A comparative study on antioxidant activities of different varieties of Solanum melongena

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ABSTRACT

Eggplant is one of the most common vegetables consumed all around the world. The present study evaluates the antioxidant potential of four different varieties of eggplant (long green, purple coloured big size, purple coloured moderate size and purple coloured small size) in terms of total phenolic content, DPPH, total reducing power, superoxide radical scavenging activity, metal chelating activity and total anthocyanin content. Extracts from purple colour small size eggplant fruit demonstrated better antioxidant activities than the other samples which may be attributed to the higher phenolic and anthocyanin content since a linear relation was observed between the TPC and the antioxidant parameters.

1. Introduction

Foods rich in antioxidants play an essential role in the prevention of chronic and degenerative diseases such as cardiovascular diseases and cancer (Ames et al., 1993). Plant foods contain an array of bioactive compounds which are actively being researched for their health care potential, including flavonoids, plant sterols/stanols, salicylates and glucosinolates. Although to date, most research on the health benefits of plant-rich diets has focused on the established vitamins, the available data are not convincing for their health care potential, especially with respect to varietal difference. Considering the established vitamins, the available data are not convincing evidence that diets rich in fruit and vegetables are associated with a lower incidence of cancer, cardiovascular, and other degenerative diseases (Ames et al., 1993).

Eggplant, Solanum melongena, is a common and popular vegetable crop grown in the subtropics and tropics (Sarker et al., 2006). The eggplant is commonly known as brinjal and aubergine in India and in Europe, respectively. Eggplant is a perennial but grown commercially as an annual crop. The unripe fruit of eggplant is primarily used as a cooking vegetable for the various dishes all over the world.

Eggplant fruit contains ascorbic acid and phenolics, both of which are powerful antioxidants (Vinson et al., 1998). Studies have shown that eggplant extracts suppress the development of blood vessels required for tumor growth and metastasis (Matsubara et al., 2005), and inhibit inflammation that can lead to atherosclerosis (Han et al., 2003). Extracts from eggplant fruit skin have been demonstrated to possess high capacity in scavenging of superoxide free radicals and inhibition of hydroxyl radical generation by chelating ferrous iron (Kaneyuki et al., 1999; Noda et al., 2000). Superoxide radicals generated in vivo are usually converted into hydrogen peroxide, and like other free radicals, can damage lipids, proteins, and DNA (Halliwell et al., 1995). From the 120 vegetable species evaluated for antioxidant activity using four different assays (2,2'-azinobis-[3-ethylbenzthiazoline-6-sulphonic acid]), 2, 2-diphenyl-1-picrylhydrazyl radical, inhibition of lipid peroxidation, and Superoxide scavenging), eggplant ranked among the top 10 species for superoxide scavenging (SOS) activity (Yang, 2006).

Nasunin, an anthocyanin isolated from the skin of purple eggplant fruit, is one phenolic compound implicated in both inhibition of hydroxyl radical generation and SOS activity (Kaneyuki et al., 1999; Noda et al., 2000).

Flavonoids isolated from S. melongena showed potent antioxidant activity (Sudheesh et al., 1999; Sadilova et al., 2006) against chromosomal aberrations induced by Doxorubicin. Various parts of the plant are useful in the treatment of inflammatory conditions, cardiac debility, neuralgia, ulcers of nose, cholera, bronchitis and asthma, analgesic and hypolipidemic (Mutalik et al., 2003; Sudheesh et al., 1997). S. melongena is also a natural source of vitamin A. It would play an important role for vision and eye health because vitamin A has been recognized as a critical factor in child health and survival (Igwe et al., 2003).

Eggplant is one of the most common vegetables used in Indian cuisines. Different varieties of eggplant are available in India on the basis of their size, shape and colour. However, literature scan reveals complete lack of data with respect to the efficacy of the eggplant, especially with respect to varietal difference. Considering...
this fact and the importance of eggplant in cuisines, the present study was undertaken to evaluate and compare preliminarily the antioxidant activity of different varieties of eggplant.

2. Materials and methods

2.1. Chemicals

Folin–ciocalteu reagent, trichloro acetic acid, aluminium chloride, ethylene diamine acetic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitro blue tetrazolium (NBT), xanthine, xanthine oxidase, quercetin, ferrozine, gallic acid, sodium bicarbonate, and sodium hydroxide were purchased from Fisher inorganic and aromatic limited (Chennai, India). Potassium ferricyanide and methanol were purchased from British drug house (India) Pvt. Ltd. and Central drug house (Gujarat, India), respectively. Disodium hydrogen phosphate and monosodium hydrogen phosphate were purchased from Sisco research laboratories Pvt. Ltd. (Mumbai, India) while sodium nitrite was purchased from Sabari M. Chemicals (Boroda, India). All the other chemicals employed were of standard analytical grade.

2.2. Plant material

Fresh eggplant (S. melongena) of different variety viz., long green, purple coloured big size, purple coloured moderate size and purple coloured small size (Fig. 1) were purchased fresh from local market, Trivandrum, Kerala.

2.3. Proximate analysis

The total moisture, ash, fat, crude fibre, protein and carbohydrate contents in four different varieties of eggplants studied were estimated by the standard procedure of the AOAC (1990).

2.4. Preparation of the extract

About 250 g of each fresh eggplant variety were cleaned and cut into pieces of approximately 1 cm³ size and extracted with methanol using soxhlet extraction method. The extraction was carried out for 6 h. The extracts were concentrated at 45 °C using a rotary evaporator (Heidolph LABOROTA 4010) and resultant residues were then made up to 50 ml and stored under refrigerated conditions until further studies.

2.5. Evaluation of antioxidant activity

2.5.1. Total phenolic content

Total phenolic contents were determined using Folin–ciocalteu reagent and expressed as gallic acid equivalents (GAE) (Singleton and Ross, 1965). Folin–ciocalteu reagent contains metals like polytungston. Phenolic content from the sample reduce the metal and change the colour from yellow to Prussian blue. The intensity of the colour is directly proportional to the phenolic content.

The extracts were diluted with the same solvent used for extraction, to a suitable concentration for analysis and 0.5 ml of commercial Folin–ciocalteu reagent was added. The contents were mixed well and kept for 5 min at room temperature followed by the addition of 1 ml of 20% aqueous sodium carbonate. After incubation at room temperature for one and half hour, the absorbance of the developed blue colour was read at 760 nm (Shimadzu UV-2450 Shimadzu corporation, Kyots, Japan) against reagent blank and results were calculated as gallic acid equivalents (mg/100 g) of sample. The reaction was conducted in triplicate and results were averaged.

2.5.2. Total anthocyanin measurement

Total anthocyanins were measured according to a modification of the method described by Fuleki and Francis (1968) and Lee et al. (2005). Two dilutions of sample were prepared, one for pH 1.0 using potassium chloride buffer (0.03 M, 19 g KCl into 980 ml distiller water) and the other for pH 4.5 using sodium acetate buffer.
The IC50 values are inversely proportional to the antioxidant activity. Antioxidant material required to scavenge 50% of free radical in the assay system. The concentration of the extract to obtain IC50 value. IC50 is defined as the amount of antioxidant’s concentration, there is an increase in the reducing power, thus increasing antioxidant material required to scavenge 50% of free radical in the assay system. The IC50 values are inversely proportional to the antioxidant activity.

2.5.3. DPPH radical scavenging activity
Free radical scavenging activity of the eggplant extracts were determined by using a stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) (Brand Williams et al., 1995). DPPH is a free radical of violet colour. The antioxidants in the sample scavenge the free radicals and turn it into yellow colour. The change of colour from violet to yellow is proportional to the radical scavenging activity.

Briefly, the assay contained 1 ml of 0.1 mM DPPH in ethanol and various concentrations of methanol extracts and standards in the same solvent and made up to 3.5 ml with methanol. The contents were mixed well immediately and then incubated for 30 min at room temperature (24–30 °C). The degree of reduction of absorbance was recorded in UV–Vis spectrophotometer at 517 nm (Shimadzu UV–Vis 2450).

The percentage of scavenging activity was calculated as: \[ \frac{(Ac - As)}{Ac} \times 100 \]
where ‘Ac’ is the absorbance of control (without extract) and ‘As’ is the absorbance of sample.

2.5.4. Superoxide radical scavenging activity
Superoxide radical scavenging activity study was performed according to the method of Martinez et al. (2001) using the xanthine–xanthine oxidase system. Xanthine is converted to uric acid by the enzyme xanthine oxidase with the formation of by-product, superoxide. Superoxide combine with nitro blue tetrazolium (NBT, 5 mg/ml buffer) and forms formazine blue colour. The antioxidant containing sample scavenge the superoxide, and thus the formation of formazine blue colour is reduced. The reduction of colour is proportional to the antioxidant content in the sample and the blue colour developed was measured at 560 nm (Shimadzu UV–Vis 2450).

About 50 µl of xanthine and 20 µl of NBT were added to varying concentrations of extracts and standard. The volume was made up to 1 ml with phosphate buffer (50 mM, pH 7.5). About 50 µl of xanthine oxidase was added to the system and mixed well to start the reaction and incubated at 37 °C for 30 min in water bath. The reaction was stopped after 30 min by adding 100 µl of 0.1 N HCl. Absorbance of blank prepared without sample and standard was considered as 100% radical. A decreased NBT reduction in the presence of added eggplant extract and standard compound was monitored. Percentage radical scavenging activity (RSA) was calculated using the formula:

\[ \text{RSA} (\%) = \frac{OD \text{ of control} - (OD \text{ of sample} - OD \text{ of sample control})}{OD \text{ of control}} \times 100 \]

2.5.5. Metal chelating activity
The chelation of ferrous ions by the extract was estimated by the method of Dinjus et al. (1994) with slight modification and compared with that of EDTA, BHT and that of ascorbic acid. The chelation test initially includes the addition of ferrous chloride. The antioxidant present in the sample chelates the ferrous ions from the ferrous chloride. The remaining ferrous combine with ferrozine to form ferrous–ferrozine complex. The intensity of the ferrous–ferrozine complex formation depends on the chelating capacity of the sample and the colour formation was measured at 562 nm (Shimadzu UV–Vis 2450).

Different concentrations of standard and extracts (100–500 µg/ml) were added to a solution of 100 µl FeCl3 (1 mM). The reaction was initiated by the addition of 200 µl ferrozine (1 mM). The mixture was finally quantified to 1.3 ml with methanol, shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically. All the test and analysis were done in duplicate and average values were taken. The percentage inhibition of ferrous–ferrozine complex formation was calculated using the formula:

\[ \text{Percentage of chelation (\%) = \frac{(Ac - As)}{Ac} \times 100} \]
where ‘Ac’ is the absorbance of control, ‘As’ is the absorbance of sample.

2.5.6. Total reducing power
Total reducing power was estimated according to Zhu et al. (2002) and Oyaizu (1986) and compared against gallic acid. In TRP, potassium ferric cyanide is reduced to ferrous cyanide by antioxidants present in the sample. With the increase in antioxidant’s concentration, there is an increase in the reducing power, thus increasing the absorbance.

The extracts (100–500 µg) were mixed with 2.5 ml phosphate buffer (0.2 mM, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The reaction system was closed and incubated at 50 °C in a water bath for 30 min. After the incubation period, 2.5 ml of 10% trichloroacetic acid was added to the assay system and the contents were mixed well. Sample (2.5 ml) was collected and mixed with 2.5 ml of distilled water and 0.5 ml 0.5% ferric chloride. The colour developed was read at 700 nm (Shimadzu UV-2450) against reagent blank.

2.6. Statistical analysis
All the experiments were carried out in triplicates. The experimental results are expressed as mean ± standard deviation of triplicate measurements and the results were processed using Microsoft Excel and Origin.

3. Result and discussion

3.1. Proximate analysis of raw materials and dry weight of the extracts
The chemical composition of four varieties of S. melongena (eggplant) is shown in Table 1.

3.2. Antioxidant activity studies
Antioxidant activities of S. melongena extract against DPPH radical, superoxide radical and metal chelating were evaluated using various assay methods. Total phenolic content (TPC), total reducing power (TRP) and total anthocyanins were also evaluated.

3.2.1. Total phenolic content (TPC)
Polyphenols are the large group of phytochemicals that are gaining acceptance as being responsible for the health benefits associated with fruits and vegetables. Because of their chemical structure, plant polyphenols can scavenge free radicals and inactive other pro-oxidants, and also interact with a number of biological relevance. Table 1 gives the total phenolic content (TPC) of the four samples. The total phenolic content (TPC) was markedly higher in Sample IV (106.98 mg/100 g) than the other three varieties [Sample I (80.31 mg/100 g), Sample II (50.79 mg/100 g) and Sample III (49.02 mg/100 g)].
3.2.2. Total anthocyanins

Anthocyanins are reported to have significant antioxidant activities and inhibitory effects on lipid peroxidation (Noda et al., 2000). Table 1 shows the anthocyanins content of all the samples of which Sample IV possessed markedly higher anthocyanin content (0.756 mg/100 g) than others. Sample I (0.525 mg/100 g) and Sample III also contained a good amount of anthocyanins (0.53 mg/100 g) approximately compared to Sample II (0.0475 mg/100 g).

3.2.3. DPPH radical scavenging activity

DPPH is a free radical compound and has been widely used to test the free radical scavenging ability of various samples. Table 2 shows the scavenging activity of different samples of eggplant on DPPH radicals at various concentrations. The scavenging activity of eggplant on DPPH radicals increased with increasing concentrations (50–250 μg). IC50 value (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of standard was observed as 183.90 μg/ml. There was an inverse relationship between IC50 and antioxidant activity. The results indicated that Sample IV possess highest DPPH scavenging activity (126.51 μg/ml) among the samples. Sample I also had better DPPH scavenging activity (185.08 μg/ml) as compared to the Sample II (220.78 μg/ml) and Sample III (228.24 μg/ml). A linear relation between the total phenolic content and DPPH activities of the extracts was observed.

3.2.4. Superoxide radical scavenging activity

The major source of free radical production in vivo is through superoxides, which are produced by the leakage of a free electron during its transport in mitochondria. Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxy radicals.

In the present study, superoxide scavenging activity of four varieties of eggplant was measured using the xanthine–xanthine oxidase system and the results were expressed as the inhibition of rate of superoxide activity. Based on the results obtained as represented in Table 2, it is clear that Sample IV has better superoxide scavenging activity as compared to other three samples, which may be again due to the higher amount of total phenolic content. Sample I exhibited a fair superoxide scavenging activity. However, when compared with standard’s superoxide scavenging activity, all the four varieties of eggplant exhibited a weaker radical scavenging activity.

3.2.5. Metal chelating activity

Foods are often contaminated with transition metal ions which may be introduced by processing methods. Bivalent transition metal ions play an important role as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Halliwell, 1997). These processes can be delayed by iron chelation and deactivation. Therefore, the ability of the extracts to chelate iron(II) ions was evaluated and expressed as % chelation capacity.

Table 2

<table>
<thead>
<tr>
<th>DPPH scavenging (μg)</th>
<th>Superoxide scavenging (μg)</th>
<th>Metal chelation (μg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample I</td>
<td>185.08</td>
<td>222.73</td>
</tr>
<tr>
<td>Sample II</td>
<td>220.78</td>
<td>270.50</td>
</tr>
<tr>
<td>Sample III</td>
<td>228.24</td>
<td>381.02</td>
</tr>
<tr>
<td>Sample IV</td>
<td>126.51</td>
<td>152.14</td>
</tr>
</tbody>
</table>

Table 2 shows the metal chelating power of different eggplant samples. From the table it is clear that chelating power of Sample IV was higher as compared to the other three samples, while Sample II showed lowest metal chelating activity among the varieties.

3.2.6. Total reducing power (TRP)

The Fe3+–Fe2+ transformation in the presence of sample extract (0.1 mg/ml) was investigated to measure reducing power of the extract. Gallic acid was used as standard. The total reducing power (TRP) of eggplant extract along with gallic acid is shown in Fig. 2. The gallic acid showed higher TRP. The total reducing power increased with increase in concentration of different sample extract. Sample IV had highest TRP among the extracts which might be due to the presence of total phenolic content (TPC) with respect to Samples I–III. The TRP was found to be proportional with the TPC of the extracts.

4. Conclusion

Eggplant, *S. melongena* is a common and popular vegetable crop grown in the sub-tropics and tropics. It contains polyphenols, flavonoids, minerals, vitamins, etc. These are reported to possess numeral medicinal proprieties as well. The present study evaluated in vitro antioxidant activities of four varieties of eggplant commonly used in Indian cooking, in terms of total phenolic content, DPPH, total reducing power, superoxide radical scavenging activity, metal chelating activity and total anthocyanins. Results from the present study showed that methanolic extract of *S. melongena* could effectively scavenge reactive oxygen species. Especially, Sample IV showed better DPPH scavenging than the standard, where as the remaining three extracts demonstrated a comparable DPPH radical scavenging activity to the standard. Sample IV (purple colour small size) demonstrated better antioxidant activities than the other samples which may be attributed to the higher phenolic and anthocyanin content since a linear relation was observed between the TPC and the antioxidant parameters.

Conflicts of interest

The authors declare that there are no conflicts of interest.
References


