparison of Kovats indices with those reported in the literature, and their identities were confirmed by computer matching of their mass spectral fragmentation patterns with those of compounds in the NBS75K-MS Library and with MS Data reported in literature (NBS75K library data of the GC–MS system (Adams, 2001).

Diphenylpicrylhydrazyl (DPPH) assay for detection of antioxidant activity

The method is based on the reduction of alcoholic 2,2-diphenylpicryl-hydrazyl (DPPH) radical solutions in the presence of a hydrogen donating antioxidant. DPPH solutions show a strong absorption band at 517 nm appearing as a deep violet color. The absorption vanishes and the resulting decolorization is stoichiometric with respect to the number of electrons captured from the antioxidant. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant (Adams, 2001). Fifty microliter of various concentrations of the oils in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After 30 min of incubation at room temperature the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (1%) was calculated in the following manner:

$$\text{Inhibition \%} = \frac{(A_{blank} - A_{sample})}{A_{blank}} x100$$

Where A_{blank} is the absorbance of the control reaction (containing all 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 plotting inhibition percentage against extract concentration. Synthetic antioxidant reagent butylated hydroxytoluene (BHT) was used as the positive control and all 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 hydroperoxides arising from linoleic acid oxidation (Burits et al., 2000; Cuendet et al., 1997). The method is based on the loss of the yellow colour of β-carotene due to its reaction with radicals which are formed by linoleic acid oxidation in an emulsion. The rate of β-carotene bleaching can be slowed down in the presence of antioxidants. This fact is used in the antioxidant activity evaluation of the essential oil in comparison with, known, synthetic and natural antioxidants, namely BHT. A stock solution of β-carotene-linoleic acid mixture is prepared as follows: 0.5 mg β-carotene is dissolved in 1.0 ml of chloroform (HPLC grade), to which 25 µl of linoleic acid and 200 mg Tween 40 are added. Chloroform is completely evaporated using a vacuum evaporator. Then 100 ml distilled water saturated with oxygen (30 min 100 ml/min) is added with a vigorous shaking; 2.5 ml of this reaction mixture is dispersed into test tubes, and 350 µl portion of the extracts prepared in ethanol at 2 g/L concentrations is added and the emulsion system is incubated up to 48 h at room temperature. Same procedure is repeated with positive control BHT and a blank. After this incubation period absorbance of the mixtures are measured at 490 nm. Antioxidative capacities of the extracts are compared with those BHT at the same concentration and blank containing only 350 µl ethanol.

Microbial strains

An antimicrobial and antifungal activity of the essential oil was evaluated against three Gram-positive and five Gram-negative bacteria, and fungus by disk diffusion method. The microorganisms used were *Staphylococcus aureus* ATCC-25923, *Escherichia coli* ATCC-35218, *Pseudomonas aeruginosa* ATCC-27853, *Salmonella thyphi* NCTC-9394, *Klebsiella pneumoniae* NCTC-5046, *Proteus vulgaris* RSHM-96022, *Bacillus subtilis* ATCC-6633, *Corynebacterium diphteriae* RSHM-633 and *Candida albicans* ATCC-10231. All these cultures were obtained from the culture collections of the Department of Health of Refik Saydam Hygiene Center Contagious Diseases Research Department (Ankara-Turkey). Bacterial strains were cultured overnight at 37°C in Mueller Hinton Agar (MHA-Oxoid-CM 337). The yeast was cultured overnight at 30°C in Sabouraud dextrose agar (Oxoid-CM41). All the experiments were carried out in triplicate and average and standard deviation (SD) were calculated for the inhibition zone diameters.

Table 1. Chemical composition of *Nepeta nuda* essential oil.

No.	Retention time (min)	Compound	Composition (%)
1	2789	Etil asetate	1.40
2	13555	Camphene	1.81
3	15183	β-pinene	10.01
4	16418	β-myrcene	2.95
5	16852	p-Mentha-1,5,8-triene	0.85
6	18531	1.8 cineole	6.40
7	19517	Trans β ocimene	0.49
8	20667	γ-terpinene	0.29
9	21908	α-terpinolen	0.78
10	24500	α-campholene aldehyde	2.56
11	24.825	*cis*-sabinol	10.11
12	28650	3-caren-10-al	1.49
13	27615	Isopulegone	12.60
14	29450	Myrcenylacetate	0.93
15	34173	Pulegone	0.35
16	35860	Calarene	2.65
17	36092	Carvone	0.27
18	41528	Geranyl acetate	1.45
19	45983	trans-caryophyllene	23.97
20	46400	Farnecene	0.32
21	46600	Nerolidol	0.75
20	47702	Allo-Aromadendrene	0.25
22	47992	α-humulene	0.80
23	51050	Germacrene-D	2.75
24	53543	Spathulenol	7.35
Total			93.46

Antimicrobial assay (disc diffusion assay)

Agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oil in question (NCCLS, 2002). A suspension of the test microorganism (0.1 ml from 10^8 cells per ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 10 µl of the oil and placed on the inoculated plates. These plates, after staying at 4°C for 2 h, were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeast. The diameters of the inhibition zones were measured in millimeters.

RESULT AND DISCUSSION

Chemical composition of the essential oil

According to GC/MS analysis, 24 (93.5%) compounds were identified in *N. nuda* essential oil. Major components of the oil were trans-caryophyllene (23.9%), isopulegone (12.6%), cis-sabinol (10.1%), β-pinene (10.0%), and spathulenol (7.3%) as listed in Table 1. We could reach limited number of reports related to the essential oil compositions of *N. nuda*, *Nepeta depauperata, Nepeta sintenisii* and *Nepeta parnassica* (Sajjadi, 2005; Mehrabani et al., 2004; Gkinis et al., 2003; Kokdil et al., 1998), but, there are no reports on antimicrobial and antioxidant activities of the members of *N. nuda* genus in the literature. Kokdil et al. (1998), reported that essential oil of *N. nuda* growing in Turkey contained caryophyllene oxide (21.8%), spathulenol (13.8%), allo-aromadendrene (9.0%) and β-caryophyllene (5.4%) as major components. Mehrabani et al. (2004) reported that *N. depauperata* originating from Iran contained spathulenol (31.8%), β-caryophyllene (12.9%) and caryophyllene oxide (10.27%) as the major components of the oil. On the other hand, it was reported that essential oil of *N. sintenisii* in Iran contained nepetalactone (23.4%), elemol (16.1%), β-farnesene (9.5%) and 1,8-cineole (8.2%), (Sajjadi, 2005). Gkinis et al. (2003), reported that essential oil of *N. parnassica* growing in Greece contained nepetalactone (22.0%), 1,8-cineole (21.1%), α-pinene (9.5%) in sample A and 1,8-cineole (34.6%), nepetalactone (17.3%), α-pinene (11.4%) in sample B. The ratio of spathulenol was lower in our study compared to other studies. It is well known that these sorts of variations are due to geographical origin, harvesting time and growing conditions.

Antimicrobial activity

Many microorganisms, which cause damage to human health, exhibit drug resistance due to inadequate use of

Table 2. Antimicrobial activity of the essential oil of N*epeta nuda* using agar disc diffusion method.

Microorganisms	Essential oil of *Nepeta nuda*[a]	Gentamycin[b]	Nystatin[c]
Staphylococcus aureus	22 ± 0.8	23 ± 0.8	-
Escherichia coli	12 ± 0.6	16 ± 1.0	-
Pseudomonas aeruginosa	6 ± 0.0	20 ± 1.1	-
Salmonella thyphi	16 ± 0.8	10 ± 0.5	-
Klebsiella pneumonia	38 ± 1.5	20 ± 0.7	-
Proteus vulgaris	11 ± 0.4	22 ± 1.4	-
Bacillus subtilis	12 ± 0.9	29 ± 1.2	-
Corynebacterium diphteriae	13 ± 0.1	23 ± 1.1	-
Candida albicans	22 ± 0.5	-	25 ± 0.9

[a]Agar disc diffusion method, diameter of inhibition zone (mm) including disk diameter of 6 mm; [b]antibacterial; [c]antifungal. Results are means of three different measurements.

Table 3. Antioxidant activity of essential oil of *Nepeta nuda* and positive control (BHT) with the free radical DPPH scavenging and β-carotene-linoleic acid methods.

Essential oil	Inhibition IC_{50} (µg/ml) by DPPH	Inhibition percent (µg/ml) by β-carotene-linoleic acid
Nepeta nuda	-	24
BHT	10.5	100

antibiotics. Thus, there is a need for the discovery of new substances from natural sources, including plants. The *in vitro* antimicrobial activity by the agar disc diffusion method of the essential oils of *N. nuda* resulted in a range of growth inhibition pattern against pathogenic microorganisms given in Table 2.

In the present study, strongest antimicrobial activity was observed against the *K. pneumoniae* and *S. typhi.* The oil showed weak antimicrobial activity against *S. aureus*, *E. coli*, *C. diphteriae, P. vulgaris, B. subtilis* and *C. albicans*. The essential oil has no activity against *P. aeruginosa.*

Antioxidant activity

Essential oils were individually assessed for their possible antioxidative activities by employing two complementary tests; DPPH free radical-scavenging and β-carotene-linoleic acid assays were given in Table 3. IC_{50} value of *N. nuda* essential oil was not found, while IC_{50} value for BHT was found to be 10.5 µg/mL. β-carotene-linoleic acid assay also identified the 24% inhibition. The essential oil of *N. nuda* showed a weak antioxidant activity.

The illnesses which are caused by the oxygenated radicals are detected in recent years. Starting the usage of natural antioxidants had been important due to the prophylaxis of these illnesses. *Nepeta* species are widely used in folk medicine because of their antispasmodic, expectorant, diuretic, antiseptic, antitussive, antiasthmatic and febrifuge activities (Baser et al., 2000; Nevall et al., 1996; Zargari, 1990).

In conclusion, this study shows that *in vitro* good antimicrobial activities (for *K. pneumonia* and *S. typhi)* and weak antioxidant activity of the *N. nuda* essential oil. These results suggest that the essential oil of *N. nuda* could be used as a natural antibacterial agent for human and infectious diseases. The results reported here can be considered as the first information on the antimicrobial and antioxidant properties of *N. nuda*. Any of these substances in *N. nuda* essential oil may be responsible for these effects. On the other hand, further detailed studies of essential oils and extracts of *N. nuda* is required to determine which of their components are more responsible for its antioxidant effect and to clarify their cytotoxicity and other biological properties.

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REFERENCES

Adams RP (2001). Identification of essential oil components by gas chromatography /quadrupole mass spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois.

Alho H, Leinonen J (1999). Total antioxidant activity measured by chemiluminescence method. Methods Enzymol 299: 3-15.

Baser KHC, Kirimer N, Kurkcuoglu M, Demirci B (2000). Essential oil of *Nepeta* species growing in Turkey. Chem. Nat. Comp. 36: 356-359.

Bhattacharjee I, Ghosh A, Chandra G (2005). Antimicrobial activity of the essential oil of *Cestrum diurnum* (L.) (Solanales: Solanaceae). Afr. J. Biotechnol. 4: 371-374.

Burits M, Bucar F (2000). Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14: 323-328.

Choi HS, Song HS, Ukeda H, Sawamura M (2000). Radical-scavenging

activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl. J. Agric. Food Chem. 48: 4156-4161.
Cuendet M, Hostettmann K, Potterat O, Dyatmiko W (1997). Iridoid glucosides with free radical scavening properties from *Fagraea blumei.* Helv. Chim. Acta. 80: 1144-1152.
Davis PH (1982). Flora of Turkey and the East Aegean Islands. Edinburgh University Press. Edinburgh pp. 264-282.
Demo A, Petrakis C, Kefalas P, Boskou D (1998). Nutrient antioxsidants in some herbs and Mediterranean plant leaves. Food Res. Int. 31: 351-354.
Evans WC (1996). Trease and Evans' pharmacognosy: W.B. Saunders Company, London p. 48.
Gkinis G, Tzakou O, Iliopoulou D, Roussis V (2003). Chemical Composition and Biological Activity of *Nepeta parnassica* Oils and Isolated Nepetalactones. Z Naturforsch 58: 681-686.
Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI (2003). Screening of antioxidant and antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. Food Chem. 83: 371-382.
Gutteridge JMC, Halliwell B (1994). Antioxidants in nutrition, health and disease. Oxford University Press, Oxford.
Halliwell B, Gutteridge JMC (1999). Free radicals in biology and medicine. Oxford University Press, Oxford.
Halliwell B (1995). How to characterize an antioxidant: an update. Biochem. Soc. Symp. 61: 73-101.
Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86: 985-990.
Hammer KA, Carson CF, Riley TV (1998). In-vitro activity of essential oils, in particular Melaleuca alternifolia (tea tree) oil and tea tree oil products, against Candida spp. J. Antimicrob. Chemother. 42: 591-595.
Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D (1993). Dietary antioxidant flavonoids and risk of coronary heart disease: The Zupthen elderly study. Lancet. 342: 1007-1014.
Ito N, Fukushima S, Hassegawa A, Shibata M, Ogiso T (1983). Carcinogenicity of butylated hydroxyanisole in F344 rats. J. Natl. Cancer Inst. 70: 343-347.
Jimenez-Arellanes A, Meckes M, Ramirez R, Tores J, Luna-Herrera J (2007). Activity against multidrug-resistant *Mycobacterium tuberculosis* in Mexican plants used to treat respiratory diseases. Phytother. Res. 17: 903-908.
Kanner J, Frankel E, Granit R, German B, Kinsella JE (1994). Natural Antioxidants in Grapes and Wines. J. Agric. Food Chem. 42: 64-69.
Kokdil G, Kurucu S, Yıldız A (1998). Essential oil composition of *Nepeta nuda* L. ssp. *nuda.* Flavour Frag. J. 13: 233-234.
Mehrabani M, Asadipour A, Saber AS (2004). Chemical Constituents of the Essential Oil of *Nepeta depauperata* Benh. From Iran. Daru. 12 (3): 98-100.
National Committee for Clinical Laboratory Standards (NCCLS) (2002). Performance standards for antimicrobial susceptibility testing, 12th informational supplement. Wayne, Pa.
Nelson RRS (1997). In-vitro activities of five plant essential oils against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium.* J. Antimicrob. Chemother. 40: 305-306.
Newall CA, Anderson LA, Phillipson JD (1996). Herbal medicines, a guide for health-care professionals. The Pharmaceutical Press. London p. 154.
Rice-Evans CA, Sampson J, Bramley PM, Holloway DE (1997). Why do we expect carotenoids to be antioxidants *in vivo*. Free Radical Res. 26: 381-398.
Robinson EE, Maxwell SRJ, Thorpe GHG (1997). An investigation of the antioxidant activity of black tea using enhanced chemiluminescence. Free Radical Res. 26: 291-302.
Sajjadi SE (2005). Analysis of the essential oil of *Nepeta sintenisii* Bornm. From Iran. Daru. 13: 61-64.
Schuler P (1990). Natural antioxidants exploited commercially. In: Hudson BJF. ed. Food Antioxidants. Elsevier Applied Science, London pp. 99-170.
Squadriato GL, Peyor WA (1998). Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite and carbon dioxide. Free Radical Bio. Med. 25: 392- 403.
Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of Salvia tomentosa Miller (Lamiaceae). Food Chem. 90: 333-340.
Vuotto ML, Basile A, Moscatiello V, De Sole P, Castaldo-Cobianchi R, Laghi E, Lucia Ielpo MT (2000). Antimicrobial and antioxidant activities of *Feijoa sellowiana* fruit. Int. J. Antimicrob. Agents 13: 197-201.
Zargari A (1990). Medicinal plants. Tehran University Publications, Tehran pp. 106-111.