Determination of Antioxidant Activities of Different *Urginea maritima* (L.) Baker Plant Extracts

Mammadov, Ramazan*+

Department of Biology, Faculty of Science and Arts, Pamukkale University, 20017 Denizli, TURKEY

Makasçı - Afacan, Ayşe

Department of Chemistry, Faculty of Science and Arts, Namuk Kemal University, Tekirdağ, TURKEY

Uysal - Demir, Derya; Görk, Çiğdem

Department of Biology, Faculty of Science and Arts, Muğla University, 48000, Kötekli / Muğla, TURKEY

ABSTRACT: *The subject handled in this study is the determination of antioxidant activities of extracts obtained from the Urginea maritima (L.) Baker plant's leaves and tubers. Extractions which were obtained using various solvents as methanol, ethanol, acetone and benzine. The antioxidant activity of the extracts was determined using β-karotene-linoleic acid system. Moreover, the free radical scavenging activity values were tested with DPPH. The concentrations of phenolic compounds in all extracts of UM, expressed as microgram of pyrocatechol equivalents (PEs) were determined with Folin-Ciocalteu reagent (FCR). The results demonstrated that the highest antioxidant activity in the U. maritima (L.) Baker showed in the tubers with ethanol solvent (%72,67) and the lowest in the benzine extract from the tubers (%31,12). The free radical scavenging activity of the methanol extract from the tubers of the U. maritima (L.) Baker plant (%66.89) was found lower than the BHT value (%91.12) but still the highest. However, the amount of total phenolic compounds of acetone extract of U. maritime (L.) Baker tubers was 6 times more than ethanol extract. This study demonstrated that U. maritime (L.) Baker species have high rate antioxidant capacity.*

KEY WORDS: *Urginea maritima (L.) Baker, Antioxidant activity, Radical scavenging activity, DPPH.*

INTRODUCTION

Man has utilized the plants in his environment in various ways since ancient times: They have eaten them, burned them to get fire, made weapons out of them and used them as building materials [1].

Secondary potential toxic compounds are formed as a result of the oxidative destruction of the plant oils and animal fat present in foods, lowering the nutritious quality and reliability in foods and causing spoilage of

* To whom correspondence should be addressed.
+ E-mail: rmammad@yahoo.com
1021-9986/10/3/51 6/$/2.60

their taste and color. Addition of antioxidants is necessary for the prevention of taste, color and vitamins from being spoiled. Synthetic antioxidats such as BHA (Butyl-Hydroxy Anisole), BHT (Butyl-Hydroxy Toluene), PG (Propyl Gallate) and TBHQ (Ter-Butyl-Hydroquinone) are widely used for the preservation of foods. However, consumers are now aware to what extent these chemicals are healthy. Therefore, it has been required to define more alternative, natural, and reliable food antioxidants such as tocopherol, despite their lower effects and higher cost [2, 3]. It has long been known that the fact that why various food and drinks are effective for certain diseases results from the presence of vitamin C, vitamin E (α-tocoferol), β-carotene and phenolic compounds in them [4, 5]. In a living system these reactive radical types are removed during normal metabolic processes either by the antioxidants received in a diet (ascorbic asid) or by endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase etc.).

Recent studies have reported that natural antioxidants obtained from medicinal plants protect againts toxic and harmful effects of free radicals [6] and have a wide range of antimicrobial [7, 8], antimutagenic [9] and antiallergic [10] antioxidant [11, 12] radical scavenging activity [13] and anticarcenogenic effects [14]. Based on all these facts, a lot of research on plant extractions has been underway. On the other hand, several studies have been carried out on *Urginea maritima* (L.) Baker plant. Some of them are: in 1996 forty-one bufadienolides were isolated from the bulbs of U. maritima from Egypt; 26 of them are novel natural compounds. Structure elucidation was performed by comparison with authentic substances or by means of ^{1}H, ^{13}C NMR and FAB mass spectroscopy. Sixteen of the glycosides derive from nine structurally new aglycones [15]. In other study carried out in southern Spain in 1998 in order to determine the insecticidal effects of the wild flora, it was found that the tuber extracts from the *U. maritima* (L.) Baker plant had a 100 % toxic effect on insects. Moreover, on the plant showed that the development of the insect larvae was inhibited at the end of the fourteenth month when the tuber of the plant with 10 % ethanol extract was mixed with fodder and given to insects. Also, the direct exposure of the plant's tubers to the sun increases the activity [16].

EXPERIMENTAL SECTION

Different parts (leaves and tubers) of *U. maritima* (L.) Baker. were collected from the natural environment of Mugla city in Turkey in October 2008 and cleaned to remove any residual compost. The air-dried leaves and tubers were ground to fine powder and then stored in an air-tight container until further use. Plant samples were identified under microscope using some reference [17], and voucher samples were stored at the herbarium of Muğla and Ege Universities.

Reagents

All reagents used were of analytical grade. Tween-20, methanol, etanol, benzen, acetone, *β*-carotene, chloroform, linoleic acid were purchased from E. Merck (Darmstadt, Germany). 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated-hydroxytoluene (BHT) were obtained from sigma Chemical Co. (St. Louis, MO). All other chemicals and solvents were analytical grade.

Plant extracts

The ground plant samples were subjected to various solvents (methanol, ethanol, acetone and benzene) in a Soxhlet device [18-20] until they discolored (approx. 4-6 h). 2 g of dried leaves and 20 mL of solvent were used for each sample [21]. The mixture was filtered, and the solvent in solution was evaporated in a rotary evaporator at 48-49 °C. The water in each extract was vacuumed with the freeze Dryer. The remaining had plant extraction was dissolved in necessary concentrations and then used.

Methods of the determination of antioxidant activity, radical scavenging and total phenolic content

The antioxidant activity of the extracts were determined with β-carotene-linoleic acid system [22]. The β-carotene solution was prepared by dissolving 2 mg of β-carotene in 10 mL of chloroform. 40 mg of linoleic acid and 400 mg of Tween 20 were added for 1 mL of the solution. The chloroform being evaporated in a rotary evaporator, the solution was mixed with 100 mL of distilled water. 4.8 mL of this emulsion was placed into test tubes that contained 0.2 mg of the sample and 0.2 of the extract solvents. For control, 0.2 mg of solvent (methanol, ethanol, acetone or benzene) was put in the test tube instead of the extract. Immediately after the

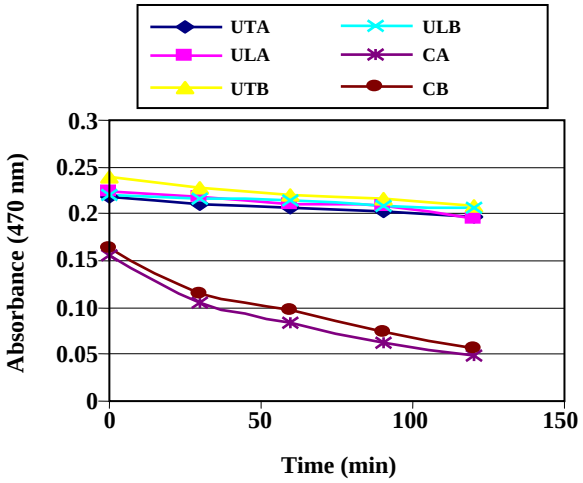

Fig. 1: Absorbance values of UTM, ULM, UTE and ULE extracts determined with β-carotene/linoleic acid model system.

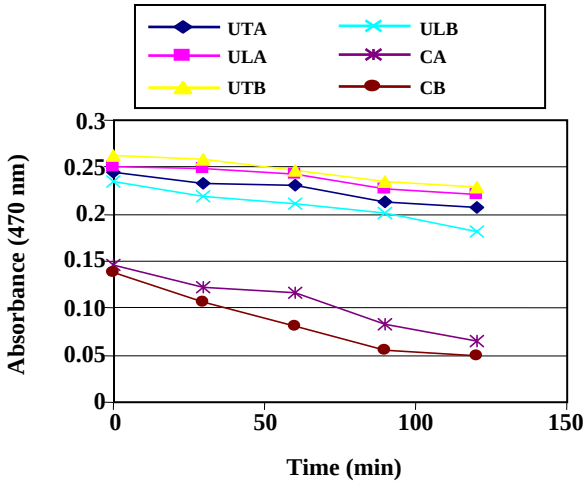

Fig. 2: Absorbance values of UTA, ULA, UTB and ULB extracts determined with β-carotene/linoleic acid model system.

emulsion was placed in test tubes the initial absorbances were measured to at 470 nm with a spectrophotometer (Shimadzu UV–1601, Japan). The tubes were left for incubation at 50 °C [23]. The total antioxidant activity was calculated using the equation below (1):

$$AA: [\ 1- (A0-At / A0^{o} - At^{o}) \times 100 \quad (1)$$

The free radical scavenging activity of the samples were determined using the free radical DPPH' [24, 25]. An aliquot of 200 µL of *U. maritima* (L.) Baker extract (0.62 - 4.96 mg/mL) and BHT (0.04 - 1.28 mg/mL) were mixed with 800 µL of 100 mM Tris - HCl buffer (pH 7.4). The mixture was then added to 1 mL of 500 µM DPPH. This was made up to a DPPH final concentration of 250 µM. The mixture was shaken vigorously and left to stand at room temperature for 30 min in a dark room. Absorbance at 517 nm was measured using a UV- spectrophotometer until the reading reached a plateau. The absorbance values of the samples were contrasted with empty control. [26, 27]. The free radical scavenging activity was calculated using the equation below (2):

$$\text{Inhibition (\%)} = 100 - [(A1\ IA_{0}\} \times 100\ ] \quad (2)$$

The concentrations of phenolic compounds in all extracts of *U. maritima* (L.) Baker expressed as microgram of pyrocatechol equivalents (PEs), were determined with Folin-Ciocalteu reagent (FCR) according to the method of *Slinkard & Singleton* [28]. 1 mL of the solution (contains 1 mg) extracts were added to 46 mL of distilled water and 1mL of FCR, and mixed thoroughly. After 3 min, the mixture was added 3 mL of sodium carbonate (2%) and shaken intermittently for 2 h. The absorbance was read at 760 nm. The concentrations of phenolic compounds were calculated to following equation that was obtained from standard pyrocatechol graph (3):

$$\text{Absorbance} = 0.002248\ \text{pyrocatechol}\ (\mu g\ ) + 0.0024 \quad (3)$$

R^2 : 0.999

RESULTS AND DISCUSSION

The total antioxidant activity of the extracts from *U. maritima* (L.) plant was determined using β-carotene-linoleic acid system. This system is based on the fact that β-carotene discolors when no antioxidant is present as a result of free radicals that form hydroperoxide from linoleic acid. Adding extracts containing antioxidants to the system neutralizes the peroxide products composed of linoleic acid, thus β-carotene maintains its characteristic yellow color. In other words, the higher the samples' absorbance, the higher the antioxidant activity (Figs. 1, 2).

A great number of plants have been studied with respect to their antioxidant activity. *Saxena et al.* [29] found out the antioxidant features of the etheric oils from *Lankana aculeata*, *Goli et al.* [30] those of *Pistachia vera,* and *Xu et al.* [31] those of *Sesamum indicum* L plants. *Tepe et al.* [32] studied the antioxidant activity of the methanol extracts from *Allium* L. species spread across Turkey. Their study showed the total antioxidant activity of these plants to be 60–70 %.

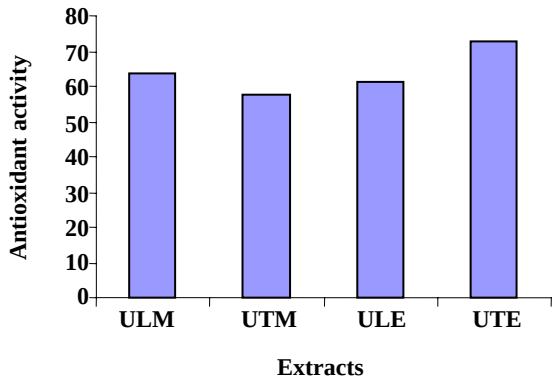

Fig. 3: The antioxidant activities in the methanol and ethanol extracts.

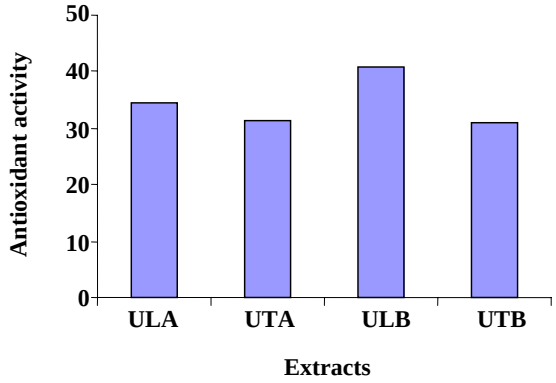

Fig. 4: The antioxidant activities in acetone and benzine extracts.

The study revealed that the highest antioxident activity in the extracts from the *U. maritima* (L.) Baker plant was 72.67 % in the ethanol solution of the tubers (UTE) addition, the antioxidant activities of the methanol extracts from the leaves and tubers of the *U. maritima* (L.) Baker plant and the ethanol extracts from its leaves produced similar results (Urginea Leaves Methanol (ULM)-63.58 %, Urginea Leaves Ethanol (ULE)-61.33 % and Urginea Tubers Methanol (UTM)-57.71%, respectively) (Fig. 3). The lowest antioxidant activity in the extracts was seen in the extract obtained with benzene (UTB-31.12 %) from the tubers of *U. maritima* (Fig.4). These results prove that the *U. maritima* (L.) Baker plant is richer than *Allium* (L.) With respect to phenolic compounds. It seems that hydroxyl groups containing phenolic compounds are found in greater amounts in the extracts from the *U. maritima* (L.) Baker plant.

The extracts were tested through DPPH, a stable radical. The results of the free radical scavenging activity was calculated as the concentration (IC_{50}) 50 % of which DPPH scavenges in 30 minutes. Low IC_{50} values show high free radical scavenging activity, which expresses the amount of the DPPH remaining or used in the medium after the reaction. The free radical scavenging capacity of the extracts depends on the ability of the antioxidant compounds in the extract to release their hydrogens and on the structural conformation of the compound. $DPPH^{\cdot}$ has a maximum wave length at 517 nm and is used as a free radical in determining the antioxidant activity in certain natural compounds. The free radical $DPPH^{\cdot}$ can easily get an electron or hydrogen radical from antioxidant molecules in order to be a stable molecule, as a result of the reaction (4) [33].

$$DPPH^{\cdot} + A\text{-}H \longrightarrow DPPH\text{-}H + A^{\cdot} \qquad (4)$$

The *Ruellia tuberosa* L plant can be given as an example of the free radical scavenging capacity in different plants. The free radical scavenging capacity of this plant is 10–40 %. In this study chemicals such as water, methanol, hexane and chloroform were used as solvents. The scavenging activity changed according to the type of the solvent. A study on the free radical scavenging capacity of peanut showed it to be 25 % [34].

The methanol extract from the UTM plant was seen to have the highest free radical scavenging activity (%66.89). Although this value is lower than that of BHT (91.12 %), it still is the highest in this study. Next come the extracts Urginea Tubers Acetone (UTA) (66.70 %), UTE (65.56 %) and UTB (17.52 %). Of the extracts obtained from the leaves of the the *U. maritima* (L.) Baker plant, the extract Urginea Leaves Acetone (ULA) had the highest free radical scavenging activity (51.04 %). The lowest result was found in the ethanol extract ULE (%33.02) (Fig. 5).

Such varying antioxidant activity in the extracts from the *U. maritima* (L.) Baker plant can be attributed to their ability to release hydrogen effectively and to their free radical scavenging. A number of factors affect the antioxidant activities in plants. Factors such as the phase the plant is in (such as blooming, seeding etc.), the extraction technique, the polarity of the solvents, the dryness or freshness of the plant material affect the plant's activity. The results obtained in this study show that the extracts obtained with various solvents from the *U. maritima* (L.) Baker plant is a convenient natural, source of antioxidants and that it can be used in medicine.

Table 1: Total phenolic content (µg of PEs[a] / mg of extract) of extracts from different parts of Urginea maritima (L.) Baker using various solvents.

Solvent	Tubers	Leaves
Benzine	27.12 ± 0.65	36.16 ± 0.97
Asetone	56.76 ± 0.19	24.06 ± 0.56
Metanol	15.28 ± 0.13	17.80 ± 0.81
Etanol	9.66 ± 0.18	23.14 ± 0.07

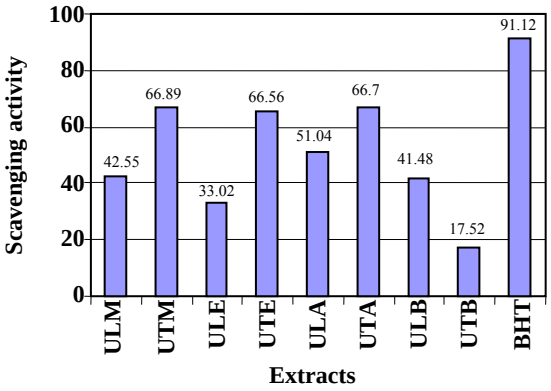

Fig. 5: The free radical scavenging capacity of the extracts with methanol, ethanol, acetone and benzine through DPPH method.

Some investigations have been reported that phenolic compounds are very important plant materials because of inhibiting effect on autoxidation of the oils [35] and their radical scavenging ability [36].

Therefore, it is important to determine the effect of the total phenolic compound on the antioxidant activity of extracts of different parts of *U. maritima* (L.) Baker. The concentration of phenolics in all of the extracts of *U. maritima* (L.) Baker tubers and leaves was expressed as mg pyrocatechol equivalentg of extracts as shown in table 1.

In petrole, acetone, methanol and ethanol extracts of *U. maritima* (L.) Baker leaves (1 mg), 36.16. 24.06, 17.30 and 23.14 µg pyrocatechol equivalent of phenols was detected. In addition, the amount of total phenolic compounds of acetone extract of *U. maritima* (L.) Baker tubers is 6 times more than ethanol extract.

CONCLUSIONS

In this study, the methanol, ethanol, benzene, and acetone extacts obtained from the leaves and tubers of *U. maritima* (L.) Baker plant were studied. As a result, the highest antioxidant activity was found in the UTE (72.67%). However, the lowest antioxidant activity was found in the UTB (31.12%). Moreover, antioxidant activity was found in the extract from *U maritima* (L.) Baker plant ULM as 63.58%, in the extract from UTM as 57.71%, and in the extract from ULE as 61.33%. The highest scavenging activity of *U.maritima* (L.). Baker plant was found to be 66.89% in the extract from UTM. Although this value is lower (91.12%) than the free radical scavenging activity of BHT, the synthetic antioxidant, it is higher than the IC_{50} value. The values of UTM extracts are followed by UTE (65.56%), ULA (51.04%), and Urginea Leaves Benzene (ULB) (41.48%). Hence, the antioxidant activity and free radical scavenging activity of *U. maritima* (L.) Baker plant were found to be high.

Received : Feb. 18, 2009 ; Accepted : Aug. 17, 2010

REFERENCES

[1] Baytop T., Therapy with Medicenal Plants in Turkey, pp.145, İstanbul University Press (1984).

[2] Sherwin E.R., Oxidation and Antioxidants in Fat and Oil processing, *J Am Oil Chem Soc.*, **55**, p. 809 (1978).

[3] Wanasundara U.N., Shahidi F., Antioxidant and Pro-Oxidant Activity of Green Tea Extracts in Marine Oils, *J Am Oil Chem Soc.*, **63**, p. 335 (1998).

[4] Abushita A.A., Hebshi E.A., Daood H.G., Biacs P.A., Determination of Antioxidant Vitamins in Tomatoes, *J Am Oil Chem Soc.*, **60**, p. 207 (1997).

[5] Aruoma O.I., Spencer J.P.E., Warre D., Jenner P., Butler J., Halliwell B., Characterization of Food Antioxidants, Illustrated using Commericial Garlic and Ginger Preparations, *Food Chem.,* **60**, p. 149 (1997).

[6] Saint-Cricq de Gaulejac N., Provost C., Vivas N., Provost C., Vivas N., Comparative Study of Polyphenol Scavenging Activities Assessed by Different Methods, *J. Agric. Food Chem.,* **47**, p. 425 (1999).

[7] Celik A., Herken EN., Arslan I, Özel MZ., Mercan N., Screening of the Constituents, Antimicrobial and Antioxidant Activity of Endemic *Origanum hypericifolium* O. Schwartz & P.H. Davis., *Natural Product Research*, **24**, p. 1568 (2010)

[8] Celik A., Mercan N., Arslan İ., Davran, H. Chemical Composition and Antimicrobial Activities of Essential Oils from *Nepeta Cadmea., Chem. of Nat. Comp.* **44**, p. 119 (2008).

[9] İkken Y., Morales P., Martínez A., Marín M.L., Haza A.I., Cambero M.I., Antimutagenic Effect of Fruit and Vegetable Ethanolic Extract Against N-Nitrosamines Evaluated by the Amfes Test, *J. Agric. Food Chem.,* **47**, p. 3257 (1999).

[10] Noguchi Y., Fukuda K., Matsushima A., Haishi D., Hiroto M., Kodera Y., Nishimura H., Inada Y., Inhibition of Df-Protease Associated with Allergic Diseases by Polyphenol, *J. Agric. Food Chem.,* **47**, p. 2969 (1999).

[11] Makasci A., Mammadov R., Dusen O., Isik H. Antimicrobial and Antioxidant Activities of Medicinal Plant Species *Ornithogalum Alpigenum* Stapf. from Turkey, *J. Med.Pl.Res.,* **4**, p. 1637 (2010).

[12] Mammadov R., Düsen O., Uysal D., Köse E., Antioxidant and Antimicrobial Activities of Extracts from Tubers and Leaves of *Colchicum Balansae* Planchon, *J. Med.Pl.Res.*, **3**, p. 767 (2009).

[13] Arslan I., Celik A. Free Radical Scavenging Activities and Essential Oil Analysis of *Salvia Cedronella* Boiss. and *Salvia fruticosa* Miller. *J. Ess. Oil Bear. Pl.*, **13**, p. 545 (2010).

[14] Przemysław S-H., Zbigniew J., Zastosowanie Roślin z Rodziny Liliaceae Farmakoterapii: Application of Plants from Liliaceae Family in Pharmacotherapy, *Farm. Polska*, **59**, p. 785 (2003).

[15] Kopp B., Krenn L., Draxler M., Hoyer A., Terkola R., Vallaster P., Robien W., Bufadienolides from *Urginea Maritima* from Egypt, *J. Phytochem.,*. **42**, p. 513 (1996).

[16] María C.B., María J.P., Benjamín R., A New 24-Nor-Oleanane Triterpenoid from Salvia Carduacea, *J. of Nat. Prod.,* **65**, p. 1513 (2002).

[17] Davis P.H., Flora of Turkey and The East Aegeon Islands, **8**, 743, .Edinburgh University Press (1984).

[18] Darwish R.M., Aburjai T., Al-Khalil S., Mahafzah, A., Screening of Antibiotic Resistance Inhibitors from Local Plant Materials Against Two Different Strains of Staphylococcus Aureus, *J. Ethnopharm.,* **79**, p. 359 (2002).

[19] Aburjai T., Darwish R.M., Al-Khalil S., Mahafzah A., Al-Abbadi A., Screening of Antibiotic Resistance Inhibitors from Local Plant Materials Against Two Different Strains of Pseudomonas Aeruginosa, *J. Ethnopharm.,* **76**, p. 39 (2001).

[20] Sokmen A., Jones B.M., Erturk,M., The in Vitro Antibacterial Activity of Turkish Medicinal Plants, *J. Ethnopharm.,* **67**, p. 79 (1999).

[21] Feresin G.E., Tapia A.A., Bustos D.A., Antibacterial Activity of Some Medicinal Plants from San Juan, Argentina, *J. Fitoterapi* **71**, p. 429 (2000).

[22] Amin I., Norazaidah Y., Emmy Hainida K.I., Antioxidant Activity and Phenolic Content of Raw and Blanched Amaranthus Species, *Food Chem.*, **94**, p. 47 (2006).

[23] Amin İ., Zamaliah M.M., Chin W.F., Total Antioxidant Activity and Phenolic Content in Selected Vegetables, *Food Chem.,* **87**, p. 581 (2004).

[24] Cuendet M., Hostettman K., Potterat O., İridoid Glucosides with Free Radical Scavenging Properties from Fagraea Blumei, *Helv. Chim. Acta,* **80**, p. 1144 (1997).

[24] Kirby A.J., Schmidt R.J., The Antioxidant Activity of Chinese Herbs for Eczema and of Placebo Herbs, *J. Ethnopharm.,* **56**, p. 103 (1997).

[26] Yen W-J., Chang L-W., Duh P-D., Antioxidant Activity of Peanut Seed Testa and its Antioxidative Component, Ethyl Protocatechuate, *LWT - Food Sci. and Technol.,* **38**, p. 193 (2005).

[27] Duh P.D., Yeh D-B., Yen G-C.,. Extraction and Identification of an Antioxidative Component from Peanut Hulls, *J Am Oil Chem Soc*., **69**, p. 814 (1992).

[28] Singh S., Saxena R., Pandey K., Bhatt K., Sinha S., Response of Antioxidants in Sunflower (*Helianthus Annuus* L.) Grown on Different Amendments of Tannery Sludge: its Metal Accumulation Ppotential, *J. Chemosphere*, **57**, p. 1663 (2004)

[29] Silinkard K., Singleton V.L.,. Total phenol Analyses: Automation and Comparison with Manual Methods, *Am. J. Enol. Viticult.,* **28**, p. 49 (1977).

[30] Goli H.A., Barzegar M., Sahari M.A., Antioxidant Activity and Total Phenolic Compounds of Pistachio (Pistachia Vera) Hull Extracts, *Food Chem.,* **92**, p. 521 (2005).

[31] Xu J., Chen S., Hu Q., Antioxidant Activity of Brown Pigment and Extracts from Black Sesame Seed (*Sesamum indicum* L.), *Food Chem.,* **91**, p. 79 (2005).

[32] Tepe B., Sokmen M., Akpulat A., Sokmen A., In Vitro Antioxidant Activities of the Methanol Extracts of Five Allium Species from Turkey, *Food Chem.,* **92**, p. 89 (2005).

[33] Fukumoto L.R., Mazza G., Assessing Antioxidant and Prooxidant Activities of Phenolic Compounds,. *J. Agric. Food Chem.,* **48**, p. 3597 (2000).

[34] Chen F-A., Wu A-B., Shieh P., Kuo D-H., Hsieh C-Y., Evaluation of the Antioxidant Activity of *Ruellia Tuberosa*, *Food Chem.,* **94**, p. 14 (2006).

[35] Ramarathnam N., Osawa T., Namiki M., Tashiro T., Studies on the Relationship between Antioxidative Activity of Rice Hull and Germination Ability of Rice Seeds, *J. Sci. Food Agricult.,* **37**, p. 719 (1986).

[36] Hatano T., Edamatsu R., Mori A., Fujita Y., Yasuhara E., Yoshida T., Okuda T., Effect of Interaction of Tannins with Co-Existing Substances. VI: Effects of Tannins and Related Polyphenols on Superoxide Anion Radical and on DPPH Radical, *CPB - Chem. Pharmace. Bull.* **37**, p. 2016 (1989).