Journal of Medicinal Plants Research Vol. 6(39), pp. 5196-5203, 10 October, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1128
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Free radical scavenging activity, phytochemical composition and nutrient analysis of *Elaeagnus umbellata* berry

Khanzadi Fatima Khattak

Food Science Division, Nuclear Institute for Food and Agriculture (NIFA), P. O. Box. 446, Peshawar, Pakistan.
E-mail: khattakkf@yahoo.com. Tel: 00-92-912964060. Fax: 00-92-912964059.

Accepted 20 September, 2011

Elaeagnus umbellata **is a deciduous shrub widely distributed in the Himalayan regions of Pakistan. The reddish berries of the plant are edible and very delicious. In the present study, the berries of the plant were investigated for proximate composition, mineral content, physicochemical characteristics, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and selected phytochemicals. The results showed that the fruit of *E. umbellata* contained 71.4% moisture, 2.9% ash, 4.0% protein, 2.3% crude fat, 5.9% fiber and 13.6% nitrogen free extract. The nutritive value of the fresh berry was 90.8 kcal/100 g. The berry contained 27.8 mg/100 g of vitamin C. The plant showed striking DPPH scavenging activity and the phytochemical analysis revealed that it contained 23.3, 3.6, 19.9, 126.5, 12.6 and 21.2 mg/g of phenolic, flavonoid, carotenoid, tannin, alkaloid and saponin contents, respectively. In short, this study showed that *E. umbellata* is a good source of various nutrients and antioxidant compounds, and it can be beneficial to consumer's health.**

Key words: *Elaeagnus umbellata,* phytochemical composition, proximate analysis, mineral contents, extraction yields, free radical scavenging activity.

INTRODUCTION

Regardless of remarkable progress in synthetic drugs, plants make a major contribution to the pharmaceutical industry (Farnsworth and Soejarto, 1991). Therapies using medicinal plants are more venerated than using synthetic chemicals because they are safe, easily available, cost effective, and there are synergistic effects of other biologically active ingredients and the presence of beneficial minerals. There is an incessant upsurge of interest in phytochemicals as these have numerous applications in traditional therapies and uses in a number of industries like those of food, nutraceutical, cosmetic, chemical, insecticide, fungicide, bactericide and especially the pharmaceuticals. About 80% of the world's population relies upon plants for their primary health care. World Health Organization fully supported the rational use of traditional plant based medicines by its member states (WHO, 2000, 2002, 2003). The curative actions of the plants are due to a relatively small number of biologically active principles, which include alkaloids, carotenoids, glycosides, terpenoids, steroids, tannins, flavonoids, vitamins, mucilages, minerals, organic acids etc (Bravo, 1998; Brown et al., 1999; Gosslau and Chen, 2004; Heber, 2004). These compounds have an extensive range of therapeutic activities.

In Pakistan, the herbal treatment is an integral part of the culture and about 70% of the population uses medicinal plants for various health disorders (Shinwari, 2010). The country has a rich medicinal flora due to its climatic diversity. There are around 6000 types of wild plants, out of which about 600 are considered medicinally important (Abbasi et al., 2010). Autumn olive (*Elaeagnus umbellata* Thunb.) is a deciduous shrub and abundantly found in the Himalayan regions of the country (Ahmad et al., 2006; Sabir et al., 2003). It is also native to China, Afghanistan, India, Japan and Korea (Ahmad et al., 2006; Parmar and Kaushal, 1982). The plant belongs to the family Elaeagnaceae and locally used as fuel wood, for fencing, fodder, basket making and shelter belts (Ahmad

et al., 2006). The edible parts of the plant are fruits and seeds. The fruit is a tasty fleshy drupe, which contains a single seed. Several species of the genus are cultivated for their fruit, including *Elaeagnus angustifolia*, *Elaeagnus multiflora* and *E. umbellata*. The fruits of various members of this genus are rich in vitamins, essential fatty acids, minerals, flavonoids and carotenoids (Matthews, 1994; Parmar and Kaushal, 1982).

The fruits of *E. umbellata* have 7 to 17 times more amount of the lycopene than tomatoes (Fordham et al., 2001) and it also contains α-cryptoxanthin, β-cryptoxanthin, β-carotene, lutein, phytoene and phytofluene. Lycopene is widely believed to protect against various forms of cancers (Collins and Perkins-Veazie, 2006). The seeds of the plant are used in raw form or cooked. It can be eaten with the fruit. The plant is medicinally very important. The flowers and seeds are astringent and stimulant in nature, and used for the treatment of coughs and cardiac ailments (Matthews, 1994; Parmar and Kaushal, 1982). The oil obtained from the seeds is used in the treatment of pulmonary infections (Matthews, 1994; Parmar and Kaushal, 1982). The fruit is considered as a food that is capable of reducing the incidence of cancer and also as a means of halting the growth of cancers (Chopra et al., 1986; Matthews, 1994). Recently, a study conducted by Sabir et al. (2007) on *E. umbellata* showed antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Furthermore, the consumers are increasingly persuaded to eat more fruits and vegetables as a contribution to a balanced diet and because a number of phytonutrients have been identified that may prevent ailments such as heart disorders and cancers (WCRF, AICR, 2007). Keeping in view the nutritional benefits, medicinal value, and natural abundance of autumn olive, the present study was designed to evaluate the proximate composition, phytochemicals ingredients and free radical scavenging activity.

MATERIALS AND METHODS

Plant materials and chemicals

The fruits of the plant were collected in the month of August from Rawlakot, Azad Kashmir and brought to the laboratory in polythene bags. Fresh samples were used for proximate and vitamin C analysis. The remaining fruits were dried in oven at 45°C and ground to the mesh size of 30 mm. The powdered samples were stored in a refrigerator at 4°C until further analysis. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid and Folin-Ciocalteau reagent were purchased from Sigma Chemical Co., USA. All the solvents and other chemicals used were of analytical grade from Sigma Chemicals Co., (USA) and Merck Darmstadt (Germany).

Proximate analysis

The fresh samples were analyzed in triplicate for moisture, crude protein, crude fibre and total mineral matter (ash) according to AOAC (1990). The moisture and total solids were determined in a drying oven at 105°C until constant weight. Determination of crude fat was carried out using petroleum ether (bp. 40 - 60°C) in a Soxtec system HT (Tecator). Estimation of crude protein (% N × 6.25) was performed by the micro-Kjeldahl method. Ash contents were determined by heating the samples at 550°C and crude fibre by digestion with acid and alkali using Fibertec system (Tecator). Carbohydrate content was given as:

100 - (Percentage of ash + Percentage of moisture + Percentage of fat + Percentage of protein + Percentage of fibre).

The nutritive value was calculated by multiplying protein and carbohydrate contents by a factor of 4 and fat by 9. All the proximate values are reported in percentage and the nutritive value is expressed in kcal/100 g.

Determination of pH, vitamin C and free acidity

For the determination of pH and free acidity, the samples (2 g) were dissolved in 40 ml of triply distilled water. The solutions were stirred at 25°C for 30 min at 200 rpm on magnetic stirrer (Gallenhamp, England) and filtered through Whatman filter paper No. 42. The filtered solutions were directly used for pH determination at room temperature using a Microcomputer pH Vision Datalogger, Model 6091, JENCO electronic Ltd., China. Three point calibrations were accomplished employing pH 7.0, 4.0 and 2.0 buffers (Fischer Scientific). Furthermore, 5 ml of the solution was taken in a flask and 2-3 drops of 1% phenolphthalein were added. Afterward, it was titrated with 0.1 N sodium hydroxide for determining the acidity. The analysis for ascorbic acid was conducted by titration method using 2,6-dichlorophenol-indophenol.

Mineral analysis

The dried sample of the plant was evaluated for calcium, potassium, phosphorus, iron, zinc, manganese and magnesium contents by wet digestion method in triplicate. About 4 g of sample was digested with a 50 ml of diacid mixture (HNO_3:$HClO_4$, 5:1, v/v) in a fume cupboard, heated initially at 80°C and then temperature was gradually increased to 250°C. After complete digestion, the sample was heated to near dryness and volume was brought up to 50 ml with double distilled deionized water and filtered through Whatman filter paper No. 42. Mineral estimation was done using atomic absorption spectrophotometer and flame photometer.

Extraction

The powdered fruit samples were separately extracted in methanol, distilled water and acetone using a Soxhlet extractor. All the extracts were filtered through Whatman No. 1 filter paper and concentrated under vacuums at 50°C. The dried extracts were then weighed. Extraction yield for each solvent was calculated by subtracting the dried weight of plant material residues after extraction from the weight of the original plant material. The extracts thus obtained were stored at 4°C and used for the estimation of certain phytochemicals and free radical scavenging activity.

Phytochemical analyses

Determination of total phenolic content

The total phenolic content of plant sample was determined using the Folin–Ciocalteu reagent (Khattak et al., 2008). In a test tube,

100 µL of the extract (5 to 0.1 mg/ml) was added to 2 ml of 2% aqueous sodium carbonate solution and mixed well. Then 100 µL of 50% Folin–Ciocalteau reagent was added to the mixture. After shaking, it was kept for 45 min and absorbance was measured at 750 nm in a spectrophotometer against a blank control. The total phenolic contents were calculated on the basis of a calibration curve of gallic acid and results were expressed as milligram gallic acid equivalents per gram (mg/g) of dry weight of the extract.

Determination of flavonoids

The total flavonoid content of the extract was determined according to colorimetric method as described by Park et al. (1997). An aliquot of 0.4 ml was added to test tubes containing 0.2 ml of 10% aluminium nitrate (w/v), 0.2 ml of 1 M potassium acetate and 8 ml of 80% ethanol. After incubation for 40 min at room temperature, the absorbance was determined at 415 nm wavelength. The total flavonoid content was expressed in milligrams of quercetin equivalents per gram of the extract.

Determination of alkaloids

The determination of alkaloid was conducted as described by Harborne (1973). In brief, 5 g of the powdered sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added. The solution was covered and incubated at room temperature for 2 h. The solution was afterward filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract and the precipitates were collected and washed with dilute ammonium hydroxide and then filtered. The residues were dried and weighed.

Determination of tannins

Tannin determination was conducted by Van–Burden and Robinson (1969) method. In brief, 1 g powdered sample was weighed into a 100 ml flask. Then 50 ml of distilled water was added and shaken for 1 h on a mechanical shaker. The solution was filtered and 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M ferric chloride in 0.1 N hydrochloric acid and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Determination of saponins

The method employed for the determination of saponins was that of Obadoni and Ochuko (2001). The ground sample (8 g) was put into a flask and 100 ml of 20% aqueous ethanol was added. The sample was heated over a water bath at 55°C for 4 h with incessant stirring. The mixture was filtered and the residue re-extracted with 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a separating funnel (250 ml). Next, 20 ml of diethyl ether was added to the concentrate and shaken vigorously and then the ether layer was discarded. This process was repeated and then 60 ml of *n*-butanol was added to the extract. Finally, the solution was heated on a water bath and after evaporation the samples were dried in the oven to a constant weight.

Determination of carotenoids

Total carotenoids were determined by the method of Jensen (1987) (1978). One gram powdered sample was extracted with 50 ml of 80% methanol solution and centrifuged at 5000 rpm for 30 min. The supernatant was concentrated to dryness under vacuum. The residue was dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10% methanolic potassium hydroxide the mixture was washed with 5% ice-cold saline water to remove alkali. The ether extract was dried over anhydrous sodium sulphate for 2 h. Finally, the extract was filtered and its absorbance was measured at 450 nm by using ether as blank.

Determination of free radical scavenging activity with DPPH

The antioxidant activity of extracts of autumn olive fruits was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Khattak et al., 2008). About 100 µL of each extract (from 100 to 2 mg/ml) was added to 1.9 ml of DPPH in methanol solution (150 µM) in a test tube and shaken vigorously. After incubation at 37°C for 35 min in the dark, the absorbance of each solution was determined at 517 nm. The corresponding blank (control) reading was also recorded. The free radical scavenging activity was expressed as percentage scavenging of the DPPH by plant extracts and calculated as:

$$\% \text{ DPPH radical scavenging activity} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of sample}} \times 100$$

The results obtained from the DPPH radical experiments were expressed as EC_{50} values. The EC_{50} value is the extraction concentration at which 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals are reduced by 50%. This is calculated from the linear regression analysis.

Statistical analysis

All determinations were obtained from triplicate measurements and results are expressed as means ± standard deviations. The data were analyzed using one-way analysis of variance (ANOVA) for mean differences. The Statistical Package for Social Sciences (SPSS, version 14.0 for windows) was used to analyze the data. Values of $p < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Humans require a wide range of organic and inorganic ingredients as supplementary caloric supplies to perform the routine biological activities. Plant materials comprise a big portion of diet and therefore their proximate composition and nutritive value is exceptionally important. Carbohydrates, fats and proteins are the main components that provide energy and are vital for growth, repair and metabolism. In this study, the proximate composition and nutritive value of *E. umbellata* berry were determined using standard procedures and the data is presented in Table 1. The results showed that the berry of the plant contained 71.4% moisture, 2.9% ash, 4.0% protein, 2.3% crude fat, 5.9% fibre and 13.6% nitrogen free extract. Earlier, Graham (1964) reported 69.4% moisture and 4.47% protein in the berry of the plant. The nutritive value of the berry determined on fresh weight basis was 90.8 kcal/100 g.

Table 1. Proximate composition and nutritive value of *Elaeagnus umbellata* fruit.

Parameter	Value
Moisture (%)	71.4 ± 1.8
Ash (%)	2.9 ± 0.3
Protein (%)	4.0 ± 0.3
Fibre (%)	5.9 ± 0.2
Fat (%)	2.3 ± 0.1
Carbohydrates (%)	13.6 ± 1.9
Energy kcal/100 g	90.8 ± 4.2

Values are means of triplicate determinations (n = 3) ± standard deviations. The analysis is based on fresh weight.

Table 2. Phytochemical analysis and physicochemical characteristics of *Elaeagnus umbellata* fruit.

Parameter	Value
Phenolic (mg/g)	23.3 ± 2.0
Flavonoids (mg/g)	3.6 ± 0.1
Carotenoids (mg/g)	19.9 ± 3.2
Tannins (mg/g)	126.5 ± 4.4
Alkaloids (mg/g)	12.6 ± 0.7
Saponins (mg/g)	21.2 ± 1.9
Vitamin C (mg/100 g)	27.8 ± 1.8
Acidity (%)	3.1 ± 0.1
pH	4.5 ± 0.1

Values are means of triplicate determinations (n = 3) ± standard deviations. The analysis is based on dry weight except for vitamin C, where fresh samples were used.

Minerals are indispensable to life as all living organisms utilize these to trigger hormones, enzymes and other organic molecules that participate in the growth, routine functions and maintenance of life processes (WHO, FAO, 2004). They cannot be produced by the body and therefore must be regularly provided in diet. The mineral content like zinc, manganese, potassium, phosphorus, iron, magnesium and calcium were estimated by wet digestion method using atomic absorption spectro-photometer and flame photometer. The values are reported in µg/g and the data is presented in Figure 1. The fruits of the plant showed 3.7, 5.3, 53.1, 91.3, 162.4, 183.3 and 232.1 µg/g of zinc, manganese, iron, calcium, magnesium, phosphorus and potassium contents, respectively. Previously, Sabir and Riaz (2005) compared five populations of *E. umbellata* from different areas of district Bagh and reported significant variation in the mineral contents. In contrast to our results, they reported comparatively low magnesium (70 - 86.6 ppm) and phosphorus (110 - 133 ppm) contents. The variations in the levels of these minerals may be due to different stages of the growth of the plant. However, the values of iron (78.5 - 95 ppm), potassium (175 - 375 ppm) and calcium (70 - 110 ppm) contents were comparable to our findings. The data showed that there were sufficient amounts of valuable minerals and consequently the plant's berry can be included in diet to have balanced nutrition.

Phytochemicals are progressively getting more importance, as recent scientific investigations ascertain how significant these nutrients are for health. Consumption of phytonutrients-rich diet defends the human body from chronic illnesses and various health disorders (Christen et al., 2008; Gosslau and Chen, 2004; He et al., 2007; Kavanaugh et al., 2007; Lee et al., 2008). The powdered sample of the berry was assessed for selected phytochemicals and the results are recorded in Table 2. The data revealed that the plant's fruit contained 23.3, 3.6, 19.9, 126.5, 12.6 and 21.2 mg/g of phenolic, flavonoid, carotenoid, tannin, alkaloid and saponin contents, respectively. Earlier, Wang and Fordham (2007) reported phenolic and carotenoid contents in the different genotypes of autumn olive ranging from 168.9 to 258.1 mg/100 g and 43.4 to 59.3 mg/100 g, respectively, on fresh weight basis. Perkins-Veazie et al. (2005) described that autumn olives are high in phenolic content (1700 mg/kg) and discussed that the astringent flavour of the berry may be due to these contents. Generally, the tannins were believed to be anti-nutritional but now it is well recognized that they have beneficial properties which depend upon their chemical structure and concentration levels. A study conducted by Schiavone et al. (2008) demonstrated that the diets containing low levels (0.15 - 0.20 %) of chestnut tannins had positive effects on the health and growth performance of broiler chicks. Earlier, Ito et al. (1999) reported seven new tannins elaeagnatins A-G, from the leaves of *E. umbellata* together with fifteen known tannins. Saponins generally reduce cancer risks, decrease blood lipids and lessen blood glucose. Saponins rich diet is useful for the inhibition of dental caries and platelet aggregation, and as an antidote against lead poisoning (Shi et al., 2004). Our study showed that fresh berry contained 27.8 mg/100 g of vitamin C. In contrast to our results, Ahmad et al. (2005) reported comparatively low vitamin C content (14.1 to 14.3 mg/100 g). This variation may be due to difference in processing and fruit maturity stage. The free acidity and pH values noted for the fruit extract were 3.1% and 4.5, respectively.

Extracts of the fruits were prepared using distilled water, methanol and acetone and the yields (%) are presented in Figure 2. The data indicated that the extraction yields were significantly ($p < 0.05$) affected by the solvent used. Methanol showed the highest extraction yield (24.1%), while the yields of aqueous and acetone extracts were 17.5 and 15.3%, respectively. The free radical scavenging activity of the plant extracts were

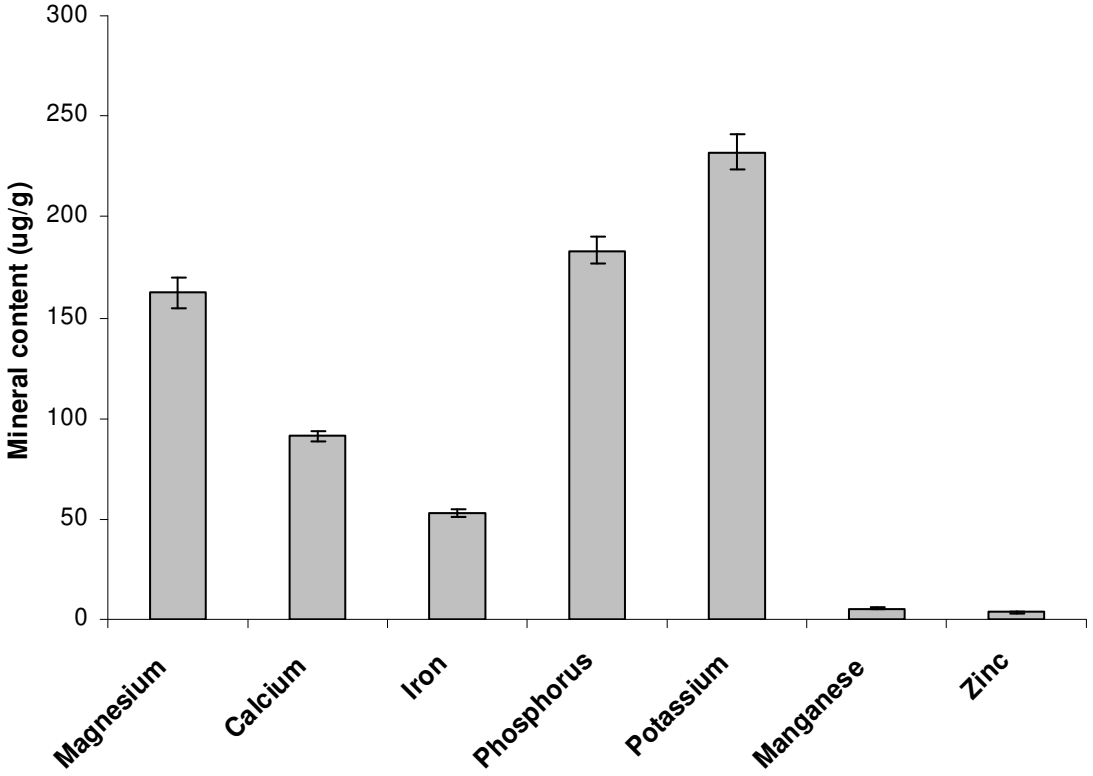

Figure 1. Mineral content of *Elaeagnus umbellata* fruit on dry weight basis. Values are means of triplicate determinations (n = 3) ± standard deviations. The vertical bars represent the standard deviation for each data point.

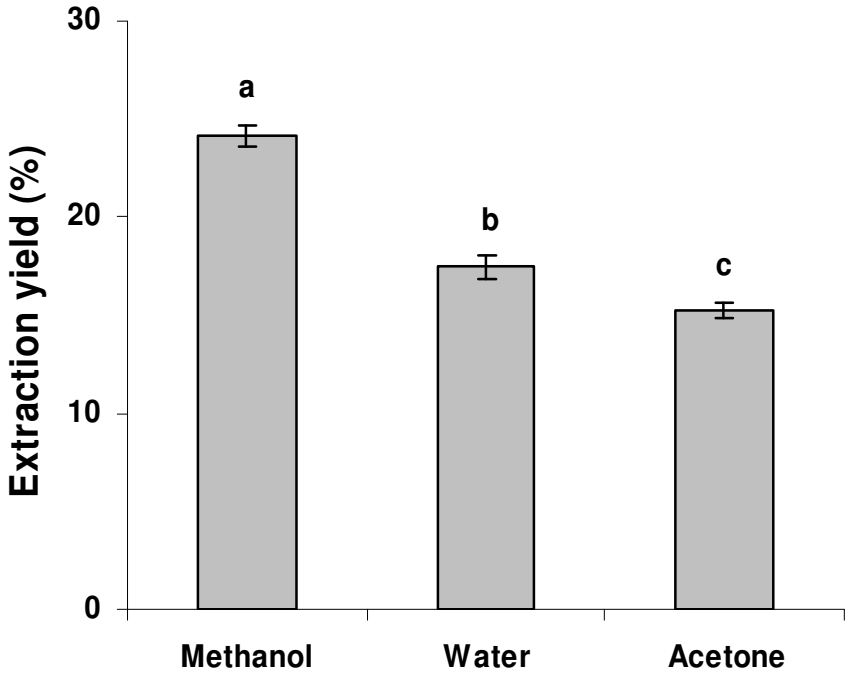

Figure 2. The extraction yields of *Elaeagnus umbellata* fruit in various solvents. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

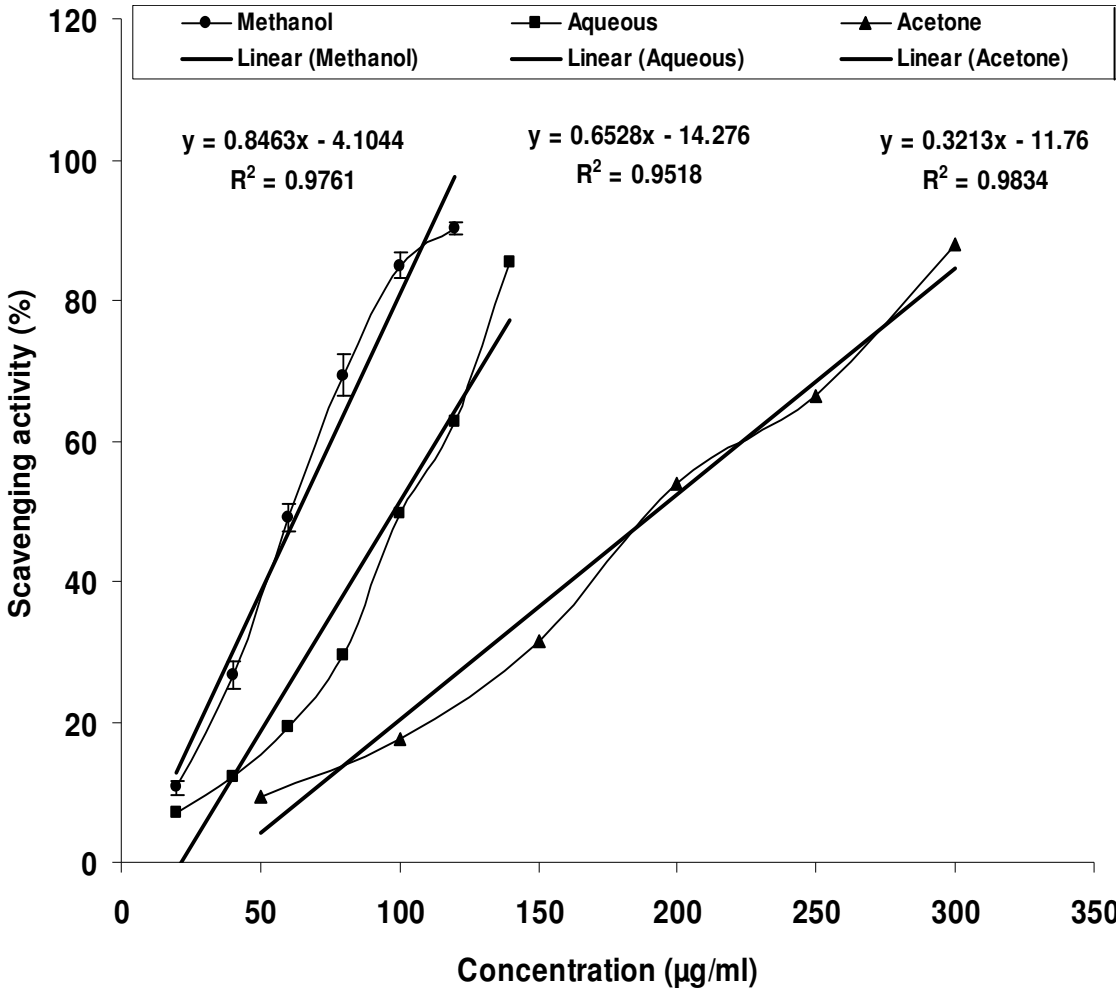

Figure 3. The DPPH scavenging activity of *Elaeagnus umbellata* fruit in acetone, methanol and water extracts. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point.

estimated using DPPH radical and the results are reported as percent scavenging activity (Figure 3). The DPPH scavenging activity was significantly ($p < 0.05$) affected by the extraction solvent. The methanol extract showed the highest scavenging activity; it exhibited 10.7, 26.7, 49.0, 69.3, 84.9 and 90.2% scavenging activity at the concentrations of 20, 40, 60, 80, 100 and 120 µg/ml, respectively. The activity was found to be dose-dependent. Similarly, both aqueous and acetone extracts showed increase in scavenging activity with increase in concentration. The results of the scavenging activity are also reported as EC_{50} values (µg/ml) in Figure 4. Low EC_{50} is indicative of high free radical scavenging activity. The values were significantly ($p < 0.05$) affected by the solvents used. The lowest EC_{50} value was noted for methanol extract (97.3 µg/ml) and highest for the acetone extract (188.0 µg/ml). Whole foods contain complex mixture of antioxidants that have wonderful synergistic effects and are useful in preventing the oxidative damage that is initiated by free radicals. Plant's antioxidants are well accepted in preventing arteriosclerosis, brain disorders, inflammations, cellular injuries, cardiovascular diseases, cancers and immune system deterioration (Ames, 1983; Steinberg, 1991).

Conclusion

Collectively, our results showed that autumn olive berry has superior food quality. The consumption of this wild berry may therefore have many benefits to human health. Owing to the availably of substantial quantity of vitamin C, phenolics, carotenoids, flavonoids and mineral like calcium, zinc, iron, manganese and magnesium, the fruits of autumn olive may be used as a dietary supplement. These data may also be supportive in allowing better food choices and improvement in nutritional and health status of the consumer.

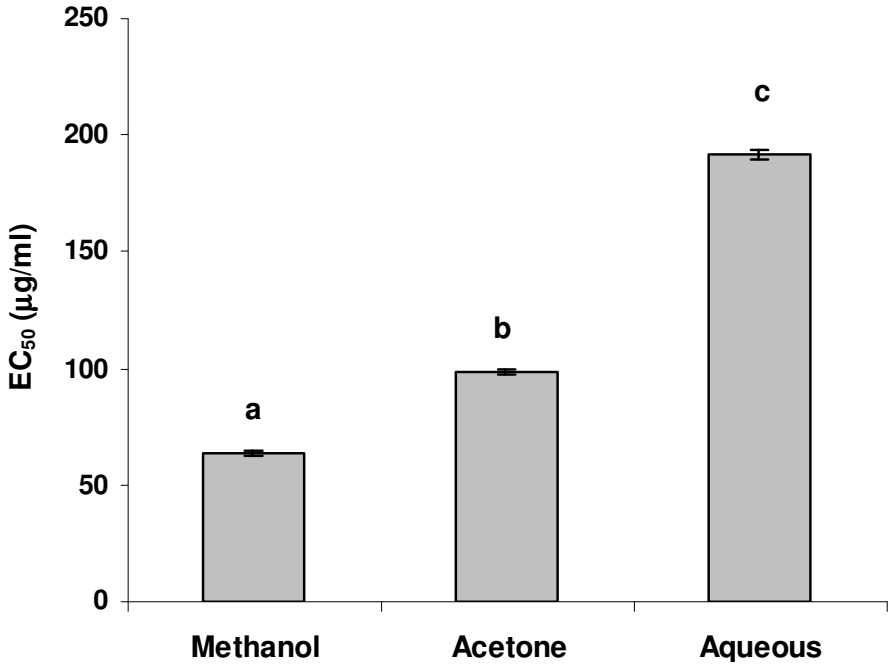

Figure 4. The EC_{50} values of the DPPH scavenging activity of *Elaeagnus umbellata* fruit. Values are means of triplicate determinations (n=3) ± standard deviations. The vertical bars represent the standard deviation for each data point. Values with different superscript letters are significantly different ($p < 0.05$).

ACKNOWLEDGEMENT

The author is thankful to the Director, NIFA for providing all the facilities to conduct the research work.

REFERENCES

Abbasi AM, Khan MA, Ahmad M, Qureshi R, Arshad M, Jahan S, Zafar M, Sultana S (2010). Ethnobotanical study of wound healing herbs among the tribal communities in Northern Himalaya ranges district Abbottabad, Pakistan. Pak. J. Bot. 42:3747-3753.

Ahmad SD, Sabir MA, Juma M, Asad HS (2005). Morphological and biochemical variations in *Elaeagnus umbellata* Thunb. from mountains of Pakistan. Acta Bot. Croat. 64:121-128.

Ahmad SD, Sabir SM, Zubair M (2006). Ecotypes diversity in autumn olive (*Elaeagnus umbellata* Thunb): A single plant with multiple micronutrient genes. Chem. Ecol. 22:509-521.

Ames BM (1983). Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. Science 221:1256-1263.

AOAC (1990). Official Methods of Analysis of the Association of Official Analytical Chemists, 15th edn. Washington, DC, Association of Official Analytical Chemists. pp. 85-89.

Bravo L (1998). Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr. Rev. 56:317-333.

Brown L, Rimm EB, Seddon JM, Giovannucci EL, Chasan TL, Spiegelman D, Willett WC and Hankinson SE (1999). A prospective study of carotenoid intake and risk of cataract extraction in US men. Am. J. Clin. Nutr. 70:517-524.

Chopra RN, Nayar SL, Chopra IC (1986). Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, New Delhi, India. pp. 70-71.

Christen WG, Liu S, Glynn RJ, Gaziano JM, Buring JE (2008). Dietary carotenoids, vitamins C and E, and risk of cataract in women: a prospective study. Arch. Ophthalmol. 126:102-109.

Collins JK, Perkins-Veazie P (2006). Lycopene: from plants to humans. HortScience 41:1135-1144.

Farnsworth NR, Soejarto DD (1991). Global importance of medicinal plants. In Akerele O et al. H (eds) Conservation of Medicinal Plants, Cambridge University Press, Cambridge, UK pp. 25-51.

Fordham IM, Clevidence BA, Wiley ER, Zimmerman RH (2001). Fruit of autumn olive; A rich source of lycopene. HortScience 36:1136-1137.

Gosslau A, Chen KY (2004). Neutraceuticals, apoptosis and disease prevention. Nutrition 20:95-102.

Graham SA (1964). The Elaeagnaceae in the Southeastern United States. J. Arnold Arbor. 45:274-278.

Harborne JB (1973) Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman and Hall. London. pp. 88-185.

He FJ, Nowson CA, Lucas M, MacGregor GA (2007). Increased consumption of fruit and vegetables is related to a reduced risk of coronary heart disease: meta-analysis of cohort studies. J. Hum. Hypertens. 21:717-728.

Heber D (2004). Vegetables, fruits and phytoestrogens in the prevention of diseases. J. Postgrad. Med. 50:145-149.

Ito H, Miki K Yoshida T (1999). Elaeagnatins A-G, C-Glucosidic Ellagitannins from *Elaeagnus umbellata*. Chem. Pharm. Bull. 47:536-542.

Jensen A (1978). Chlorophyll and carotenoids. In Hallebust JA, Craigie JS (eds) Handbook of Physiochemical and Biochemical Methods. Cambridge University Press, Cambridge, UK pp. 5-70.

Kavanaugh CJ, Trumbo PR, Ellwood KC (2007). The U.S. Food and Drug Administration's evidence-based review for qualified health claims: tomatoes, lycopene, and cancer. J. Natl. Cancer Inst. 99:1074-1085.

Khattak KF, Simpson TJ, Ihasnullah (2008). Effect of gamma irradiation on the extraction yield, total phenolic content and free radical-scavenging activity of *Nigella staiva* seed. Food Chem. 110:967-972

Lee Y, Lee HJ, Lee HS, Jang YA, Kim C (2008). Analytical dietary fiber database for the national health and nutrition survey in Korea. J. Food Compos. Anal. 21:S35-S42.

Matthews V (1994). The New Plantsman. Royal Horticultural Society. Vol 1.
Obadoni BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Glob. J. Pure Appl. Sci. 8:203-208.
Park YK, Koo MH, Ikegaki M, Contado JL (1997). Comparison of the flavonoid aglycone contents of *Apis mellifera* propolis from various regions of Brazil. Arq. Biol. Tecnol. 40:97-106.
Parmar C, Kaushal MK (1982). *Elaeagnus umbellata* Thunb. In Wild Fruits of Sub-Himalayan region. Kalyani Publishers. New Delhi, India pp. 23-25.
Perkins-Veazie PM, Black BL, Fordham IM, Howard LR (2005). Lycopene and total phenol content of autumn olive (*Elaeagnus umbellata*) selections. HortScience 40:883-893.
Sabir SM, Ahmad SD, Hussain IM, Tahir KM (2007). Antibacterial activity of *Elaeagnus umbellata* (Thunb.) a medicinal plant from Pakistan. Saudi Med. J. 28:259-263.
Sabir SM, Ahmad SD, Lodhi N (2003). Morphological and biochemical variation in Sea buckthorn *Hippophae rhamnoides* L. ssp turkestanica, a multipurpose plant for fragile mountains of Pakistan. South Afr. J. Bot. 69:587-592.
Sabir SM, Riaz K (2005). Morphological, biochemical and elemental analysis of *Elaeagnus umbellata*, a multipurpose wild shrub from Pakistan. J. Appl. Hortic. 7:113-116.
Schiavone A, Guo K, Tassone S, Gasco L, Hernandez E, Denti R, Zoccarato I (2008). Effects of a natural extract of chestnut wood on digestibility, performance traits, and nitrogen balance of broiler chicks. Poult. Sci. 87:521-527.
Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y (2004). Saponins from edible legumes: chemistry, processing and health benefits. J. Med. Food 7:67-78.
Shinwari ZK (2010). Medicinal plants research in Pakistan. J. Med. Plants Res. 4:161-176.
Steinberg D (1991). Antioxidants and atherosclerosis, a current assessment. Circulation 84:1420-1425.
Van–Burden JP, Robinson WB (1969). Formation of complexes between protein and tannin acid. J. Agric. Food Chem. 17:772-777.
Wang SY, Fordham IM (2007). Differences in chemical composition and antioxidant capacity among different genotypes of autumn olive (*Elaeagnus umbellata* Thunb). Food Technol. Biotechnol. 45:402-409.
WCRF, AICR (2007). Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. World Cancer Research Fund, American Institute for Cancer Research, Washington DC. pp. 1-394.
WHO (2000). General Guidelines for Methodologies on Research and Evaluation of Traditional Medicines. World Health Organization, Geneva. pp. 1-70.
WHO (2002). WHO Traditional Medicine Strategy 2002-2005. World Health Organization, Geneva. pp. 1-54.
WHO (2003). WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. World Health Organization, Geneva. pp. 1-72.
WHO, FAO (2004). Vitamin and Mineral Requirements in Human Nutrition. 2nd edn, World Health Organization and Food and Agriculture Organization of the United Nations, Hong Kong. Available online: http://whqlibdoc.who.int/publications/2004/9241546123.pdf (Retrieved on August 11, 2011).