

Antioxidant Activity and Total Phenolic Content of Different Skin Coloured Brinjal (*Solanum melongena*)

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ABSTRACT: The aim of this study was to determine the antioxidant activity and the total phenolic content (TPC) of *Solanum melongena* of different skin colours/patterns: purple with no lines (S1), light purple with lines (S2), dark purple with lines (S3), pink coloured (S4) and purple with green lines (S5). The antioxidant activity was evaluated using different analytical techniques: DPPH and ABTS free radical scavenging assays, ferric reducing antioxidant power (FRAP) assay and ferric thiocyanate (FTC) test. The results showed that there was a significant difference ($P < 0.0001$) between the skin colour/pattern and antioxidant activity. Total phenolic content (TPC) and FRAP values of brinjal extracts varied from 48.67 ± 0.27 to 61.11 ± 0.26 mg GAE/100 g fresh weight and 4.19 ± 0.11 to 7.46 ± 0.26 mmol of FeSO₄/g fresh weight, respectively. Brinjal with dark purple lines (S3) showed the highest antioxidant activity as quantified by FRAP and TPC while brinjal with light purple lines (S2) showed the least. Purple brinjal with no lines (S1) displayed the highest DPPH radical scavenging activity with an IC₅₀ value of 3.51 ± 0.62 mg/ml while S3 demonstrated the strongest total antioxidant activity as measured by ABTS assay with an inhibition of 40.45%. In the FTC assay, the percent inhibition of linoleic acid oxidation ranged from 15.11 ± 1.31 to 26.74 ± 2.85 .

Keywords: Antioxidant activity, lipid peroxidation, phenolic content, skin colour, *Solanum melongena*

INTRODUCTION

Brinjal (*Solanum melongena* L., Solanaceae), one of the most widespread vegetable consumed around the world contains a variety of phytochemicals such as flavonoids which provide a myriad of health benefits. Brinjal fruit is reported to be a rich source of ascorbic acid and phenolics, both of which are powerful antioxidants (Vinson *et al.*, 1998). Brinjal extracts have been reported to successfully suppress the development and growth of tumours, lung cancer (Matsubara *et al.*, 2005), inhibit inflammation (Keli *et al.*, 1996), and cardiovascular diseases (Knekt *et al.*, 1996 and 1997). Brinjal has received an increased interest among consumers and researchers worldwide because of its health benefits and is ranked amongst the top 10 vegetables in terms of antioxidant capacity (Cao *et al.*, 1996).

Hydroxycinnamic acid conjugates are the main class of phenolics present in brinjal. Of these, chlorogenic acid (5-O-caffeoylquinic acid and its isomers) typically accounts for 70-95% of total phenolics in brinjal flesh (Whitaker and Stommel, 2003). The beneficial effects of

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chlorogenic acid and related compounds present in minor quantities in brinjal are numerous. Furthermore, they are reported to impart anti-tumoral activities (Sawa *et al.*, 1998; Triantis *et al.*, 2005).

Brinjal is one of the commonest vegetables consumed in Sri Lanka. The unripe fruit is primarily used as a cooking vegetable for various dishes. Different varieties of brinjals of different size, shape and colour are available in the market. The most widely grown variety in Sri Lanka is the elongated ovoid or slender ones with a dark purple skin. Extract of brinjal with purple skin has been shown to possess a high capacity in the scavenging of superoxide radicals and inhibition of hydroxyl radical generation by chelating ferrous iron (Kaneyuki *et al.*, 1999; Noda *et al.*, 2000). Despite the beneficial effects associated with brinjal, only a limited literature is available on the antioxidant efficacy, especially with respect to colour difference. Considering this fact and the importance of brinjal in cuisines, the present study was carried out to evaluate and compare the *in vitro* antioxidant activity of brinjal with respect to skin colour.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and solvents used were of analytical grade. Folin Ciocalteu's reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), 2, 2'-azobis (2-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2'-azobis (2-methylpropanimidamide) dihydrochloride (AAPH), 2, 4, 6-tris (2-pyridyl)-1, 3, 5-triazine (TPTZ), linoleic acid, thiobarbaturic acid were purchased from Sigma Aldrich Chemical Company, USA while other chemicals used were purchased from HIMEDIA Chemicals Ltd. UK.

Fresh matured brinjals with different skin colour *viz.*, purple with no lines (S1), light purple with lines (S2), dark purple with lines (S3), pink coloured (S4) and purple with green lines (S5) were purchased fresh from Pambahinna, Belihuloya (Figure 1).

Preparation of water extracts

Approximately 50 g of fresh and disease free brinjal pods were washed with pure water and sliced. The slices were well blended in a laboratory blender and filtered through a cotton mesh. The resulting extracts were stored at -20 °C until further analysis. The extracts were appropriately diluted with distilled water and used for chemical analysis.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of the extracts was determined colourimetrically as described by Kriengsak *et al.* (2006) with some modifications. The results were expressed as mg gallic acid equivalents (GAE) per 100 g of fresh weight.

Determination of DPPH radical scavenging activity

The DPPH assay was performed according to the method described by Brand-Williams *et al.* (1995) with modifications. DPPH solution (2 ml) was added to different volumes (150, 300, 450, 600, 750, 900 μ l) of aqueous brinjal extract and diluted with distilled water until the

volume reached 4 ml and allowed to stand for 30 min in dark at ambient temperature. The absorbance was read at 517 nm. Ascorbic acid was used as the reference antioxidant and the antioxidant capacity was expressed as IC₅₀ value.



Fig. 1. Different skin colours of brinjal used for the study

Determination of ABTS radical scavenging activity

The total antioxidant capacity of the extracts was determined using ABTS radical scavenging assay. The ABTS^{•+} was generated by reacting ABTS with AAPH, which acts as the radical generator. Each extract (150 μ l, 25 mg/ml) was mixed with 2.85 ml of ABTS radical solution and absorbance was read over 6 minutes at 1 min interval at 734 nm. The radical scavenging activity after lapse of 6 min was calculated as percentage of ABTS^{•+} discolouration (Robereta *et al.*, 1999).

Determination of antioxidant activity using FRAP assay

Ferric reducing antioxidant power (FRAP) assay was performed according to the method explained by Kriengask *et al.* (2006) with modifications. The FRAP reagent included 10 mM TPTZ, 20 mM FeCl₃, and 0.3 M acetate buffer at 1:1:10 (v/v) ratio. FRAP reagent (3 mL) at 37 °C was mixed with 150 μ l of the extract. After a lapse of 4 min, the absorbance was read at 593 nm against a reagent blank and the results were expressed as mmol of Fe²⁺/g fresh weight using a Fe²⁺ (0.05-0.50 mmol) standard curve.

Ferric Thiocyanate (FTC) test

Brinjal extracts were mixed with 4.1 ml of linoleic acid (2.5% in ethanol 99.5%), 8 ml of phosphate buffer (0.02 M, pH 7) and 3.9 ml distilled water. The mixture was placed in a dark oven maintained at 40-45 °C. Following incubation, 0.1 ml of the solution was drawn and 9.7 ml of 75% ethanol and 0.1 ml of 30% NH₄SCN were added. Precisely 3 min after the

addition of 0.1 ml of 0.002 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance was read at 500 nm and the inhibition of lipid oxidation was measured (Huda-Faujan *et al.*, 2007).

Statistical analysis

Each antioxidant activity assay was performed four times using the same extract in order to determine their reproducibility and results were expressed as mean \pm SD. Statistical analysis was performed using the SAS 9.1.3 version.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

Total Phenolic Content (TPC) reported as the gallic acid equivalent is presented in Table 1. *S. melongena* was found to have varying levels of TPC, ranging from 48.67 \pm 0.27 to 61.11 \pm 0.26 mg GAE/100 g on fresh weight basis. The TPC was markedly higher in S3 and S1 than the other three samples. S2 had the lowest total phenolic content which was significantly lower than all other extracts. Plant phenolics have received much attention as potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelators. Therefore, it is worthwhile to determine the total amount of phenolic content in the plant chosen for the study.

Table 1. Total phenolic content and antioxidant activity of five different varieties of brinjal

Extract	TPC (mg/100 g)	DPPH (IC ₅₀) (mg/ml)	ABTS (% inhibition)	FRAP (mmol /g)	FTC (% inhibition)
S1	60.94 \pm 0.52	3.51 \pm 0.62 ^c	27.33 \pm 0.57 ^b	6.77 \pm 0.03 ^b	26.74 \pm 2.85 ^a
S2	48.67 \pm 0.26	4.78 \pm 0.65 ^a	17.78 \pm 0.38 ^c	4.19 \pm 0.11 ^d	15.11 \pm 1.31 ^d
S3	61.11 \pm 0.26	3.78 \pm 0.90 ^c	40.45 \pm 1.17 ^a	7.46 \pm 0.26 ^a	25.36 \pm 1.01 ^{a,b}
S4	56.00 \pm 0.53	4.87 \pm 1.47 ^a	23.90 \pm 0.07 ^b	4.64 \pm 0.37 ^c	24.50 \pm 0.87 ^b
S5	54.38 \pm 0.51	3.96 \pm 1.28 ^b	24.02 \pm 0.24 ^b	4.81 \pm 0.35 ^c	20.65 \pm 0.57 ^c

Data are presented as Mean SD of four estimations.

Means followed by the same letters in a column are not significant ($p=0.0001$).

Phenolic compounds such as N-caffeoylputrescine, 5-caffeoylquinic acid, and 3-acetyl-5-caffeoylquinic acid were identified from brinjal pulp (Singh *et al.*, 2009). Noda *et al.* (2000) also reported that nasunin, delphinidin-3-(*p*-coumaroylrutinoside)-5-glucoside, was a representative anthocyanin in eggplant peel. Generally, the peel contains higher amounts of phenolics, anthocyanins, and flavonols than pulp tissue (Tomás-Barberán *et al.*, 2001; Jang *et al.*, 2010). Compared to pulp, brinjal peel contains higher amounts of those compounds. Especially, the anthocyanin content in the peel is much higher than the pulp (Jung *et al.*, 2011). Significantly higher TPC in S1 and S3 compared to S2 on fresh weight basis may be mainly due to the high anthocyanin content of the brinjal peel (Brown, 2005).

Nisha *et al.* (2009), in their work found the TPC of brinjal to be in the range of $49.02 \pm 1.3 - 106.98 \pm 2.2$ mg gallic acid equivalents/100 g which correlates well with our findings. They studied the antioxidant activity of four different varieties of brinjal and the purple colour small size brinjal exhibited better antioxidant activity which was attributed to the higher phenolic and anthocyanin contents. In another study, where 69 *S. melongena* varieties were studied the TPC ranged between 280 to 834 mg/kg (Prohens *et al.*, 2007). According to a recent study on 12 indigenous commonly consumed vegetables of Bangladesh reported that TPC of *S. melongena* was 271 mg GAE/100 g of fresh sample in green variety and 394 mg GAE/100 g in violet one (Sharmin *et al.*, 2011).

DPPH and ABTS radical scavenging activities

The DPPH and ABTS radical scavenging assays measure the relative antioxidant efficacy of natural extracts in scavenging free radicals (DPPH[•] and ABTS^{•+}, respectively) generated in the assay medium (Apak *et al.*, 2007). The degree of discolouration is an indication of the scavenging capacity of the extracts. The extracts were able to reduce the stable violet DPPH radical to yellow DPPH-H over a range of concentrations (0.94-5.63 mg/ml). An increase in sample concentration significantly increased the DPPH radical scavenging activity of samples in a dose dependent manner as shown in Figure 2.

The IC₅₀ value represents the amount of antioxidative constituents required to scavenge 50% of free radical in the assay system. The lower the IC₅₀ value, the greater is the antioxidant activity. The results showed that there was a significant difference ($P < 0.0001$) between the samples and DPPH radical scavenging activity. The IC₅₀ values of the extracts ranged from 3.51 ± 0.62 to 4.87 ± 1.47 mg/ml (Table 1). The highest antioxidant activity was recorded in S1 followed by S3 and S5. The least antioxidant activity was recorded in S2 and S4. The IC₅₀ values for S2 and S4 were 4.78 ± 0.65 and 4.87 ± 1.47 mg/ml respectively and were not significantly different. The IC₅₀ value of ascorbic acid was observed to be very low (1.86 µg/ml) indicating significantly higher antioxidant activity compared to different brinjal varieties used in the study. Difference in scavenging activities might be due to the presence of different phenolic compounds and the difference in the total phenolic.

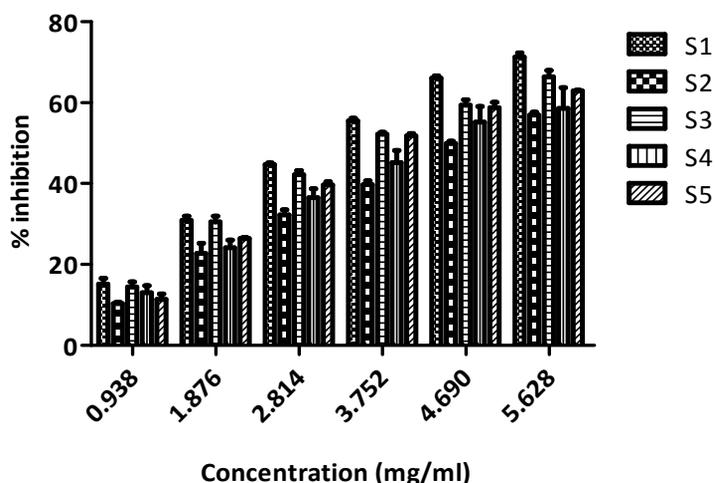


Fig. 2. DPPH radical scavenging activity of brinjal extracts at various concentrations. Each bar represents mean + SD (n=4).

Generation of $ABTS^{*+}$ involves direct production of the blue/green $ABTS^{*+}$ chromophore through the reaction between ABTS and potassium persulfate/AAPH. Addition of antioxidants to the pre-formed radical cation reduces it to ABTS, based on the concentration of the antioxidant and the duration of the reaction. Thus, an increase in time and concentration significantly increases the radical scavenging activity. The brinjal extracts showed potent antioxidant activity in scavenging the ABTS radicals over 6 minutes. The S3 extract displayed the highest total antioxidant activity of $40.45\% \pm 1.17$ at a concentration of 25 mg/ml which was significantly higher than all other tested extracts. Total antioxidant capacity of S2 was significantly lower than all other extracts while, the activity of S1, S4 and S5 was not significantly different ($p > 0.0001$) (Table 1). Ascorbic acid showed the highest total antioxidant capacity of $85.79\% \pm 1.91$ at a concentration of 0.01 mg/ml.

FRAP assay

The FRAP assay measures the reducing potential of an antioxidant reacting with ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex and producing a coloured ferrous tripyridyltriazine (Fe^{2+} -TPTZ) (Kriengsak *et al.*, 2006). The FRAP assay showed a positive correlation between reducing power and phenolic content in brinjal extracts. The ferric reducing antioxidant power activity of *S. melongena* is presented in Table 1. The S3 extract showed the highest FRAP value of 7.46 ± 0.26 mmol of Fe^{2+} /g fresh sample. The antioxidant potential of brinjal extracts as measured by FRAP followed the order of $S3 > S1 > S5 = S4 > S2$. On the contrary, the FRAP value for ascorbic acid (1.43 ± 0.04 mmol of Fe^{2+} /g) was significantly lower than that of brinjal extracts. It is clear from the results that the FRAP activity of S3 and S1 was the highest among all the brinjal extracts studied.

Ferric Thiocyanate (FTC) test

The antioxidant potential of brinjal extracts was evaluated by measuring inhibition of lipid peroxidation in linoleic acid system. The percent inhibition of linoleic acid peroxidation of water extracts of brinjal is illustrated in Figure 3. From the analysis, it is evident that all samples had been oxidized when stored over seven days at 40-45°C. Absorbance of each sample increased progressively with the time of incubation. Lower absorbance values indicate higher antioxidant activities.

The results showed that there was a significant difference ($p < 0.0001$) between skin colour and antioxidant activity as measured by FTC assay. The percent inhibition increased from day 1 to day 3 and the subsequent measurements were not coherent. The highest antioxidant activity was observed on day 3 for all extracts, except for S1, where the highest activity was observed on day 4 ($30.28\% \pm 0.85$). Of the five extracts tested, the highest antioxidant activity was observed in S1 which exhibited $26.74\% \pm 2.85$ inhibition on day 3 and the lowest value was recorded in S2 ($15.11\% \pm 1.31$) at a concentration of 25 mg/ml. The antioxidant activity of $25.36\% \pm 1.01$ was shown by S3 which was not significantly different ($p > 0.0001$) from those of S1 and S4. The higher inhibition potential of S1 and S3 against linoleic acid peroxidation can be attributed to the presence of higher amounts of phenolic bioactives (Zainol *et al.*, 2003).

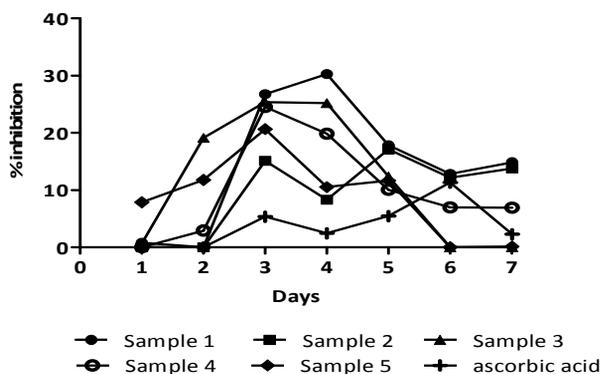


Fig 3. Antioxidant activity of brinjal extracts and ascorbic acid determined by the FTC method.

The inhibition of lipid oxidation by the reference ascorbic acid was $5.37\% \pm 1.08$ at a concentration of 0.01 mg/ml. This was significantly ($p < 0.0001$) lower than all brinjal extracts. Until day 3, ascorbic acid did not show any inhibition potential. The absorbance values were higher than control, indicating pro-oxidant activity.

The brinjal extracts, S1, S3 and S5 started to show inhibition of lipid peroxidation from the first day while the other samples started to inhibit oxidation from day 2. This observation is in line with the study carried out by Huda-Faujan *et al.* (2009), where the absorbance of control was lower than the samples till day 4. After day 5, S3 and S5 did not show any inhibition while the activity of the other extracts also declined. The effectiveness of the reference antioxidant was generally lower than that of the extracts. Formation of peroxides ceased during the incubation period due to non-availability of linoleic acid. Also, the intermediate products might have been converted to stable end-products. The non-availability of hydroperoxides resulted in the stoppage of oxidation of ferrous sulphate. Hence, the optical density declined.

Relationship between phyto-chemical content and antioxidant activities

Previous studies have demonstrated the strong relationship between total phenolic content and antioxidant activities found in different crops (Yang *et al.*, 2004; Kriengsak *et al.*, 2006). In the present study, a significant correlation was observed between total phenolic content found in S1-S5 and the DPPH, ABTS, FRAP and FTC assays (Figure 4). These results indicated that total phenolic content may be in part responsible for the antioxidant activities of brinjal tested.

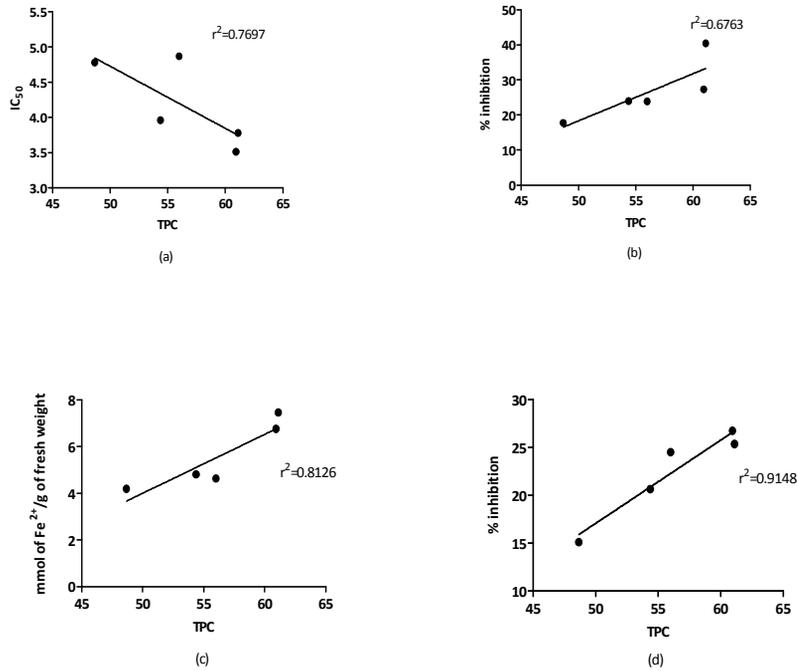


Fig. 4. Correlation between TPC and (a) DPPH radical scavenging activity (b) ABTS radical scavenging activity (c) FRAP assay and (d) FTC of five brinjal types

Anthocyanins are the main phenolic compounds in eggplant peel (Jung *et al.*, 2011). The total anthocyanin content in purple colour brinjal variety was found to be markedly higher than the green variety (Nisha *et al.*, 2009). Whitaker and Stommel (2003) examined the phenolic acid content of the fruit flesh of seven commercial brinjal cultivars and found significant differences in the phenolic content and composition among cultivars and in tissue from stem, middle, and blossom end segments. Therefore, the high diversity in the variety of phenolic compounds as well as the proportions of phenolic compounds contained in individual brinjal varieties probably explains the differences in the antioxidant activities of S1–S5 observed in the present study.

CONCLUSION

The present study demonstrated that all aqueous extracts of brinjal exhibit potent antioxidant activity and apparently, the antioxidant properties of all five types of brinjal positively correlate with their polyphenolic content. From all these observations it appears that S1 and S3, intensely coloured skins, shows greater antioxidant activity than lighter ones.

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