ANALELE UNIVERSITATII BUCURESTI
Department of Physical Chemistry
4-12 Regina Elisabeta Blvd, District 3, Bucharest
phone: +40-21-3143508; fax: +40-21-3159249
*p*ISSN: 1220-871X *e*ISSN: 1844-0401

ARS DOCENDI PUBLISHING HOUSE
Sos. Panduri 90, District 5 Bucharest
phone/fax: +40-21-4102575
e-mail: arsdocendi@yahoo.com

QUANTITATIVE DETERMINATION OF ANTIOXIDANT POTENTIAL OF *ARTEMISIA PERSICA*

Ayoub Rashid ch, M. Zahid Qureshi, Syed Ali Raza *, J. William and M. Arshad

abstract: An experimental protocol was designed to investigate the total flavonoids, phenolics and antioxidant potential of methanol extract of Artemisia persica. For initial screening of antioxidant activity DPPH on TLC was employed. After the qualitative confirmation of antioxidant potential, spectroscopic measurements were made through DPPH assay. Free fatty acids (FFA), Peroxide values (PV) and Iodine values were monitored in refined, bleached and deodorized (RBD) sunflower oil. Plant extract was found to possess strong antioxidant potential in all mentioned assays. Total phenolic and flavonoid contents were 407 mg/g and 308 mg/g respectively.

key words: lipid oxidation; antioxidant activity; *Artemisia persica*, phenolic content; flavonoid content.

received: March 03, 2010 *accepted*: June 25, 2010

1. Introduction

Lipid oxidation may be the primary cause of deterioration in quality of food products. It can seriously interfere with the efficiency of processing steps and therefore, leads to potential economic losses [1]. Lipid oxidation also produces reactive oxygen species (ROS), which have been implicated in carcinogenesis, inflammation, early aging and cardiovascular diseases [2]. It also decreases the organoleptic value of foods and imparts rancid and unpleasant flavours to the raw and end-use oil and fat products, thus making them unacceptable to consumers [1]. Lipid oxidation can be minimized by adding appropriate amount of antioxidants. Traditionally, chemically synthesized compounds, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are used as antioxidants in oil products [3]. The use of BHA and BTH is proved to be carcinogenic. Therefore, there is an increasing interest in the antioxidant activity of natural compounds [4-5]. Tert-butylhydroquinone (TBHQ) is not allowed in Japan, Canada and Europe. Similarly, BHA has also been removed from the generally recognized as safe (GRAS) list of compounds. [6]. Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods [7]. Most of their properties are due to

* Department of Chemistry, GCU, Lahore, Pakistan, *corresponding author e-mail*: chemstone@yahoo.com

essential oils produced by their secondary metabolism [8]. Plant essential oils as antioxidants were researched in detail with the view to investigating their protective role for highly unsaturated lipids [9].

A great number of aromatic and other medicinal plants contain chemical compounds that exhibit antioxidant properties which are included with Artemisia persica that belongs to Asteraceae family, Artemisia genus. It is common in North districts of Chitral, Baltistan, Gilgit and Kashmir from 2500-3500 msl in sandy soils mixed with limestone gravel and rocky hill slopes. It is used as febrifuge, tonic and vermifuge [10].

Present research work is designed to investigate the antioxidant activity of methanol extract of Artemisia persica. As no earlier reports are available at antioxidant activity of Artemisia persica in context of Pakistan, so it is very essential to develop natural antioxidants to meet up the challenges and demands especially in case of lipid oxidation to avoid carcinogenic synthetic antioxidants.

Experimental Part

Materials and methods

Plant collection: Both the stems and leaves of the plant were collected in July 2009 from the District Gilgit of Kashmir. The plant was identified at the department of botany and Voucher specimen (GCU Lahore).

Extract preparation

Both stems and leaves were air dried at room temperature to constant weights. The dried plant materials were ground separately to powder. Thirty grams of ground plant materials was taken to obtain the extract by Soxhlet apparatus using methanol as solvent. Filtrate was concentrated to dryness under reduced pressure at 40ºC using a rotary evaporator. Each extract was resuspended in methanol to make 2mg/ mL stock solution [11].

Determination of total flavonoid content

The total flavonoids content of each plant extract was estimated by Zhishen et al method [12]. Based on this method, each sample (0.5 mL) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of a NaNO2 solution (15%). After 6 min, 0.15 mL of an AlCl3 solution (10%) was added and allowed to stand for 6 min, then 2 mL of NaOH solution (4%) was added to the mixture. Immediately, water was added to bring the final volume to 5 mL and the mixture was thoroughly mixed and allowed to stand for another 15 min. The absorbance of the mixture was then determined at 510 nm versus the water blank. Results were expressed as rutine trihydrate equivalents (mg rutin trihydrate/ g dried extract).

Determination of total polyphenol content

Total phenolic content of each lyophilized extract was determined with the Folin–Ciocalteu method [13]. We mixed 2.5 mL fresh Folin reagent with 0.5 mL of different concentrations of each extract and immediately added 2 mL Na2CO3 (7.5%) to each one and left for 90

min at 30°C. The absorbance was measured at 765 nm. Results were expressed as gallic acid equivalents (mg gallic acid/g dried extract).

Antioxidant activity:

Initial Screening of Antioxidant Potential of methanol extract

DPPH assay on TLC. DPPH assay with TLC was used to measure the antioxidant activity of essential oil. Method of Bektas was followed [14]; 1:10 dilution of methanol extract was made in methanol. Five microlitres of this dilution was applied on the TLC plate. Plate was developed by methanol and ethyl acetate in ratio of 1:1. Then the plate was sprayed with 0.2% of DPPH reagent in methanol and stayed for 30 min at room temperature. Purple colour of DPPH reagent bleaching by yellow spots is the indication of positive antioxidant activity.

DPPH radical scavenging activity. DPPH radical scavenging activity of each plant extract was determined according to the method of Blois [15]. Briefly, 3 mL of extract was added to 1 mL of DPPH (1, 1-diphenyl-2-picrylhydrazyl) solution (0.2 mM in methanol) as the free radical source. The mixture was shaken and kept for 30minutes at room temperature. The decrease of solution absorbance due to proton donating activity of components of each extract was determined at 517nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Vitamin C was used as the positive control. The DPPH radical scavenging activity was calculated using the following formula: DPPH Radical Scavenging Activity (%) = [(A0 – A1 /A0) ×100], where A0 is the absorbance of the control, and A1 is the absorbance of extract or standard sample.

Analysis of rancidity parameters in sunflower oil. Refined, bleached and deodorized (RBD) sunflower oil samples were used to investigate the antioxidant activity of methanol extract in lipids. Free fatty acid (FFA) values and Peroxide values (PV) and Iodine values (IV) were determined by following the recommended methods of AOCS [16]. All these parameters were good indicators of lipid oxidation. Many scientists monitored the phenomenon of lipid oxidation to judge the extent of oxidation and antioxidant potential of plant extracts [17-20]. Sunflower oil was selected due to its high use in food as it is a rich source of linoleic acid and it easily can undergo rancidity due to high degree of unsaturation. [21].

Determination of antioxidant activity in sunflower oils

Storage of samples. Seven refined, bleached and deodorized sunflower oil samples (SFO) were stored in triplicate in transparent polyethylene bottles of 250 mL capacity each. Out of total twenty one bottles, seven bottles contained 120 mL blank deodorized, refined and bleached SFO (blank). Other seven bottles contained 200 ppm of BHA per 120 mL deodorized, refined and bleached sunflower oil samples (SFO). Remaining seven bottles contained 200 ppm of methanol extract of A. persica per 120 mL of RBD sunflower oil. [22]. All these samples were stored at ambient conditions. All the investigations in triplicate were made on weekly basis. Data was analyzed by GraphPad Prism 3.0 (Graph Pad Software, Inc. San Diego, USA).

Results and Discussions

Total Flavonoid and Phenolic Contents

Medicinal plants are rich sources for natural occurring antioxidants. Among these substances, the phenolic compounds have the ability to scavenge free radicals, super oxide and hydroxyl radicals through single-electron transfer reactions [23-24]. In this study, we evaluated the antioxidant activity of crude extracts of *Artemisia persica*. The total phenolic and flavonoid contents of crude extract was determined and expressed in terms of gallic acid and rutin trihydrate equivalents (Table 1).

Table 1 *Total flavonoid and phenolic contents of Artemisia persica crude extract.*

Sample	Total Phenolics (mg gallic acid/g dried extract)	Total Flavonoids (mg rutin trihydrate/ g dried extract)
Extract of *Artemisia persica*	407	308

DPPH assay on TLC: Purple colour of DPPH reagent was bleached by yellow spots was the indication of positive antioxidant activity.

DPPH Assay: The extent of decrease in the absorbance of DPPH in the presence of antioxidants correlates with the free radical scavenging potential of the antioxidant. These scavenging activities might be due to the presence of different phenolic contents. The scavenging ability of extract is 91.826% which is very close to tannic acid 93.99% and gallic acid 93.84%.

Table 2 *Percentage inhibition of extract of Artemisia persica.*

Sample	Inhibition %
Extract of *Artemisia persica*	91.826%
Gallic Acid	93.99%
Tannic Acid	93.84%

Tannic acid and gallic acid are taken as standard.

Rancidity parameters: Formation of free fatty acids might be an important measure of rancidity of foods. FFAs are formed due to hydrolysis of triglycerides and may get promoted by reaction of oil with moisture [19]. Table 3 shows the changes in free fatty acid values. FFA value of blank RBD sunflower oil was found to be 0.055 ± 0.01. After one week the FFA values were promoted to 0.076 ± 0.001. While the FFA value for the BHA containing sample after one week of storage protocol was found to be 0.058 ± 0.001. This value was same as methanol extract containing sample. After the completion of seven week storage protocol FFA value for blank solution was increased to 0.298 ± 0.002. This change in FFA contents was significant according to statistical analysis. FFA value for BHA containing sample was increased from 0.058 ± 0.001 (1^{st} week value) to 0.101 ± 0.002 at the end of seven week experimental protocol. Changes in BHA containing RBD sunflower oil samples showed the significant blockage of oxidation phenomenon as compared to blank RBD sunflower oil sample. Similar findings were observed in case of methanol extract containing RBD sunflower oil sample. In this case FFA value was jumped from 0.058 ± 0.001 (1^{st} week value) to 0.102 ± 0.001 (7^{th} week value). These findings explored the strong antioxidant ability methanol extract of *Artemisia persica*.

Peroxide value is a widely used measure of the primary lipid oxidation indicating the amount of peroxides formed in fats and oils during oxidation. [20]. Changes in peroxide values are showed in table 4. Peroxide value of blank RBD sunflower oil sample was 0.85 ± 0.01. It was increased to 7.01 ± 0.01 at the end of seven week trial. These changes were significant indicating the noticeable phenomenon of lipid oxidation. Peroxide value of BHA containing RBD sunflower oil was found to be 0.87 ± 0.01 after one week. It was subjected to 0.97 ± 0.01 at the completion of seven week analysis. Investigations in case of methanol extract containing RBD sunflower oil samples expressed the peroxide value increase from 0.88 ± 0.02 (1st week value) to 0.99 ± 0.01. Changes in case of BHA and methanol extract containing RBD sunflower oil samples were very minor indicating the strong antioxidant activity of BHA and methanol extract of *Artemisia persica*. Decrease in iodine value (IV) is an authentic tool to monitor lipid oxidation [25]. Magnitude of variation in iodine values was measured according to recommended methods of AOCS [16]. Changes in iodine values were presented in Table 5.

Iodine value for blank RBD sunflower oil sample was calculated as 141 ± 1.20. It was subjected to 109 ± 2.50. These variations were statistically significant. While variation in BHA and methanol extract containing RBD sunflower oil samples was not huge indicating the presence of antioxidants in the form of BHA and methanol extract. RBD sunflower oil samples having 200ppm of BHA showed a change from first week value, 140 ± 3.25 to 134 ± 2.50 at the ending of experiments. The Iodine values of first and last week for methanol extract containing RBD sunflower oil samples were 140 ± 2.50 and 132 ± 2.50 respectively. Iodine values for first two weeks in BHA containing RBD sunflower oil samples were the same (Table 5).

Table 3 *Free fatty acid values in terms of % ages of various RBD sunflower oil samples.*

Number of weeks	Blank sample	BHA sample	Methanol extract samples
1st	0.076 ± 0.001	0.058 ± 0.001	0.058 ± 0.001
2nd	0.095 ± 0.001	0.061 ± 0.001	0.062 ± 0.002
3rd	0.142 ± 0.002	0.069 ± 0.003	0.070 ± 0.001
4th	0.196 ± 0.002	0.077 ± 0.001	0.077 ± 0.001
5th	0.235 ± 0.003	0.086 ± 0.001	0.085 ± 0.002
6th	0.284 ± 0.001	0.095 ± 0.002	0.094 ± 0.003
7th	0.298 ± 0.002	0.101 ± 0.002	0.102 ± 0.002

Original value of blank= 0.058 ± 0.001
t-test significant in case of blank
t-test non-significant in case of BHA and methanol extract containing SFO samples

Table 4 *Peroxide values (meq kg-1) of various RBD sunflower oil samples.*

Number of weeks	Blank sample	BHA sample	Methanol extract samples
1st	1.10± 0.02	0.87± 0.01	0.88± 0.02
2nd	2.48± 0.01	0.89± 0.02	0.90± 0.01
3rd	3.46± 0.01	0.90± 0.01	0.92± 0.01
4th	4.25± 0.03	0.91± 0.01	0.94± 0.01

5th	5.40± 0.01	0.93± 0.02	0.95± 0.01
6th	6.31± 0.02	0.95± 0.01	0.97± 0.03
7th	7.01± 0.01	0.97± 0.01	0.99± 0.01

Original value of blank = 0.85 ± 0.01
t-test significant in case of blank
t-test non-significant in case of BHA and methanol extract containing SFO samples

Table 5 *Iodine vales.*

No of weeks	Blank sample	BHA sample	Methanol extract samples
1st	139 ± 2.50	140 ± 3.25	140 ± 2.50
2nd	135 ± 3.50	140 ± 3.50	139 ± 3.15
3rd	130 ± 2.50	139 ± 2.50	139 ± 2.50
4th	125 ± 1.50	138 ± 3.05	138 ± 2.25
5th	119 ± 3.25	137 ± 2.03	136 ± 208
6th	113 ± 2.55	136 ± 2.05	134 ± 2.05
7th	109 ± 2.50	134 ± 2.50	132 ± 2.50

Original value of blank = 141 ± 1.20
t-test significant in case of blank
t-test non-significant in case of BHA and methanol extract containing SFO samples

Conclusions

Above mentioned analytical investigations reveal that methanol extract of Artemisia persica is an excellent antioxidant for lipid containing foods. Its activity was quite comparable with the synthetic antioxidant BHA. Antioxidant activity of methanol extract was probably due to presence of flavonoids and phenolics compounds which are present in the extract [26]. After checking the DNA toxicity of methanol extract of Artemisia persica, it can be cultivated in Pakistan. This can lead to great health and economic benefits. Further studies can lead us to evaluate the antioxidant activity of essential oil (which contain eugenol, carvacrol) [27] and could be exploited on commercial scale.

REFERENCES

1. Min, D.B., Lee, H.O. (1998) Lipid oxidation of edible oil, Food lipid. In: Akoh, C.C. & Min, D.B. (Eds.): *Chemistry, Nutrition and Biochemistry* **88**, 283-96.

2. Siddhuraju, P., Beeker, K. (2003) Antioxidant properties of various solvent extracts of total phenolic constituents from three different agro climatic origins of Drumstick tree (Moringa oleifera Lam.) leaves. *Journal of Agriculture and Food Chemistry* **51**, 2144-55.

3. Whysner, L., Wang, C.X., Zang, E., Iatropoulos, M.J., Williams, G.M. (1994) Dose response of promotion by butylated hydroxyanisole in chemically initiated tumours of the rat forestomach. *Food and Chemical Toxicolology* **32**(30), 215-22.

4. Amakura, Y., Umino, Y., Tsuji, S., Ito, H., Hatano, T., Yoshida, T. (2002) Constituents and their antioxidative effects in eucalyptus leaf extract used as a natural food additive. *Food Chemistry* **77**, 47-56.

5. Orhan, I., Aydin, A., Colkesen, A., Isimer, A.I. (2003) Free scavenging activities of some edible fruit seeds. *Pharmaceutical Biology* **41**, 163-5.

6. Farag, R.S., Badei, A.Z.M.A., El Baroty, G.S.A. (1989) Influence of thyme and clove essential oils on cottonseed oil oxidation. *Journal of the American Oil Chemists' Society* **66**(6), 800-4.

7. Hulin, V., Mathot, A.G., Mafart, P., Dufossé, L. (1998) Les proprietés anti-microbiennes des huiles essentielles et composés d'arômes. *Science Aliments* **18**, 563-82.

8. Adam, K., Sivropoulou, A., Kokkini, S., Lanaras, T., Arsenakis, M. (1998) Antifungal activities of Origanum vulgare subsp. hirtum, Mentha spicata, Lavandula angustifolia, and Salvia fruticosa Essential Oils against Human Pathogenic Fungi. *Journal of Agriculture and Food Chemistry* **46**, 1739-45.

9. 9. Deans, S.G., Svoboda, K.P., Gundidza, M., Brechany, E.Y. (1992) Essential oil profiles of several temperate and tropical aromatic plants: their antimicrobial and antioxidant activities. *Acta Horticulture* **306**, 229-32.

10. Luximon-Ramma, A., Bahorun, T., Soobrattee, A. M., Aruoma, O. I. (2005) Antioxidant activities of phenolic, proanthocyanidin and flavonoid components in Extracts of Acacia fistula, *J. Agric. Food Chem*. **50**, 5042-7.

11. Sanchez-Moreno, C. (2002) Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci Tech Int* **8**, 121-37.

12. Zhishen, J., Mengcheng, T., Jianming, W. (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* **64**(4), 555-9.

13. Slinkard, J., Singleton, V.L. (1979) Total phenol analysis: automation and comparison with manual methods. *AJEV* **28**, 49-55.

14. Bektas, T., Dimitra, D., Atalay, S., Munevver, S., Moschos, P. (2005) Antimicrobial and antioxidant activites of essential oil and various extracts of Salvia tomentosa Miller. *Food Chemistry* **90**, 333-40.

15. Zhishen J, Mengcheng T, Jianming W. (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* **64**, 555-9.

16. ***AOCS (1989): *Official and Recommended Practices of the American oil*; Chemists Society, 5th edition. Champaign, I.L., 48-62.

17. Anwar, F., Jamil, A., Iqbal, S., Sheikh, M.A. (2006) Antioxidant activity of various plant extracts under ambient and accelerated storage of sunflower oil. *Grasas Y Aceities* **57**(29), 189-97.

18. Anwar, F., Chatta, S.A.S., Hussain, A.I. (2007) Assessment of oxidative deterioration of soybean oil at ambient and sunlight storage. *Grasas Y Aceities* **58**(4), 390-5.

19. Frega, N., Mozzon, M., Lercker, G. (1999) Effect of free fatty acids on the oxidative stability of vegetable oil. *Journal of the American Oil Chemists' Society* **76**, 325-9.

20. Gulcan, O., Bedia, H. (2007) Antioxidant activities of Satureja cilicica essential oil in butter and in vitro. *Journal of Food Engineering* **79**, 1391-6.

21. Shahidi, F., Janitha, P.K., Wanasundara, P.D. (1992) Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition* **32**(1), 67-103.

22. Duh, P.D., Yen, G.C. (1997) Antioxidant efficacy of methanolic extracts of peanut hulls in soybean and peanut oils. *Journal of the American Oil Chemists' Society* **74**, 745-8.

23. Kamatha, V.G., Chandrashekarb, A., Rajini, P.S. (2004) Antiradical properties of sorghum (Sorghum bicolor L. Moench) flour extracts. *J Cereal Sci* **40**, 283-8.

24. Hollman, P.C.H., Katan, M.B. (1999) Dietary flavonoids: intake, health effects and bioavailability. *Food Chem Toxicol* **37**, 937-42.

25. Naz, S., Sheikh, H., Saddiqi, R., Sayeed, S.A. (2004) Oxidative stability of olive, corn and soybean oil under different conditions. *Food Chemistry* **88**, 253-9.

26. Oyedeji, O.A., Adayan, A.J. (2005) Chemical composition and antibacterial activity of essential oil of Centella asiatica growing in South Africa. *Pharmaceutical Biology* **43**(3), 249-52.

27. Sadeghpour O., Asgharib G., Ardekanic M.R.S. (2004) Composition of Essential Oil of Artemisia persica Boiss. from Iran, *Iranian Journal of Pharmaceutical Research* **3**, 65-7.