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A comparative study on antioxidant and DNA protective activity of different skin coloured brinjal (*Solanum melongena*)

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Abstract

The aim of this study was to investigate the *in vitro* antioxidant activity and DNA damage inhibition potential of aqueous extract of *S. melongena* with different skin colours. Water extracts of brinjal with four different skin colours: moderately purple (S1), light purple (S2), dark purple (S3) and purple with green lines (S4) were tested for their antioxidant and radical scavenging activities. The total phenolic content (TPC) was quantified using Folin-Ciocalteau's method. The effectiveness of brinjal extracts in preventing radical induced DNA damage was also determined. There was a significant difference ($p<0.0001$) between the skin colour andantioxidant activity. Brinjal with S3skin colour showed the highest TPC and antioxidant activity measured by FRAP while, S2 showed the least. S1 displayed the highest percentage of DPPH radical scavenging activity with an IC_{50} value of 3.51±0.62 mg/ml while, S3 demonstrated the strongest total antioxidant capacity with an inhibition percentage of 40.45±1.17. In the FTC (Ferric Thiocyanate) and egg yolk model, S1 and S3 showed better antioxidantactivity than S2 and S4. The *in vitro* freeradical quenchingand antioxidant results well correlated with the *in vitro* lipid peroxidation assays. All extracts were able to effectively retain DNA against AAPH induced radical damage at the concentration levels (25 and 75 mg/ml) tested. All the extracts showed moderate to potent antioxidant activity, among which S3 and S1, intensely coloured skins, demonstrated better antioxidant activity which may be attributed to the higher phenolic content since a linear relation was observed between the TPC and the antioxidant parameters.

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Keywords:Solanum melongena; skin colour; antioxidant; DNA protection; lipid peroxidation

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1. Introduction

Brinjal (*Solanum melongena*) is one of the most widespread vegetables consumed all around the world which contains a variety of phytochemicals such as phenolics and flavonoids that provide important health benefits. Brinjal extracts have been reported to successfully suppress the development and growth of tumours, metastasis, inhibit inflammation, lung cancer and heart disease[1]. Brinjal is now receiving more interest from consumers and researchers worldwide because of its health benefits and is ranked amongst the top 10 vegetables in terms of antioxidant capacity[2]. The beneficial effects of brinjal can be attributed to the presence of plant bioactives, mostly phenolics. Brinjal cultivars vary in shape and colour, the most common ones being dark purple or violet. Extract from purple eggplant skin has been shown to possess a high capacity in the scavenging of superoxide radicals and inhibition of hydroxyl radical generation by chelating ferrous ion[3]. Therefore, the present work aims at evaluating the in vitro antioxidant and DNA damage prevention activity of brinjal with respect to skin colour.

2. Methodology

2.1. Sample collection and preparation

Fresh eggplants with different skin color viz., moderately purple (S1), light purple (S2), dark purple (S3) and purple with green (S4), were purchased fresh from Pambahinna, Belihuloya (Figure 1). Water extracts of brinjal (0.25 g/ml) was prepared and stored under 0^{o}C until analysis.

2.2. Antioxidant activity

Total phenolics content was determined by the Folin–Ciocalteu method, which was adapted from Singleton and Ross[4] with slight modifications. The DPPH assay was done according to the method of Brand-Williams et al.[5] with some modifications. For ABTS assay, the procedure followed the method of Arnao et al.,[6] with some modifications. The FRAP assay was done according to Al-Farsi et al,[7] with some modifications. The lipid peroxidation assays, Ferric thiocyanate (FTC) and Thiobarbituric acid reactive substances (TBARS) were done according to the methods described by Osawa and Namiki[8] and Ibehetetal.[9] respectively.

2.3. DNA protectant activity

The DNA damage protective activity of S. melongena extract was performed using cattle blood DNA. The eggplant extracts (25 mg/mL and 75 mg/mL) were mixed with blood DNA (0.3 µg/ µL) and were incubated for 10 min at room temperature followed by the addition of 3 µL of AAPH (300 mM). The final volume of the mixture was made up to 15 µL with TE and incubated for 30 min at 37oC and then placed on ice for 10 min to stop the reaction. The reaction mixture was mixed with 2 µL of loading dye (0.5% bromophenol blue, 0.5% xylene cyanol, 50% glycerol in water). The DNA was analyzed on 0.8% agarose gel using ethidium bromide staining and photographed in Gel Doc. Images were analyzed using UN-SCAN-ITTM gel analysis software (Silk Scientific, Inc. Utah, USA).

2.4. Statistical analysis

Each antioxidant activity assay was done four times from the same extract in order to determine their reproducibility and results are shown as mean ± SD. Antioxidant potential of different assays were determined by applying Graph pad prism 5-software. Statistical analysis was performed using the SAS 9.1.3 version.

3. Result and Discussion

The TPC value of brinjal extracts varied from 48.67±0.27 - 61.11±0.26 mg GAE/100 g fresh weight. The total phenolic content was markedly higher in S3 and S1 than the other two samples (Table 1).

In the DPPH free radical scavenging activity, water extracts of brinjal were evaluated for their free radical scavenging activity with ascorbic acid as the standard compound. Table 1 indicates the IC_{50} value for each sample and standard. The IC50 value of standard ascorbic acid was observed to be very low (1.86±0.17 µg/ml) indicating highest antioxidant activity compared to the test samples. The IC_{50} values for other samples ranged from 3.51± 0.62 - 4.78±0.65 mg/ml. Highest antioxidant activity/ the lowest IC_{50} value was recorded in S1 followed by S3 and S4. The least activity was recorded in S2.

In the ABTS free radical scavenging assay, the S3 extract displayed the highest total antioxidant activity of 40.45±1.17% at a concentration of 25 mg/ml. Total antioxidant capacity of S2 was significantly lower than all other extracts. Ascorbic acid had the highest total antioxidant capacity of 85.79±1.91% at a concentration of 0.01 mg/ml.

The ferric reducing ability of brinjal extracts was in the range of 4.19±0.11 –7.46±0.26 mmol of Fe (II)/g fresh weight (Table 1). The S3 extract had the highest ability to reduce Fe (III) followed by S1, S4 and S2. The FRAP value for ascorbic acid was significantly higher than the brinjal extracts.

In the FTC method, the optical density of control and samples increased up to day three and then decreased, except in S1, where the highest antioxidant activity was observed in day 4 (30.27±0.85). Of the four extracts tested, the highest antioxidant activity was observed in extract S1, which exhibited 26.74±2.85 % inhibition on day 3 and the lowest value was recorded in S2 at a concentration of 0.25 g/ml. The antioxidant activity of S3 was non-significantly lower

than S1 and higher than S4. The inhibition of lipid peroxidation by ascorbic acid (standard) was 5.37±1.08% at a concentration of 0.01 mg/ml (Table 1). Surprisingly, this was significantly lower than all the tested brinjal extracts.

A modification of TBARS assay was used to determine the level of lipid peroxides formed using egg yolk homogenate as lipid-rich media. The brinjal extracts were examined for their ability to act as radical scavenging agents in comparison among them and BHT. The S1 and S3 extracts exhibited almost the same antioxidant power which was significantly higher than those of S2 and S4 and significantly lower than BHT (47.72±1.75%).

Extensive DNA fragmentation due to oxidation by peroxyl radicals was seen on agarose gel as shown in Figure 2. In lane 10 (DNA+AAPH), where the reaction mixture did not contain any antioxidant, DNA was completely damaged and was seen by the enhanced mobility of DNA as compared to the control DNA (lane 1). The effectiveness of the eggplant extracts to prevent AAPH induced DNA damage was associated to its peroxyl radical scavenging activity. At an extract concentration of 75 mg/mL the highest and the lowest DNA retention potential was displayed by S2 and S1 respectively while at 25 mg/mL S1 showed the highest and S2 showed the least activity.

Table 1. Total phenolic content, antioxidant activity, lipid peroxidation activity and DNA protectant activity of four different skin colored eggplant

Results reported are mean values of four determinations ± SD. Means in each column sharing the same superscript are not significantly different (P> 0.0001, *P>0.05) from one another.

	TPC (mg/100 g)	DPPH (IC_{50}) (mg/ml)	ABTS (% inhibition)	FRAP (mmol /g)	FTC (% inhibition)	Egg yolk model (% inhibition)	*DNA retention capacity	
							25 mg/mL	75 mg/mL
S1	60.94±0.52[a]	3.51±				10.04±0.48[a]	68.66±4.31[a]	62.17±5.09[c]
S2	48.67±0.26[c]	4.78±				4.62±2.75[c]	45.61±2.42[b]	73.50±2.93[a]
S3	61.11±0.26[a]	3.78±				10.08±0.28[a]	67.95±6.22[a]	69.88±1.58[a,b]
S4	54.38±0.51[b]	3.96±1.28[b]	17.29±0.18[c]	4.81±0.35[c]	20.65±0.57[b]	6.47±0.16[b]	49.83±7.49[b]	66.05±3.03[b,c]

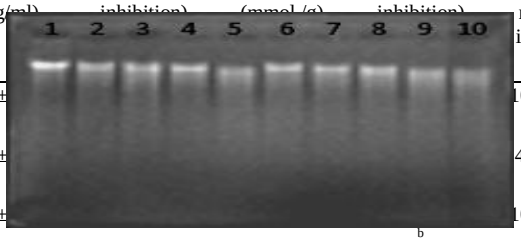

Figure 1. Effect of eggplant extracts in preventing peroxyl –radical induced DNA damage. The numbered lane represent, 1) DNA+ PBS; 2) DNA+AAPH+S1 (75 mg/mL); 3) DNA+AAPH+S1 (25 mg/mL); 4) DNA+AAPH+S2 (75 mg/mL); 5) DNA+AAPH+S2 (25 mg/mL); 6) DNA+AAPH+S3 (75 mg/mL); 7) DNA+AAPH+S3 (25 mg/mL); 8) DNA+AAPH+S4 (75 mg/mL); 9) DNA+AAPH+S4 (25 mg/mL); 10) DNA+AAPH

4. Conclusion

Results from the present study showed that water extract of *S. melongena* could effectively scavenge reactive oxygen species. Especially, S1 (purple with no lines) and S3 (dark purple with lines) demonstrated better antioxidant activities than the other samples which may be attributed to the higher phenolic content since a linear relation was observed between the TPC and the antioxidant parameters. In conclusion, it can be said that antioxidant properties of brinjal differed based on skin colour. A more detailed study on the bioactive compound in these plant extracts that contribute to these biological activities as well as their possible mechanism of action are therefore suggested.

References

1. Nisha P, Abdul NP, Jayamurthy PA. Comparative study on antioxidant activities of different varieties of *Solanum melongena*. Food ChemToxicol ;2009;47:2640-2644.
2. Cao G, Sofic E, Prior R. Antioxidant capacity of tea and common vegetables. J Agric Food Chem ;1996;44:3426-3431.
3. Kaneyuki T, Noda Y, Traber MG, Mori A, Packer L. Superoxide anion and hydroxyl radical scavenging activities of vegetable extracts measured using electron spin resonance. BiochemMolBiolInt ;1999;47:979–89.
4. Singleton VL, Ross JA. Colorimetry of total phenolic with phosphomolybdate–phosphotungstic acid reagent. Am J EnolVitic ;1965;16:144–58.
5. Brand WW, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. LWT-Food Sci Tech ;1995;28:25–30.

6. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem ;2001;73:239–44.
7. Al-Farsi M, Alasalvar C, Morris A, Baron M, Shahidi F. Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. J Agric Food Chem ;2005;53:7592-99.
8. Osawa T, Namiki M. A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. J AgricBiolChem ;1981;45(3):735-9.
9. Ibeh BO, Maxwell E, Bitrus HJ. Phytochemical compositions and in vitro antioxidant capacity of methanolic leaf extract of AxonopusCompressus (P. Beauv.). Eur J Med Plants ;2013;3(2):254-65.