Evaluation of Antioxidant Activity of Essential Oil from *Artemisia vulgaris*

Lok Ranjan Bhatt, Jae Sug Lee[1], Seung Hwa Baek*

Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University,
1 : Department of Hair Design, Cheongyang College

Artemisia vulgaris essential oil exhibited a strong metal chelating activity but withlow reducing power, and radical scavenging activity. However, a gradual increase in the radical scavenging activity was obtained with increasing concentration and reaction time.

Key words : *Artemisia vulgaris* essential oil, antioxidant activity, metal chelating activity, reducing power

Introduction

Plant volatile oils are known to have potential natural agents for food preservation as their effectiveness against a wide range of microorganisms has been well established. Essential oils have been reported to have a range of biological properties. The major properties amongst are their antibacterial, antifungal and antioxidant properties[2)]. Recently many essential oils have been qualified as natural antioxidants[1,4,15)] and are suggested as a potentialsubstitute of synthetic antioxidants. Furthermore, essential oils and their components are gaining aninterest because of their relatively safe status, wide acceptance by consumers, and exploitation for potential multi-purpose functional use[11,14)].

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 manyritual 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). 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[6)]. So far a few species of Nepalese plants have been studied for their antioxidant activity and there is no previous report on antioxidant activity of *Artemisia vulgaris* essential oil. Therefore, the present study was conducted to identify antioxidant propertiesof essential oil of this plant. We evaluated antioxidant capacity of *Artemisia vulgaris* essential oil using different in vitro methods.

* To whom correspondence should be addressed at : Seung Hwa Baek, Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan 570-749, Korea.

· E-mail : shbaek@wonkwang.ac.kr, · Tel : 063-850-6225

· Received : 2006/12/26 · Accepted : 2007/02/09

Materials and Methods

1. Chemicals

Octadecyl-functionalized silica gel, butylated hydroxy anisole(BHA), α-tocopherol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) linoleic acid, gallic acid (GA),ascorbic acid, ferrozine, potassium ferricyanide, trichloroacetic acid (TCA), ferrous chloride, 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.

2. Plant material

Artemisia vulgaris essential oil (steam distillation of the leaves) was obtained from Gharelu Herb Processing Centre, Chapagaun, Lalitpur in Nepal in March 2004. The oil has a pale yellow colour with powerful, fresh-camphoraceous aroma.

3. Antioxidant activity

1) DPPH radical-scavenging activity

The hydrogen atoms or electrons donation ability of the *Artemisia vulgaris* essential oil was measured from the bleaching of purple coloured methanol solution of DPPH[8)]. Briefly, 0.5 mM DPPH solution in methanol was prepared and 1mL of this solution 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 has reached its plateau. Butylated hydroxyanisole (BHA), α-tocopherol (α-toco) and ascorbic acid (AA) were used as positive controls.

2) Metal chelating activity

The ferrous ions chelating activity was estimated as described by Dinis et al.[4]. Briefly, different concentrations (0.5 - 8 mg/mL) of extracts 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 total volume was adjusted to 4mL with methanol. Then the mixture was shaken vigorously and left standing at room temperature for 10 min. 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 (%) = [(A0 − A1)/A0]×100,

Where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the sample and standards. The control contains $FeCl_2$ and ferrozine, complex formation molecules.

3) Reducing power determination

The reducing power of samples and standards was determined following Oyaizu[12]. Different amounts of samples (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 [$K_3Fe(CN)_6$] (2.5L, 1 %). The mixture was incubated at 50 °C for 20min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10min 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%), and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power.

4) 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[7]. 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 weight sample 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.

5) 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 analysis of variance (ANOVA) using Origin (Micro cal Software, Inc.) and were considered significant at $p<0.05$.

Results and Discussion

1. DPPH radical-scavenging activity

Essential oil 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[8] and the poor activity of *A. vulgaris* essential oil 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[8,9].

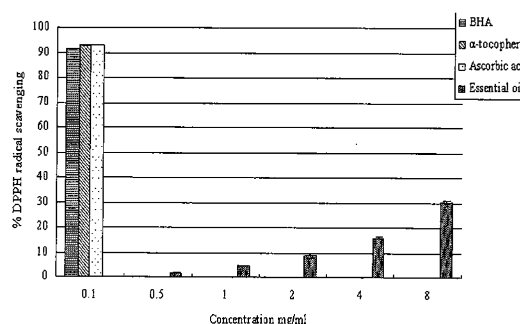

Fig. 1. DPPH radical scavenging activity of essential oil. Each value is expressed as mean ± SD (n=3), mean are sgnificantly different. p<0.001

The present study showed that the reaction rate of

essential oil was very slow reaching its plateau after 220 minutes. At 30 minute incubation, essential oil (8 mg/mL) exhibited only 30.13% radical scavenging activity where as at 240 minutes; it showed 84.44 % inhibition of DPPH radical and remained constant thereafter(Fig. 2). This shows that in spite of its slow reaction rate, essential oil could scavenge free radicals.

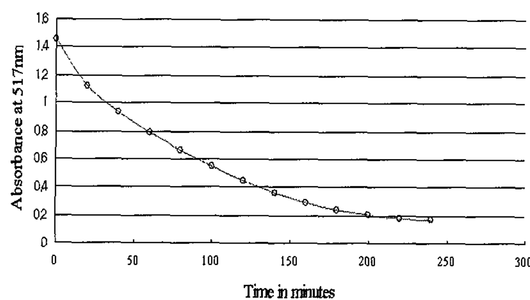

Fig. 2. Bleaching of DPPH radical by *Artemisia vulgaris* essential oil.

2. Reducing power determination

The reducing properties are generally associated with the presence of reductones[13], which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom[5]. Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. In the present study, essential oil 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). 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.

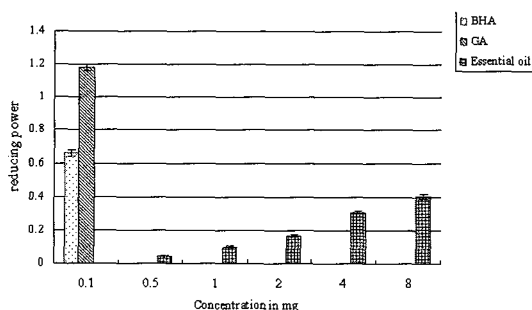

Fig. 3. Reducing power of essential oil. Each value is expressed as mean ± SD (n=3), mean are significantly different, $p<0.01$

3. 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. Among the transition metals, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of color reduction therefore allows the estimation of the metal chelating activity of the coexisting chelator. Essential oil is able to chelate ferrous ion in a concentration dependent chelating manner (r^2=0.9969). At the concentration of 8 mg/mL, essential oil chelated 75 % of ferrous ions. This showed the presence of strong ferrous ion chelating component in essential oil. However, the activity was much lower than that of EDTA.

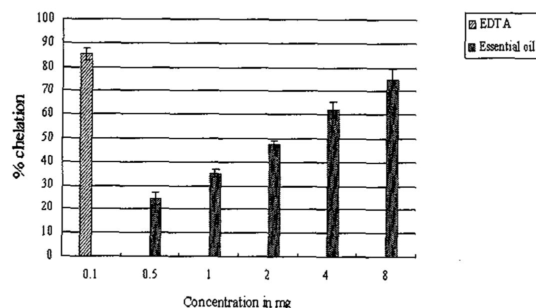

Fig. 4. Metal chelating activity of essential oil. Each value is expressed as mean ± SD (n=3), means are significantly different. $p<0.05$

4. 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 a gradual increase in the absorbance of control which reached the highest level after 3 days and declined thereafter. As shown in Fig. 5, essential oil 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. Similar to our results, previous studies also reported weak to medium antioxidant and DPPH radical scavenging activities of essential oils of *Artemisia* species[8,9]

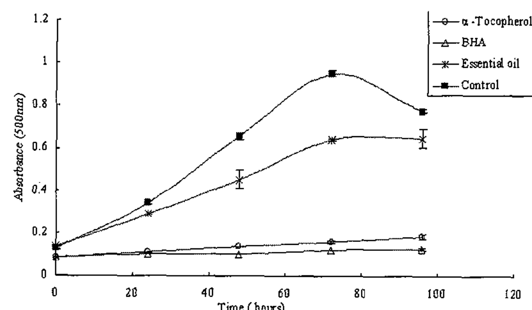

Fig. 5. Inhibition of lenoleic peroxidation by *Artemisia vulgaris*(1 mg/mL final concentration) essential oil by FTC method. Each value is expressed as mean ± S.D (n=4), means are significantly different, $p<0.05$

Conclusions

The present results revealed that the *A. vulgaris* essential oil possessed a strong metal chelating activity with low reducing power, radical scavenging and antioxidant activity. However, DPPH assay showed that oil possessed a radical scavenging activity with slow reaction rate. Yet, possible reason behind its kinetic could not be determined in the present study. It can be concluded that the antioxidant activity of oil might be in part due to its metal chelating activity. Further study should be focused on its *in vivo* potential in animal models and its reaction mechanisms.

References

1. Aeschbach, R., LoÈ liger, J., Scott, B.C., Murcia, A., Butler, J., Halli-well, B. and Aruoma, O.I. Antioxidant action of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem. Toxic. 32: 31-36, 1994.
2. Deans, S.G., Waterman, P.G. In: R.K.M. Hay & P.G. Waterman(Eds.), Volatile Oil Crops: Their Biology, Biochemistry and Production. London, Longman, p 113, 1993.
3. Dinis, T.C.P., Madeira, V.M.C., 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.
4. Gianni, S., Silvia, M., Mariavittoria, M., Martina, S., Stefano, M., Matteo, R., 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.
5. Gordon, M.H. The mechanism of antioxidant action in vitro. In: B.J.F. Hudson, Editor, Food antioxidants, London, Elsevier Applied Science pp 1-18, 1990.
6. Khaled, F. El-Massry, A., El-Ghorab, H., Farouk, A. Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chem. 79: 331-336, 2002.
7. Kikuzaki, H. and Nakatani, N. Antioxidant effects of some ginger constituents. J. Food Sci. 58(6):1407-1410, 1993.
8. Kordali, S., Cakir, A., Mavi, A., Kilic, H., 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: 1408-1416, 2005a.
9. Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A., 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, A. dracunculus, Artemisia santonicum, and Artemisia spicigera Essential Oils. J. Agric. Food Chem. 53(24):9452-9458, 2005b.
10. Medicinal plants of Nepal, Bulletin of the Department of Medicinal Plant Number 3, His Majestys' Government of Nepal, Ministry of Forest and Soil Conservation, Department of Plant Resources, Thapathali, Kathmandu, Nepal, 1997.
11. Ormancey, X., Sisalli, S., Coutiere, P. Formulation of essential oils in functional perfumery, Parfums, Cosmetiques, Actualites 157: 30-40, 2001.
12 Oyaizu, M. Studies on product of browning reaction prepared from glucose amine, Jpn. J. Nutr. 44: 307-315, 1986.
13. 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.
14. Sawamura, M. Aroma and functional properties of Japanese yuzu (*Citrus junos* Tanaka) essential oil, Aroma Res. 1: 14-19, 2000.
15. 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.