Natural Product Sciences
12(4) : 226-231 (2006)

Antioxidative and Antimicrobial Activities of Essential oil from *Artemisia vulgaris*

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Abstract – *Artemisia vulgaris*, one of the most religious plants in Nepal, is used in the treatment of various ailments. In this study, antioxidative activity of essential oil from *A. vulgaris* was evaluated, using different *in vitro* methods and antimicrobial activity by disc diffusion method against skin disease microorganisms such as *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, Candida albicans* and *Propionibacterium acnes*. The essential oil exhibited a concentration-dependent antioxidant activity. It showed strong metal chelating activity with low reducing and antioxidant power. However, gradual increase in radical scavenging activity was obtained with increasing concentration and reaction time. It also possessed a broad spectrum of antimicrobial activity and notable susceptibility was observed against *S. pyogenes* and *P. acnes*
Keywords – *Artemisia vulgaris,* essential oil, antioxidative activity, antimicrobial activity, skin disease microorganism

Introduction

Plant volatile oils are known to be potential natural agents for food preservation as their effectiveness against a wide range of microorganisms has well been established. Essential oils have been reported to have a range of biological properties. The major properties amongst are their antibacterial, antifungal and antioxidant properties (Deans and Waterman, 1993). Recently many essential oils have been qualified as natural antioxidants (Aeschbach *et al.*, 1994; Yanishlieva *et al.*, 1999; Gianni *et al.*, 2005) and are suggested as a potential substitute of synthetic antioxidants. Furthermore, essential oils and their components are gaining an interest because of their relatively safe status, wide acceptance by consumers, and exploitation for potential multi-purpose functional use (Ormancey *et al.*, 2000; Sawamura, 2000).

Artemisia vulgaris Linn (family: *Compositae*) is a common shrub found in mountains of Nepal (1,500-3,600 m). It is one of the most religious plants in Nepal and is offered in many ritual celebrations. It is also used extensively in spiritual treatment of patient. Infusion of leaves and flowering tops are used in nervous and spasmodic affections, asthma and diseases of brain (Medicinal plants of Nepal, 1997). Many *Artemisia* species have a characteristic scent or taste, caused by monoterpenes and sesquiterpenes, which in many cases are the reason for their application in folk medicine (Khaled *et al.*, 2002).

Up until now, there has been no sufficiency previous report on antioxidant and antimicrobial activity of essential oil from *A. vulgaris*. Therefore, in this study, we evaluated antioxidant capacity of essential oil from *A. vulgaris*, using different in vitro methods and antimicrobial activity against skin disease microorganisms.

Experimental

Chemicals – Butylated hydroxy anisole (BHA), α tocopherol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), linoleic acid, ascorbic acid, ferrozine, potassium ferricyanide, trichloroacetic acid (TCA), ferrous chloride and sodium phosphate (monobasic and dibasic) were purchased from Sigma-Aldrich Co. (St.Louis). Ethylenediaminetetraacetic acid (EDTA) was purchased from Yakuri Pure Chemicals, Osaka, Japan, ferric chloride from Junsei Chemical Company, Japan. All other reagents were of analytical grade. Microbial media such as nutrient broth, brain heart

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infusion broth and potato dextrose broth was obtained from Difco, USA. Recordings were made in a UV-vis Diode Array Spectro-photometer, Hewlett Packard 8453. IR spectrum was recorded on a JASCO FT/IR-5300 spectro-meter using KBr disk. ^{1}H-NMR spectrum was recorded on a JEOL FT-500 MHz NMR spectrometer. Tetramethylsilane was used as an internal standard.

Plant material – Fresh leaves of *A. vulgaris* from its natural habitat were collected from Lalitpur district, Nepal during their late vegetative stage (July 2003). 500 g of leaves were hydro distillated for 4 hours, using Clevenger type apparatus to give 0.2% yield. The obtained essential oil was dried over anhydrous sodium sulphate and after filtration stored at 4 °C until analyzed. The oil has pale yellow colour with powerful, fresh-camphoraceous aroma. Essential oil sample was deposited in Natural product research laboratory, Wonkwang University, Korea.

Antioxidative activity

DPPH radical-scavenging activity – The hydrogen atoms or electrons donating ability of the essential oil from *A. vulgaris* was measured from the bleaching of purple coloured methanol solution of DPPH (kordali *et al.*, 2005a). Briefly, one mL of 0.5 mM DPPH solution in methanol was mixed with 3 mL of sample solution in ethanol. After 30 minute incubation at room temperature, the absorbance was measured at 517 nm against blank. The percentage DPPH radical scavenging activity was determined using following equation.

DPPH radical scavenging (%) = [(control absorbance – sample absorbance) / control absorbance] × 100

Moreover, to understand its kinetic behavior, the decrease in absorbance was studied until the reaction reached its plateau. Butylated hydroxyanisole (BHA), α-tocopherol and ascorbic acid were used as positive controls.

Metal chelating activity – The ferrous ions chelating activity was estimated as described by Dinis *et al.* (1994). Briefly, different concentrations (0.5-8 mg/ml) of the essential oil from *A. vulgaris* in 0.4 ml methanol were added to a 50 µl solution of $FeCl_2$ (2 mM). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL) and the total volume was adjusted to 4 mL with methanol. Then the mixture was shaken vigorously and left standing at room temperature for 10 min. The absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula:
Metal chelating effect (%) = $[(A_0 - A_1)/A_0] \times 100$, Where A_0 is the absorbance of the control, and A_1 is the absorbance of the samples and standards. The control contains $FeCl_2$ and ferrozine, the complex formation molecules.

Reducing power determination – The reducing power of the essential oil from *A. vulgaris* and standards was determined following Oyaizu (1986). Different amounts of the essential oil (0.5-8 mg/ml) in 1 ml methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 1000 g. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increase in the absorbance of the reaction mixture indicated increase in the reducing power.

Determination of total antioxidant activity by ferric thiocyanate (FTC) method – The lipid peroxidation assay was carried out as described in the modified method of Kikuzaki and Nakatani (1993). FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation. The peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment. In this method, the concentration of peroxide decreases as the antioxidant activity increases. Briefly, a mixture of 20 mg essential oil from *A. vulgaris* in 4 mL absolute ethanol (Merck) 4.1 mL of 2.52% linoleic acid (Sigma) in absolute ethanol, 8 mL of 0.05 M phosphate buffer (pH 7.0), and 3.9 mL of water was placed in a vial with a screw cap and then incubated at 40 °C in the dark. To 0.1 mL of this solution was added 9.7 mL of 75% ethanol and 0.1 mL 30% ammonium thiocyanate (Sigma). Precisely 3 min after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm for every 24 hours until the absorbance of the control reached maximum. The control and standard were subjected to the same procedures as the sample except that for the control, only the solvent was added, and for the standard, sample was replaced with 4 mg of Butylated hydroxy anisole (BHA) and α tocopherol.

Antimicrobial activity

Test microorganisms – The antimicrobial activity of essential oil from *A. vulgaris* was measured using skin disease microorganisms. The list of microorganisms used is given in Table 1. Microorganisms were provided by KCTC (Korean Collection for Type Cultures, Korea).

Disc diffusion method – The antimicrobial activity of essential oil from *A. vulgaris* was carried out by the disc diffusion method (Murray *et al.*, 1995) using 100 µL of suspension containing 10^8 CFU/mL of bacteria and 10^6

Table 1. The list of microorganisms used in this study

microorganisms (KCTC No.)	medium	incubation condition
Staphyloscoccus aureus (KCTC 1927)	nutrient Agar (Difco USA)	aerobic 37 °C, 24 hr
Staphylococcus epidermidis (KCTC 1917)	nutrient Agar (Difco USA)	aerobic 37 °C, 24 hr
Streptococcus pyogenes (KCTC 3096)	brain Heart Infusion broth (Difco USA)	aerobic 37 °C, 24 hr
Candida albicans (KCTC 7965)	potato Dextrose Agar (Difco USA)	aerobic 37 °C, 24 hr
Propionibacterium acnes (KCTC 3314)	brain Heart Infusion Agar (Difco USA)	anaerobic jar 37 °C, 48 - 72 hr

CFU/mL of yeast spread on nutrient agar or brain heart infusion agar and potato dextrose agar medium, respectively. The discs (Whatman, 8 mm in diameter) which impregnated with 19 mg of essential oil were placed on the inoculated agar. Control disc containing only 19 mg of n-hexane employed to dissolve the essential oil showed no inhibition in a preliminary test. Commercial disc (BBL, USA) of tetracycline, amikacin and bovobiocin were used as positive reference standards to determine the sensitivity of strain in tested each microbial species. The inoculated plates were incubated at 37 °C for optimum incubation time of each strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms in comparison to a control of negative and reference standards.

Statistical analysis – The data are results of triplicate experiments. Microsoft Excel was used to compute means, standard deviation, correlation and regression. Differences among all sample means were determined by one- way analysis of variance (ANOVA) using Origin (Micro cal Software, Inc.). P value less than 0.05 was considered as statistically significant.

Results and Discussion

Antioxidative activity

DPPH radical-scavenging activity – Essential oil from *A. vulgaris* exhibited DPPH radical scavenging activity in concentration dependent manner ($r^2 = 0.9045$); however, the activity was much lower than that of reference standards (Fig. 1). Antioxidants are believed to intercept the free radical chain of oxidations and to contribute hydrogen from the phenolic hydroxyl groups themselves. But, essential oil of *Artemisia* species were reported to contain less phenolic components (Kordali *et al.*, 2005a) and their poor antiradical activity might be due to the less availability of hydrooxy group to scavenge the free radicals. Essential oil of some *Artemisia* species was reported to have moderate to weak DPPH radical scavenging activity (Kordali *et al.*, 2005a and b).

The present study showed that the reaction rate of

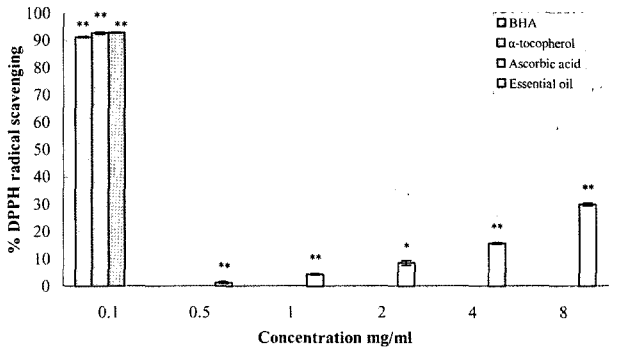

Fig. 1. DPPH radical scavenging activity of essential oil from *Artemisia vulgaris*, each value is expressed as mean ± SD (n = 3), Means within each bar are significantly different, $^{*}p < 0.05$, $^{**}p < 0.01$.

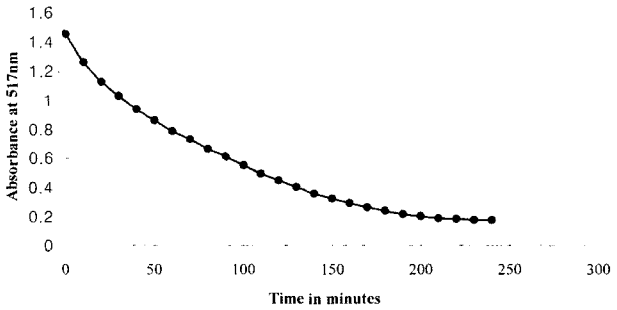

Fig. 2. Bleaching of DPPH radical by essential oil from *Artemisia vulgaris*.

essential oil was very slow, which reached its plateau after 220 minutes. As mentioned by Brand-William *et al.* (1995), in the case of slower reaction kinetic behavior, antiradical power determined at 30 minutes would be erroneous because the reaction would be still progressing. In this study also, at 30 minute incubation, essential oil (8 mg/mL) exhibited only 30.13% radical scavenging activity where as at 220 minutes; it showed 84.44% inhibition of DPPH radical and remains constant thereafter (Fig. 2). This clearly showed that in spite of its slow reaction rate, essential oil could scavenge the free radicals.

Reducing power determination – The reducing properties are generally associated with the presence of reductones (Pin-Der-Duh, 1998), which have been shown

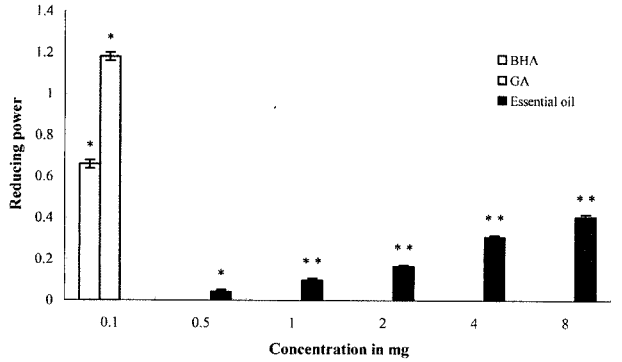

Fig. 3. Reducing power of essential oil from *Artemisia vulgaris*, each value is expressed as mean ± SD (n = 3), means within each bar are significantly different, * $p < 0.05$, ** $p < 0.01$.

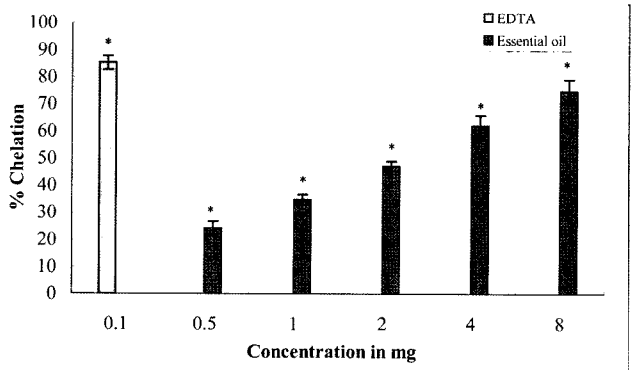

Fig. 4. Metal chelating activity of essential oil from *Artemisia vulgaris*, each value is expressed as mean ± SD (n = 3), means within each bar are significantly different, * $p < 0.05$.

to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. In the present study, essential oil form *A. vulgaris* reduced iron (III) in a concentration-dependent manner ($r^2 = 0.97$). However the activity was very low in comparison to reference standards BHA and Gallic acid (Fig. 3). A direct correlation between antioxidant activities and reducing power has been reported (Tanaka *et al.*, 1988). In this study a high positive correlation obtained between reducing power and DPPH radical scavenging ($r^2 = 0.9459$) showed a direct correlation between DPPH scavenging activity and reducing power.

Metal chelating activity – The production of highly reactive oxygen species (ROS) such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals is also catalysed by free iron through Haber-Weiss reaction. This "free" (loosely bound) iron can catalyse the formation of very injurious compounds, such as the hydroxyl radical (· OH) from compounds such as hydrogen peroxide, which are normal metabolic byproducts (Fenton reaction) In the present study, essential oil from *A. vulgaris* enabled to chelate ferrous ion in a concentration-dependent manner ($r^2 = 0.9969$). At the concentration of 8 mg/ml, essential oil chelated 75% of ferrous ions (Fig. 4). This showed the presence of strong ferrous ion chelating component in essential oil. However, the activity of EDTA was more pronounced than essential oil.

The key property of chelators is that the metal ion bound to the chelator is chemically inert and hence metal induced lipid peroxidation could be prevented. The result indicated that essential oil could in part act as secondary antioxidant by delaying or minimizing the free iron induced lipid peroxidation.

Determination of total antioxidant activity by ferric thiocyanate (FTC) method – The amount of peroxides formed during the initial stages of lipid oxidation was measured by FTC method for every 24 h, over a period of 96 hours. There was gradual increase in the absorbance of control from day 1 and reached at the highest level after 3 days and declined thereafter. As shown in figure 5, essential oil from *A. vulgaris* poorly inhibited the peroxide formation from the linoleic acid during the autoxidation process in comparison to reference standards. At 72 hour of incubation, the effectiveness of essential oil (final concentration 1 mg/mL), tocopherol (final concentration 0.02 mg/mL) and BHA (final concentration 0.02 mg/mL) in inhibiting the linoleic acid was 32.51%, 83.32% and 87.31% respectively (Fig. 5). Similar to our results, previous studies also have reported weak to medium antioxidant and DPPH radical scavenging activities of essential oils of *Artemisia* species (Kordali *et al.*, 2005a and b).

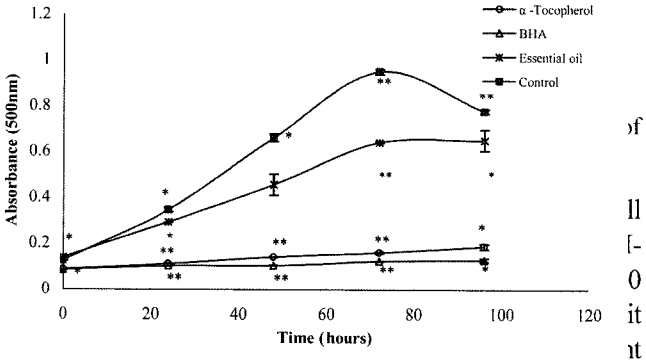

Fig. 5. Inhibition of linoleic peroxidation by essential oil from *Artemisia vulgaris* (1 mg/mL final concentration) essential oil by FTC method. Each value is expressed as mean ± SD (n = 3), means are significantly different, ** $p < 0.01$, * $p < 0.05$.

Antimicrobial activity – The antimicrobial activity of essential oil from *A. vulgaris* against skin disease microorganisms is shown in Table 2. The essential oil was

by interchain pressure (full line, τ_a=1462s).

In this contribution, we reconsider the Molecular Stress

Table 2. Antimicrobial activity of essential oil from *Artemisia vulgaris* against bacterial skin pathogens based on disc diffusion method

	inhibition zone (mm)				
	S. aureus	*S. epidermidis*	*S. pyogenses*	*C. albicans*	*P. acnes*
essential oil (9.5 mg)	16	15	18	14	18
essential oil (19 mg)	22	21	23	18	22
tetracyclin (30 μg/disc)	32	8	29	–	65
novobiocin (5 μg/disc)	28	31	24	–	18
amikacin (30 μg/disc)	19	20	30	–	21

shown to possess a broad spectrum of antimicrobial activity. The results revealed that essential oil exhibited variable levels of antimicrobial activity against tested microbial strains. In particular, the highest sensitivity was observed against *S. pyogenes* (inhibition zone 18 mm/9.5 mg, 23 mm/19 mg) and *P. acnes* (inhibition zone 18 mm/ 9.5 mg, 22 mm/19 mg). According to the literature (Deans, 1991; Beratta *et al.*, 1998; Marino *et al.*, 2001; Mimica-Dukic *et al.*, 2003), Gram-positive bacteria seemed to be more sensitive to essential oils examined than that of Gram-negative bacteria. Most of tested skin disease microorganisms are made up of Gram-positive bacteria and are strongly inhibited with the essential oil. The activity observed in the non-polar phase can be attributed to the presence of the phenolic compounds (Gora and Lewandowski, 1996). The active volatile components are probably responsible for the antimicrobial activity of the essential oils. It has been shown that phenolic components of essential oils exhibit the strongest antimicrobial activity, followed by camphor (Mario *et al.*, 1998) and 1,8 cineole (Carsn and Riley, 1969).

Analytical data – We standardized essential oil from *A. vulgaris* by nuclear magnetic resonance (NMR) spectrometry (Varian Unity 500, 500 MHz, Janpan). The essential oil was analysed by ^{1}H-NMR spectroscopy of $CDCl_3$ solubles. It gave the main biological compound, which the signals were clearly visible in the ^{1}H-NMR spectrum. Perhaps, the obvious were chemical shifts were between weak 5.6 ppm and 5.7 ppm indicative of lactone of exocyclic-α-methylene group, and olefinic groups between 4.6 ppm and 5.4 ppm in germacranolide-type compounds. It showed IR absorptions at 3474 (OH), 3073 (olefinic C-H stretching), 1746 (αβ-unsaturated five-ring lactone) and 1657 cm^{-1} (non-unconjugated alkene group) (Jakupovic *et al.*, 1992; Bohlmann *et al.*, 1984; Williams *et al.*, 1995).

In conclusion, these results suggested that essential oil from *A. vulgaris* could better act as secondary antioxidant by iron chelation rather than chain termination by radical-scavenging activity. Nevertheless, essential oil enabled to scavenge the free radicals, though the reaction rate was slow. Further study should be focused on its *in vivo* potential in animal models and identification of individual components responsible for the antioxidant activity. The essential oil also shows inherent antimicrobial activity and may ultimately be used therapeutically in this respect. Nevertheless, further studies examining some metabolic pathway are necessary.

Acknowledgment

We thanks to Ghrelu Hern Processing Centre, Chapagaun, Lalitpur, Nepal for allowing one of us (LR Bhatt) to use their lab for steam distillation of essential oil. Authors are thankful to Dr Lokendra Raj Sharma and Mr. puran Pd Kurmi, Departement of plant Resources (DPR), Ministry of Forest & Soil Conversation, Nepal for their help in identification of plant material and to Dr Prem Ratna Sthapit, Act. Director General DPR for allowing permission to use the plant material for the study. This work was supported by wonkwang university in 2006, Korea.

References

Aeschbach, R., LoÈ liger, J., Scott, B.C., Murcia, A., Butler, J., Halliwell, B., and Aruoma, O.I., Antioxidant action of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. *Food Chem. Toxic.*, **32**, 31-36 (1994).

Beratta, M.T., Dorman, H.J.D., Deans, S.G., Biondi, D.M., and Ruberto, G., Chemical composition, antimicrobial and antioxidant activity of laurel, sage, rosemary, oregano and coriander essential oils. *J. Essent. Oil Res.*, **10**, 618-627 (1998).

Bohlmann, F., Jakupovic, J., Robinson, H., and King, R., Heliangolides and germacrolides and Disynaphia multicrenulata. *Phytochem.*, **23**, 1435-1437 (1984).

Brand-Williams, W., Cuvelier, M.E., and Berset, C., Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.*, **28**, 25-30 (1995).

Carson, C.F. and Riley, T.V., Antimicrobial activity of the major components of the essential oil *Melaleuca arternifolia*. *J. Appl. Bacteriol.*, **78**, 264-269 (1995).

Deans, S.G., Evaluation of antimicrobial activity of essential (volatile)

oils. In Modern Method of Plant Analysis. Essential Oils and Waxes; Liskens HF, Jackson JF, Eds.;SpingerVerlag: Berlin Germany, **12**, 309-320 (1991).

Deans, S.G. and Waterman, P.G., In: R.K.M. Hay, & P.G. Waterman (Eds.), Volatile Oil Crops: Their Biology, Biochemistry and Production. London, Longman 113 (1993).

Dinis, T.C.P., Madeira, V.M.C., and Almeida, L.M., Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys.*, **315**, 161-169 (1994).

Gianni, S., Silvia, M., Mariavittoria, M., Martina, S., Stefano, M., Matteo, R., and Renato, B., Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem.*, **91**, 621-632 (2005).

Gora, J., Lis, A., and Lewandowski, A., Chemical composition of the essential oil of cultivated summer savory (*Satureja hortensis* L. cv. Saturn). *J. Essent. Oil Res.*, **8**, 427-428 (1996).

Gordon, M.H., The mechanism of antioxidant action *in vitro*. In: B.J.F. Hudson, Editor, Food antioxidants, Elsevier Applied Science, London, 1-18 (1990).

Jakupovic, J., Ganzer, P., Pritschow, L., Lehmann, F., Bohlmann, F., and King, R.M., Sesquiterpene lactones and other constituents from Vrsina species. *Phytochem.*, **31**, 863-880 (1992).

Khaled, F., El-Massry, A., El-Ghorab, H., and Farouk, A., Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. *Food Chem.*, **79**, 331-33 (2002).

Kikuzaki, H. and Nakatani, N., Antioxidant effects of some ginger constituents. *J. Food Sci.*, **58**(6), 1407-1410 (1993).

Kordali, S., Cakir, A., Mavi, A., Kilic, H., and Yildirim, A., Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish *Artemisia* species. *J. Agric. Food Chem.*, **53**, 1408-16 (2005a).

Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A., and Yildirim, A., 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*, *Artemisia dracunculus*, *Artemisia santonicum*, and *Artemisia spicigera* Essential Oils. *J. Agric. Food Chem.*, **53**(24), 9452-9458 (2005b).

Marino, M., Bersani, C., and Comi, G., Impendance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *Int. J. Food Microbiol.*, **17**(3), 187-195 (2001).

Mario, D.I.M., Alessandra, T.P., Antonio, F., and Carmela, C., In vivo activity of Salvia officinalis oil against *Botrytis cinerea*. *J. Essent. Oil Res.*, **10**, 157-160 (1998).

Medicinal plants of Nepal, Bulletin of the Department of Medicinal Plants Number 3, His Majestys' Government of Nepal, Ministry of Forest and Soil Conservation, Department of Plant Resources, Thapathali, Kathmandu, Nepal, 1997.

Mimica-Dukic, N., Bozin, B., Sokovic, M., Mihajlovic, B., and Matavulj, M., Antimicrobial and antioxidant activity of three *Mentha* species essential oils. *Planta Med.*, **69**, 413-419 (2003).

Murray, P.R., Baron, E.J., Pfalletr, M.A., Tenover, F.C., and Yolke, R.H., Manual of clinical microbiology, 6th ed., ASM, Washington, DC. 1995.

Ormancey, X., Sisalli, S., and Coutiere, P., Formulation of essential oils in functional perfumery,. *Parfums Cosmetiques Actualites,* **157**, 30-40 (2001).

Oyaizu, M., Studies on product of browning reaction prepared from glucose amine. *Jpn. J. Nutr.*, **44**, 307-315 (1986).

Pin-Der-Duh, X., Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free-radical and active oxygen. *J. Am. Oil Chem. Soc.*, **75**, 455-461 (1998).

Sawamura, M., Aroma and functional properties of Japanese yuzu (*Citrus junos* Tanaka) essential oil. *Aroma Res.*, **1**, 14-19 (2000).

Tanaka, M., Kuie, C.W., Nagashima, Y., and Taguchi, T., Application of Antioxidative Maillard Reaction Products from Histidine and Glucose to Sardine Products. *Nippon Suisan Gakkaishi,* **54**, 1409-1414 (1988).

Williams, D.H. and Fleming, J., Spectroscopic methods in organic chemistry, New York: Mcgraw-Hill, 28-62 (1995).

Yanishlieva, N.V., Marinova, E.M., Gordon, M.H., and Raneva, V.G., Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chem.*, **64**, 59-66 (1999).

(Accepted October 17, 2006)