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ANTIOXIDANT ACTIVITY AMONG DIFFERENT PARTS OF AUBERGINE (*SOLANUM MELONGENA* L.)

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Abstract

Methanolic (80%) extracts of various parts (green crown, peel and flesh) of selected varieties of round and long aubergine were explored for total phenolic content (TPC) and antioxidant activity using a number of colorimetric assays. The results showed that TPC methanolic extracts, of different parts of selected varieties of aubergine, ranged from 16.72-25.00 mg GAE/100g DW. The highest amounts (22.05-25.00 mg GAE/100g DW) were obtained in round aubergine extracts and lower in long aubergine extracts (16.72-20.43 mg GAE/100g mg GAE/100g DW). Similarly, the methanolic extracts of round aubergine exhibited better inhibition of oxidation of linoleic acid (59.34-64.00 %) as compared to that of long aubergine (56.91- 60.56 %). The analysis of variance data showed that the difference in peroxide inhibition capacity and reducing power of different parts of aubergine was significant ($p<0.05$). The highest DPPH scavenging activity (70.01%) was achieved with methanolic extracts of peels of round aubergine. The present study suggests that round aubergine contained higher antioxidant components and potential as compared to the long variety. A positive correlation was observed between the phenolic component and free radical scavenging potential of methanolic extracts of different parts of aubergine suggesting its use as a bioactive functional food.

Introduction

Vegetables, especially those which can be eaten raw, can promote youthfulness and improve health status by reducing the incidence of chronic diseases such as cancer and cardiovascular disease (Bazzano *et al*., 2002; Riboli & Norat, 2003; Wan Hassan, 2007). According to The World Health Organization and, Food and Agricultural Organization (2003), the recommended daily consumption of at least 400 g of fruit and vegetables is essential for the prevention of cardiovascular, atherosclerosis, carcinogenesis, accelerated ageing cancer, diabetes and obesity. These beneficial effects of plants and vegetables are attributed to their antioxidant contents (Lako *et al*., 2007; Naczk & Shahidi, 2006; Gull *et al*., 2012; Vemanu, 2013).

Antioxidants are the key species which retard the process of oxidation to keep the optimum level of reactive oxygen species (ROS) and reactive nitrogen species (RNS) thus preventing a large number of chronic diseases. ROS/RNS produced during normal aerobic conditions, are essential for numerous metabolic processes including cell signaling, energy production, gene transcription and immune defense. (Halliwell & Gutteridge, 2000; Seifried *et al*., 2007). However, an increase in the concentration of these species caused by different environmental and nutritional factors may cause a vast variety of diseases including lipid and protein damage (cancer), atherosclerosis, diabetes mellitus, neurodegenerative disorders, liver damage by certain toxins (aflatoxins), hypertension, AIDS and aging (Valko *et al*., 2007; Biglari *et al*., 2008; Mushtaq *et al*., 2012; Khatoon *et al*., 2012).

Natural antioxidants including carotenoids, flavonoids, cinnamic acids, benzoic acids, folic acid, ascorbic acid, tocopherols and tocotrienols are the secondary metabolites produced by plants for their sustenance. The bioactive species Beta-carotene, ascorbic acid and alpha tocopherol have the enhanced potential as free radical scavengers. Vegetables are the potential source of natural antioxidants and play a significant role in reducing the risk of certain types of cancer, cardiovascular diseases and other chronic diseases (Ajila *et al*., 2007).

Aubergine, otherwise known as eggplant, brinjal, melongene or guinea squash (*Solanum melongena* L.) and botanically classified as berry, is a delicate perennial often cultivated in tropical and subtropical climates, mainly China, India Egypt, Pakistan and Iran (Doijode, 2001; Tsao and Lo, 2006). Aubergine is one of the few important cash vegetables which prevails during hot and wet climates when other vegetables have comparatively high prices and so is beneficial for impoverished consumers. (Rashid *et al*., 2003).

Aubergine fruit is fleshy with a meaty texture; edible with a bitter taste and easily distinguished into three parts: peel, flesh and green crown. Earlier, epidemiological reports show that aubergine extracts have successfully suppressed the development and growth of tumours, metastasis (Matsubara *et al*., 2005), inhibited inflammation, which can lead to atherosclerosis, and reduced the risk of stroke (Keli *et al*.,1996), lung cancer and heart disease (Knekt *et al*., 1996; 1997). Aubergine is now receiving more interest from consumers and researchers worldwide because of its health benefits. The beneficial effects of aubergine can be attributed to the presence of life saving plant bio-actives, mostly phenolics (Barreira *et al*., 2008; Gorinstein *et al*., 2009; Muller *et al*., 2011; Gull *et al*., 2012). The present work investigates polyphenols, metal chelating and free radical scavenging potential of extracts of different parts of selected varieties of aubergine.

Materials and Methods

Collection of samples: Aubergine (*Solanum melongena*) samples were obtained from the botanical garden, University of Agriculture, Faisalabad. The collected samples of aubergine (long and round) were further identified and authenticated by the Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Pretreatment of samples: The peel, flesh and green crown of the samples were separated manually with a sharp knife, dried under ambient conditions, ground into fine powder and stored in airtight polythene bags for further use and analysis.

Extraction of antioxidant component: Each of the powdered samples (10 g) of the different parts (peel, flesh and green crown) of selected aubergine (long and round) varieties were extracted with 100mL of 80% aqueous methanol by agitation for 24 hrs at ambient conditions using an orbital shaker (Gallenkamp, UK). All extracts were filtered using Whatman filter paper No. 1 and residues were re-extracted twice in the same manner. The extracts were then combined and concentrated by evaporation under reduced pressure at 25°C, using a rotary evaporator (EYELA, SB-651, Rikakikai Co. Ltd. Tokyo, Japan). The crude, dried extracts were weighed to calculate the yield (mg g^{-1} of DW) and stored in a refrigerator (-4ºC) for further analysis.

Determination of total phenolic content (TPC): Total phenolic contents (TPC) in peel, flesh and green crown of round and long aubergine was assessed using a colorimetric method as described by Chaovanalikit and Wrolstad (2004). Dry mass of each extract (50 mg) was mixed with 0.5mL of Folin–Ciocalteu reagent, diluted with 7.5mL deionized water and kept at ambient temperature for 10 min. To this, 1.5mL of 20% sodium carbonate (w/v) was added, heated at 40°C for 20 min and the mixture was then cooled in an ice bath. The Folin-Ciocalteau method was chosen due to its sensitivity, low interference and fastness to quantify the phenolics contents (Sultana *et al*., 2007). Finally, the absorbance was measured at 755 nm (Hitachi U-2001 spectrophotometer, model 121-0032). The results were expressed as gallic acid equivalents (GAE) per dry matter (DW). All samples were analyzed in triplicate and the results were averaged.

Antioxidant activity determination in linoleic acid system: The antioxidant activity of extracts was determined in terms of % inhibition of peroxidation in linoleic acid system following a reported method of Iqbal and Bhanger (2005). Extracted material (5 mg) of each sample was added to a solution containing linoleic acid (0.13 ml), 99.8% ethanol (10 ml) and 0.2 M (10 ml) sodium phosphate buffer (pH 7). The mixture was diluted to 25 ml with distilled water, incubated at 40 °C and the degree of oxidation was measured following the thiocyanate method (Gulçin *et al*., 2003; Yen *et al*., 2000), with 10 ml of ethanol (75%), 0.2 ml aqueous ammonium thiocyanate (30%) solution, 0.2 ml of sample solution and 0.2 ml of ferrous chloride ($FeCl_2$) solution (20 mM in 3.5% HCl) being added sequentially. After 3 min of stirring, the absorption values of the mixtures were measured at 500 nm and the readings were taken as peroxide contents. A control reading was performed with linoleic acid without the extracts. Synthetic antioxidants, butylated hydroxytoluene (BHT) and ascorbic acid (200 ppm) were used as positive controls. The maximum peroxidation level was observed at 360 h (15 days) in the sample which contained no antioxidant component and was used as a test point. Percent inhibition of linoleic acid peroxidation was calculated using the following formula:

100 – [(Abs. increase of sample at 360 h/Abs. increase of control at 360 h) ×100] (Eq.1)

Determination of reducing power: The reducing power of methanolic extracts of peel, flesh and green crown was determined according to the procedure described by Yen *et al*., (2000) with modification. Equivalent volume of extracts of different parts of round and long aubergine containing 2.5-10.0 mg of extract, was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%). The mixture was incubated at 50°C for 20 min, after which 5mL of 10% trichloroacetic acid was added and centrifuged for 10 min at 5°C in a refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). The upper layer of the solution (5.0mL) was diluted with 5.0mL of distilled water and 1.0mL ferric chloride (0.1%). Absorbance was measured at 700 nm to evaluate the reducing power of the aqueous methanol extracts of different parts of aubergine. The measurements were performed in triplicate and the results averaged.

DPPH radical scavenging assay: Free radical scavenging activities of the methanolic extracts of different parts of round and long aubergine were measured by using the procedure described by Iqbal & Bhanger (2005). 1.0mL of each extract containing 0.025 mg mL^{-1} of extract in methanol was mixed with 5.0mL of freshly prepared solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (0.025 g L^{-1}). Absorbance at 0, 0.5, 1, 2, 5 and 10 min was measured at 515 nm. The remaining amounts of DPPH free radical were calculated from the calibration curve and absorbance measured at the 5th minute was used to compare radical scavenging activity of each extract.

Statistical analysis: All the samples were analyzed in triplicate and data was reported as mean (n = 2 ×3) ±SD. The results were analyzed using ANOVA using Minitab 2000 Version 13 statistical software (Minitab Inc. Pennsylvania, USA) at 5% significance level.

Results and Discussion

Yield of bioactive constituents: The yield of bioactive constituents from different parts (peel, flesh and green crown) of round and long aubergine extracted in 80% aqueous methanol solvent ranged from 12.88-32.2g /100g of dry weight (DW). The maximum mean extract yield (29.8%) was obtained for peel of round aubergine while the minimum (11.2%) was observed in the green crown of long aubergine as expressed in Fig. 1.

It is evident from Fig. 1 that the peel of both long and round varieties contained higher amounts of aqueous methanol extractable bioactive components as compared to flesh and green crown respectively. Furthermore, for investigated parts, round aubergine was found to have higher extractable biological constituents as compared to long aubergine.

The analysis of variance results showed that there was non-significant difference in the methanolic extraction yield among round and long varieties of aubergine under the given extraction conditions, whereas variation among different parts (peel, flesh and green crown) was significant ($p<0.05$). When compared with earlier reports available in the literature, the extraction yield for different parts of aubergine using 80% aqueous methanol was higher (Jeong *et al*., 2004; Oh *et al*., 2008; Jung *et al*., 2011) for eggplant than when using ethanol suggesting that methanol is a better solvent for the extraction of antioxidant and phenolic components from aubergine. Furthermore, the extractable bioactive components were found to be higher than apple and guava (Gull *et al*, 2012) extracted under the same conditions.

Total phenolic contents (TPC): Polyphenols are the bioactive molecules distributed largely in fruits, vegetables and herbs which contribute directly to the overall antioxidant activities of plant material by acting as free radical terminators (Othmon *et al*., 2007; Chahardehi *et al*., 2009). In human non-infectious diseases i.e. aging, atherosclerosis, diabetes mellitus, neurodegenerative disorders, hypertension and stroke, the antioxidant defense system consists of both endogenous and exogenous antioxidant systems which work together at the molecular level and protect cell membranes, lipoproteins and nucleic acids. The endogenous antioxidant system consists of certain enzymes which are primarily of physiological origin whereas exogenous antioxidants include entities entering the body through the diet. Increased intakes of these natural supplement antioxidants, mainly phenolics, help to maintain the balance between antioxidant and oxidants in living organisms (Halliwell *et al*., 1995; Valko *et al*., 2007; Biglari *et al*., 2008).

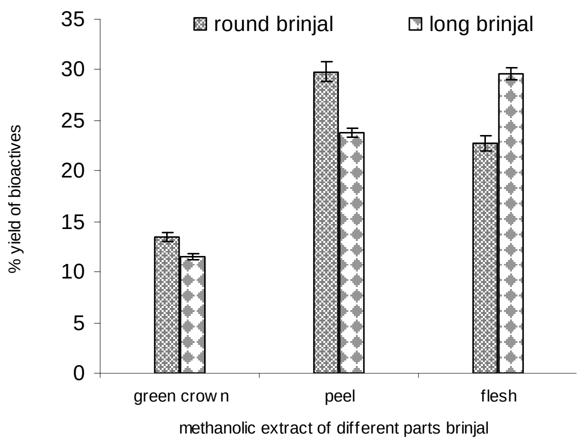

Fig. 1. Bioactive components extracted from aubergine using aqueous methanol.

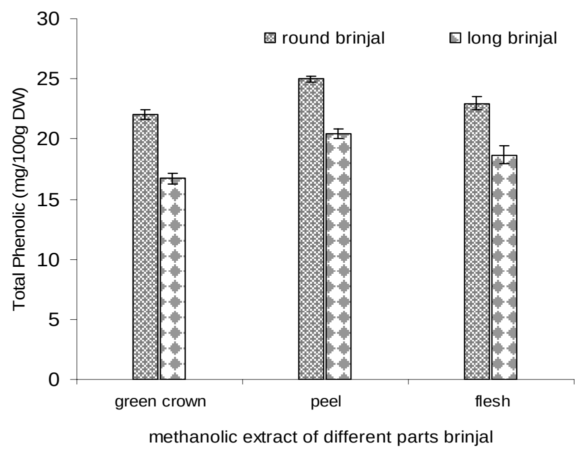

Fig. 2. Comparison of total phenolic contents in different parts of aubergine.

Fig. 2 shows that methanolic extract of round aubergine peel contained higher amounts of polyphenols (25.0±0.36 mg/100g DW) followed by flesh (22.94±0.71 mg/100g DW) and green crown (22.05±0.25 mg/100g DW). Long aubergine values were, peel (20.43±0.25 mg/100g DW), flesh (19.68±0.56 mg/100g DW) and green crown (16.72±0.39 mg/100g DW). The overall total phenolic contents (16.72±0.39-25.0±0.36 mg/100g DW) in methanolic extracts of both varieties (round and long aubergine) were found to be higher than those reported earlier in garlic (9 mg GAE /g) and onion (15.87 mg GAE/g) (Gorinstein *et al*., 2009) but were lower than cauliflower (274mg/100g) (Wu *et al.,* 2004) and cucumber (12.38±0.99) (Al-Mamary., 2002). The high percentage of phenolic antioxidant components as observed in the present work are consistent in ranking aubergine as one of the top ten oxygen free radical absorbing vegetables (Cao *et al*., 1996) and methanol as a good solvent for the extraction of antioxidant attributes (Turkmen *et al.*, 2006). The ANOVA results (Table 1) indicate significant variation of total phenolic contents (TPC) among different parts (peel, leaves and green crown), as well as the varieties (round and long), of aubergine tested.

Total reducing power of aubergine extracts: The potential of a material to reduce ferric/ferricyanide complex to its ferrous (Fe^{2+}) state is considered an important aspect of antioxidant activity of extracts. In this assay, the amount of Fe^{2+} is quantitatively monitored by measuring the intensity of Perl's Prussian blue colour complex at 700 nm. The increase or decrease in absorbance is directly related with reducing power of the material; ultimately antioxidant potential of the material.

The reducing power of different parts (peel, flesh and green crown) of both varieties (round and long) of aubergine extracted in 80% aqueous methanol were investigated for their total reducing power at different concentrations. The overall Fe^{3+} reducing potential of both varieties of aubergine ranged from 0.351± 0.02 – 0.849±0.05 with the lower value in the long green crown and the higher value in round aubergine peel. The results further showed that the reducing power of extracts of different parts of aubergine increased with an increase in extract concentration as shown in Table 1.

The results of the present study showed that all the parts of round aubergine have high Fe^{3+} reducing potential in terms of ferric/ferricyanide complex. Jung *et al*., (2011) investigated the reducing power of peel, pulp and leaves of egg plant in water and 70% ethanol. The results reported were (0.2-0.7) which were found to be lower than the values observed during this study (0.35-0.84), suggesting that aqueous methanol is a better solvent for the extraction of active bioactives as compared to water and ethanol.

Table 1. Reducing power of methanolic extracts of different parts of aubergine.

Variety	Part	Reducing power of 70% methanolic extracts (Absorbance)			
		2.5mg/mL	5 mg/mL	7.5 mg/mL	10 mg/mL
Round	Peel	0.807 ± 0.043[a]	0.819 ± 0.052[a]	0.832 ± 0.034[a]	0.849 ± 0.054[a]
	Flesh	0.741 ± 0.041[a]	0.755 ± 0.044[a]	0.768 ± 0.048[a]	0.777 ± 0.054[b]
	Green Crown	0.637 ± 0.054[b]	0.649 ± 0.035[b]	0.665 ± 0.033[b]	0.681 ± 0.037[c]
Long	Peel	0.684 ± 0.033[b]	0.696 ± 0.042[b]	0.711 ± 0.024[a]	0.727 ± 0.044[a]
	Flesh	0.711 ± 0.042[a]	0.728 ± 0.054[a]	0.741 ± 0.038[a]	0.766 ± 0.063[a]
	Green Crown	0.651 ± 0.051[b]	0.669 ± 0.045[b]	0.678 ± 0.043[a]	0.693 ± 0.039[b]

Means followed by different letters are significantly ($p<0.05$) different

Antioxidant activity in linoleic acid system: The antioxidant potential of extracts of different parts (peel, flesh and green crown) of both varieties (round and long) was evaluated by measuring inhibition of peroxidation in linoleic acid system (Iqbal & Bhanger, 2005). The percentage inhibition of linoleic acid peroxidation for aqueous methanol extracts of different parts of both varieties (round and long) of aubergine are illustrated in Fig. 3. Percent inhibition of peroxidation in linoleic acid system by 80% methanolic extract was found to be in the order, round peel (64.00±0.45) > round flesh (61.63±0.71) > long peel (60.56±0.25) > green crown (59.34±0.45) > long flesh (57.14±0.56) when butylated hydroxytoluene (BHT) and ascorbic acid were used as positive controls. The values of percent inhibition are comparable with BHT and vary significantly ($p<0.05$) amongst the different parts tested of both varieties (long and round).

The higher inhibition potential of round aubergine peel against linoleic acid peroxidation can be attributed to the presence of higher amounts of phenolics bioactives (Zainol *et al.,* 2003). The values of percentage inhibition of linoleic acid peroxidation of the extracts from different parts of aubergine were found to be comparable to those reported in chestnut fruit (Barreira *et al*., 2008) and higher than those reported for Cassia fistula (Siddhuraju *et al.*, 2002).

Free radical scavenging activity using DPPH radical: Results obtained for free radical scavenging potential of different parts of round and long aubergine presented in Fig. 4 indicated that scavenging activity of aubergine in 70% methanol ranged from 55.3-70.1% and 50.0-64.5% for round and long variety, respectively. The highest DPPH free radical scavenging activity was achieved for methanolic extract of peel from round aubergine and the lowest DPPH free radical scavenging activity was observed for green crown from long aubergine. This may be attributed to the fact that round aubergine contains large amounts of phenolic compounds, as shown in Fig. 4, which have the ability to donate the hydrogen ion, ultimately increasing the free radical scavenging activity of the extract. These values of scavenging activity were compared with the values of BHT and BHA (71.56 and 66.78%). The values of free radical scavenging activity determined in the present investigation were comparable to BHT and BHA and also those reported in different vegetables and other fruits (Abdou, 2011, Shad, 2012).

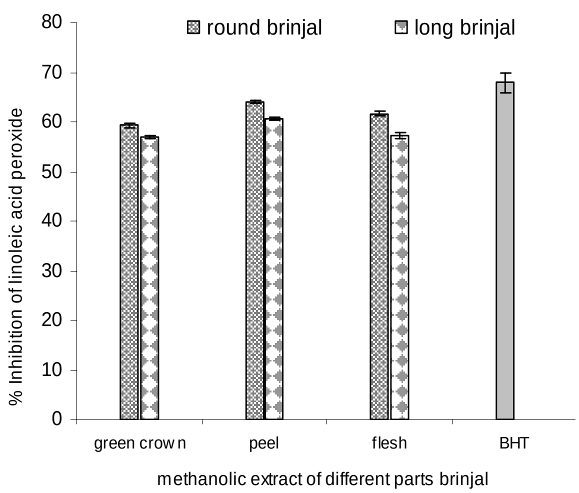

Fig. 3.Inhibition of linoleic acid of aubergine extracts.

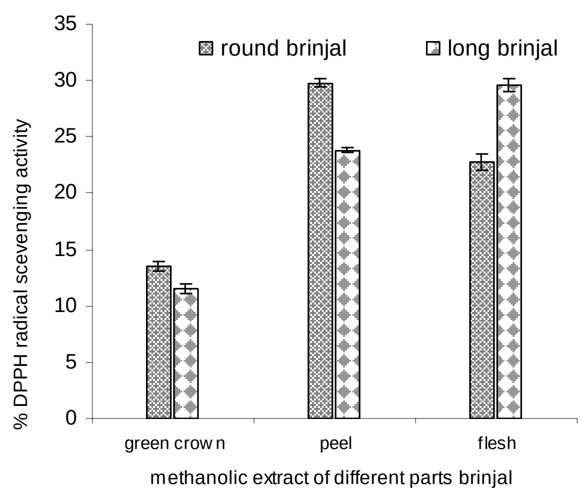

Fig. 4. DPPH radical scavenging activity of different parts of aubergine.

Correlation between different antioxidant assays: A number of assays accepted for the assessment of antioxidant activity of plant materials such as scavenging of DPPH radical, inhibition of lipid peroxidation in linoleic acid system and reducing potential towards ferric/ferrous ions can be used for the determination of antioxidant activity of methanolic extracts of aubergine. A strong correlation was observed between the estimation of phenolic bioactive and antioxidant activities which were evaluated with the help of these assays as shown in Table 2 and as reported earlier (Jayaprakash *et al*., 2008; Sultana *et al*., 2007; Rawat *et al*., 2011). Table 2 shows a highly significant correlation (r = 0.907) between TPC and Inhibition of linoleic acid peroxidation. Similarly, the relationship between TPC and DPPH scavenging potential of extract was also found to be significant (r = 0.776). These findings suggest a strong relationship between phenolic bioactives and antioxidant activity of plant materials. These findings are comparable with earlier reports by Sultana *et al*., (2008) and Anwar *et al*., (2010) which demonstrated that extracts with higher TPC also showed strong activity against linoleic acid peroxidation. Finally, variation in correlation coefficient among different antioxidant assays indicates that a single assay is insufficient to evaluate the total antioxidant activity of a specific plant material (Singleton & Rossi, 1965; Zhishen *et al*., 1999).

Table 2. Comparison of different assays used for the determination of antioxidant activity of aubergine.

Variable	TPC	DPPH	Percent Inhibition	Reducing Power
TPC	-			
DPPH	0.776* p>0.000	-		
% Inhibition	0.907* p>0.003	0.875* p>0.000	-	
Reducing power	0.651* p>0.003	0.151ns p>0.549	0.441ns p>0.067	-

*Significant at $p<0.05$

Conclusion

Methanolic extracts of different parts of both long and round varieties of aubergine showed considerable antioxidant activities and significant levels of phenolic antioxidants. The findings of the present study are consistent with the idea of ranking aubergine in the top ten high antioxidant containing vegetables. The maximum antioxidant activity was achieved from extract of peel of round aubergine and minimum in the case of green crown of long aubergine. It can be concluded that aubergine represents an excellent source of natural antioxidants and can be considered as useful source of nutrition for human health.

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