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Phenolics and carotenoids contents and radical scavenging capacity of some selected solanaceous medicinal plants

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Abstract

Plants being an important source of medicine play significant role in human health. The aim of the present study was to evaluate the total phenolics and carotenoids contents, and free radical scavenging capacity of leaves and fruits of selected five solanaceous medicinal plants, namely *Solanum melongena* (brinjal), *Solanum torvum* (tit begun), *Solanum virginianum* (kantikari), *Solanum sisymbrifolium* (sada kantikari) and *Solanum nigrum* (futi begun). Carotenoids content in the leaves and fruits of solanaceous plants varied significantly among the species. Leaf phenolics content ranged between 147.40 (*S. melongena)* and 585.15 (*S. virginianum*) mg gallic acid equivalent (GAE)/100 g fresh weight, while fruit phenolics content varied from 50.52 (*S. nigrum*) to 105.02 (*S. virginianum*) mg GAE/100 g fresh weight. IC_{50} values for scavenging 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) radical ranged between 31.52 (*S. nigrum*) and 33.55 (*S. melongena*) mg mL^{-1} in leaf, while in fruit it ranged between 27.90 (*S. virginianum*) and 33.11 (*S. melongena*) mg mL^{-1}. The highest carotenoids content (0.370 mg g^{-1} fresh weight) was measured from *Solanum nigrum* leaf. *S. virginianum* leaf contained about 4−fold high phenolics content than that in *S. melongena*. *S. nigrum* leaf had about 15−fold high carotenoids content (0.370 mg g^{-1} fresh weight). compared to *S. torvum* and *S. virginianum* fruits (0.024 mg g^{-1}FW in each). Because of the highest fruit phenolics and carotenoids content along with the lowest IC_{50} values for scavenging DPPH, *S. virginianum* fruit can be considered as superior for its health beneficial biochemical constituents.

Introduction

Medicinal plants have been used in Ayurvedic, Unani and folk medicine since ancient times in Bangladesh. Even in the present day, use of medicinal plants in primary health care system is very important, especially in remote rural communities and poorly accessible areas.The family Solanaceae represents one of the most economically and medicinally important families of angiosperms. The genus *Solanum* is a hyper-diverse taxon of this family. There are about 2000 species of *Solanum* in the world that are mainly distributed in the tropical and sub-tropical areas, with a small number in the temperate areas (Jennifer and James,1997). Eggplant is one of the most common vegetables in Bangladesh. Wild species of *Solanum* have been extensively used in folk-medicine for their protective effects on the liver and anti-secretory gastric properties (Sabir and Rocha, 2008). *S. torvum* a wild ally of *S. melongena* with promising multidisease resistant traits has great economic, medicinal and genetic importance (Hao *et al*., 2015). It is an important medicinal plant in tropical and subtropical countries and is widely used like food and folk medicine around the world. Literatures shows that *S. torvum* is mainly used for the treatment of fever, wounds, tooth decay, reproductive problems and arterial hypertension (Jaiswal, 2012). *S. nigrum* has been extensively used for traditional medicine in India and other parts of world to cure liver disorder, chronic skin ailments, inflammatory conditions, painful periods, fevers, diarrhoea, eye disease, hydrophobia etc. (Saleem *et al*., 2009). *S. virginianum* used traditionally in cold, fever, migraine, headache, dental infections, cough, and pain in chest, reduces the pain and swelling in arthritis (Sundar and Pillai, 2016). *S. sisymbrifolium* is a densely prickled perennial shrub used to treat pain, inflammation. A number of compounds from the plant have already been reported to possess hypotensive activity (Gupta *et al.*, 2014).

Sporadically some of these important medicinal plants have been studied separately by the scientists for their phenolics content, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) radical scavenging capacity and pigment content. In the present study these medicinal plants were taken to assess their total soluble phenolics, anti-oxidative power to scavenge free radical compounds and carotenoids content. This would provide a better understanding of their relative importance as natural antioxidants in the defense against oxidative stress. Keeping this view in mind laboratory studies were done to assess and compare total phenolics content in different medicinal plants; to determine radical scavenging ability of plant extracts as an index of antioxidative power of these plants against oxidative stress; and to quantify carotenoids content in these plant samples.

Materials and Methods

Plant materials and experimental sample collection

Cultivated eggplant (*Solanum melongena* L.*)* and its four wild relatives, namely tit begun (*Solanum torvum* Sw.*),*

kantikari *(Solanum virginianum* L.*),* sada kantikari *(Solanum sisymbrifolium* L.*)* and futi begun *(Solanum nigrum* L.*)* were studied in this research. They were collected from the Botanical Garden, Bangladesh Agricultural University, Mymensingh. For each plant genotype studied, three different healthy plants from three different colonies were considered as three replicates. At morning time, part of the shoot with leaves were dissected from each colony and immediately placed in a zip lock bag to check moisture loss. Soon after collection samples were brought to the laboratory for chemical analyses. As tender leaves and mature fruits are often been used for consumption, both tender leaves and relatively mature fruits of each species from each replication were separated and chopped separately to produce working samples for leaves and fruits.

Phytochemical and biochemical characterization

Total phenolics content assay

Total phenolics compound assayed with the method modified after Albano and Miguel (2011). Exactly 5 g of working samples for leaf or fruit were taken to a 250 mL beaker and 100 mL ice cooled methanol were added to it and then the samples were homogenized for 2 minutes using OV-5 Homogenizer, VELP, Italy. The mixture was kept for 30 minutes in the dark condition and centrifuged for 5 minutes at 1500 rpm and then its supernatant was treated as working sample extract. An aliquot amount of extract was used for determining total phenolics content or DPPH scavenging activity.

Gallic acid was used here as standard. Exactly, 330 µL different concentrations of gallic acid solutions or suitable amount of plant extracts were taken into a 50 mL test tube. Then 0.16 mL of Folin-Ciocalteu reagent and 3 mL of Na_2CO_3 (10%) solution was added to 1 mL of gallic acid solution. The mixture was kept in dark condition for half an hour at room temperature (25^0C). Then absorbance was measured at 760 nm. The absorbance value is the reflection of the total phenolic content of the compound. After plotting the absorbance in ordinate against the concentration a linear relationship was obtained which was used as a standard curve for the determination of the total phenolics content of the test samples.

DPPH radical scavenging assay

Free radical scavenging activity of the medicinal plant 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 2.7 mL of 0.1 mM DPPH in methanol and made up to 3 mL with 300 µL plant extracts (working sample). The contents were mixed well immediately and then incubated for 30 min at room temperature (25°C). The degree of reduction of absorbance was recorded at 517 nm using DR 6000 UV-Spectrophotometer.

The percentage of scavenging activity was calculated as:
$(Ac – As)/ Ac \times 100$

where 'Ac' is the absorbance of control (without extract) and 'As' is the absorbance of sample with plant extract. Percentage of radical scavenging activity was plotted against the corresponding concentration of the extract to obtain IC_{50} value. IC_{50} is defined as the amount of antioxidant material required to scavenge 50% of free radical in the assay system. The IC_{50} values are inversely proportional to the antioxidant activity (Nisha *et al*., 2009)

Pigments determination

This protocol describes how the contents of chlorophyll-a, b and total carotenoids can be determined in a whole pigment extract of green plant tissue by spectrophotometer (Lichtenthaler, 1987). From the fresh composite leaf or fruit samples, 50 mg were taken in glass bottles and 200 µL distilled water were added to it. Then 16 mL ethanol were added and shaken properly and finally the content was kept in dark condition for 24 h. Absorbance reading was taken in the following day in a spectrophotometer (DR 6000, Hach, USA) at 470, 649, 664 and 750 nm wave lengths. Afterward, amount of total carotenoids (sum of carotene and xanthophyll) were calculated using the following formulae:

Carotenoids $(\mathbf{C_{x+c}})$=$(4.785\ A_{470}+3.657\ A_{664}-12.76\ A_{649})\times 16.2/FW$
Where,
A_{649} = Absorbance at 649 nm
A_{664} = Absorbance at 664 nm
A_{470} = Absorbance at 470 nm
FW = Fresh weight of plant tissue (mg)

Statistical analyses

Mean values of each parameter studied for each group of medicinal plants were subjected to one way ANOVA analysis using Minitab 17.3 to determine whether significant differences in the group existed or not. In case of having significant *F-ratio,* means were subjected to Tukey's post-hoc test to observe the significant differences among the mean values.

Results

Total soluble phenolics

Methanolic extracts of leaves and fruits of different eggplants viz. *Solanum torvum* (tit begun*), Solanum virginianum* (kantikari*), Solanum sisymbrifolium* (sada kantikari*), Solanum nigrum* (futi begun*)* and *Solanum melongena* (cultivated brinjal*)* were tested to determine total phenolics as gallic acid equivalent (GAE) per 100 g fresh weight (Fig. 1). Phenolics content in leaves varied

significantly among the tested eggplant and its four wild relatives, and it ranged from 147.40 to 585.15 mg GAE/ 100 g fresh weight. The highest amount of phenolic content was recorded in *S. virginianum* (585.15 mg/ 100 g FW) followed by *S. torvum* leaves (510.511 mg/ 100 g FW). The lowest amount of phenolic content was found in the leaves of *S. melongena* (147.399 mg/ 100 g FW). Phenolics contents in *S. nigrum* and *S. sisymbrifolium* were 163.07 and 187.63 mg/ 100 g FW, respectively. Considering the value of phenolics content in *S. melongena* leaves (147.40 mg) as 100%, the relative value of phenolics content in the leaves of *S. virginianum, S. sisymbrifolium, S. torvum* and *S. nigrum* were 397.0, 127.3, 346.4 and 110.6%, respectively.

Phenolics content in fruits of eggplant and its four wild relatives also varied significantly and ranged from 50.52 to 105.02 mg GAE/ 100 g fresh weight (Fig. 1). Phenolics content was recorded maximum in *S. virginianum* fruits (105.02 mg GAE/100 g FW) followed by *S. sisymbrifolium* fruit (89.60 mg GAE/100 g FW). The minimum amount of phenolics was found in the fruits of *S. nigrum* (50.52 mg GAE/100 g FW). Fruit of *S. melongena* and *S. torvum* contained phenolic 67.99 and 55.56 mg GAE/100 g FW, respectively. Phenolics content in the fruit of *S. torvum, S. virginianum, S. sisymbrifolium* and *S. nigrum* were 81.7, 154.5, 131.8 and 74.3%, respectively of that in *S. melongena* (67.99 mg GAE/100 g FW) treated as 100%.

DPPH radical scavenging capacity

The IC_{50} value (the amount of antioxidant material required to scavenge 50% of free radical in the assay system) of leaf extract to scavenge DPPH radical varied significantly among the tested of *Solanum spp.* and ranged from 31.52 (*Solanum nigrum*) to 36.85 (*Solanum torvum*) mg mL^{-1} leaf methanolic extract (Fig. 2). *S. virginianum, S. sisymbrifolium* and *S. nigrum* leaf extracts showed statistically the lowest IC_{50} values as 31.52, 32.59 and 31.52 mg mL^{-1} leaf extract, respectively and thus having highest radical scavenging capacity. Whereas *Solanum torvum* leaf extract exhibited the highest IC_{50} (36.85 mg mL^{-1} leaf extract) and thus having the lowest radical scavenging capacity. Considering the IC_{50} value of *S. melongena* leaf (33.10 mg mL^{-1} leaf extract) as 100%, the relative radical scavenging capacity of *S. torvum, S. virginianum, S. sisymbrifolium and S. nigrum* were 109.8, 94.4, 97.1 and 93.9%, respectively.

The IC_{50} value of fruit extract for DPPH radical scavenging capacity varied significantly in the fruits among the tested *Solanum spp.* and ranged from 27.78 to 33.11 mg mL^{-1} fruit methanolic extract. As like the results of leaf extracts, *S. virginianum, S. sisymbrifolium* and *S. nigrum* fruit extracts shared statistically the lowest IC_{50} values as 27.90, 28.35 and 27.78 mg mL^{-1} fruit extract, respectively, and thus having highest radical scavenging capacity. In contrast, IC_{50} values of *S. torvum* (31.05 mg mL^{-1}) and *S. melongena* (33.11) fruit extracts were maximum with identical statistical rank and thus having their lowest radical scavenging capacity. Considering the value of IC_{50} of *S. melongena* fruit (33.11 mg mL^{-1} leaf extract) as 100%, the relative IC_{50} values in *S. torvum, S. virginianum, S. sisymbrifolium and S. nigrum* were 93.7, 84.28, 85.62 and 83.89%, respectively.

Carotenoids content

Total carotenoids content in leaves varied widely among the tested species of *Solanum* genus andits ranged from 0.027 to 0.370 mg g^{-1} FW (Fig. 3). *S. nigrum* leaf showed the highest amount of carotenoids content as 0.370 mg g^{-1} FW followed by the second highest in *S. torvum* (0.266 mg g^{-1} FW). The lowest value of carotenoids content was found in *S. sisymbrifolium* leaf (0.027 mg g^{-1} FW). The carotenoids content were intermediate in as *S. virginianum* (0.190 mg g^{-1} FW) and *S. melongena* (0.244 mg g^{-1} FW). *S. torvum, S. virginianum, S. nigrum* and *S. sisymbrifolium* had carotenoids content 111.1, 79.5, 154.3 and 11.3%, respectively considering that had in *S. melongina* leaf as 100%.

Carotenoids content in fruits varied widely among the tested genotypes and ranged from 0.0084 to 0.024 mg g^{-1} FW (Fig. 3). *S. torvum* and *S. virginianum* fruits contained maximum amount of carotenoids (0.024 mg g^{-1} FW in each), while. *S. melongena* fruit contained minimum amount of carotenoids (0.0084 mg g^{-1} FW). Carotenoids contents in fruits of *S. nigrum* (0.011 mg g^{-1} FW) and *S. sisymbrifolium* (0.0085 mg g^{-1} FW) were in between those. Considering the value of carotenoids content in *S. melongena* fruit as 100%, the relative carotenoids content in *S. torvum, S. virginianum, S. nigrum* and *S. sisymbrifolium* were 298.0, 305.6, 145.7 and 106.7%, respectively.

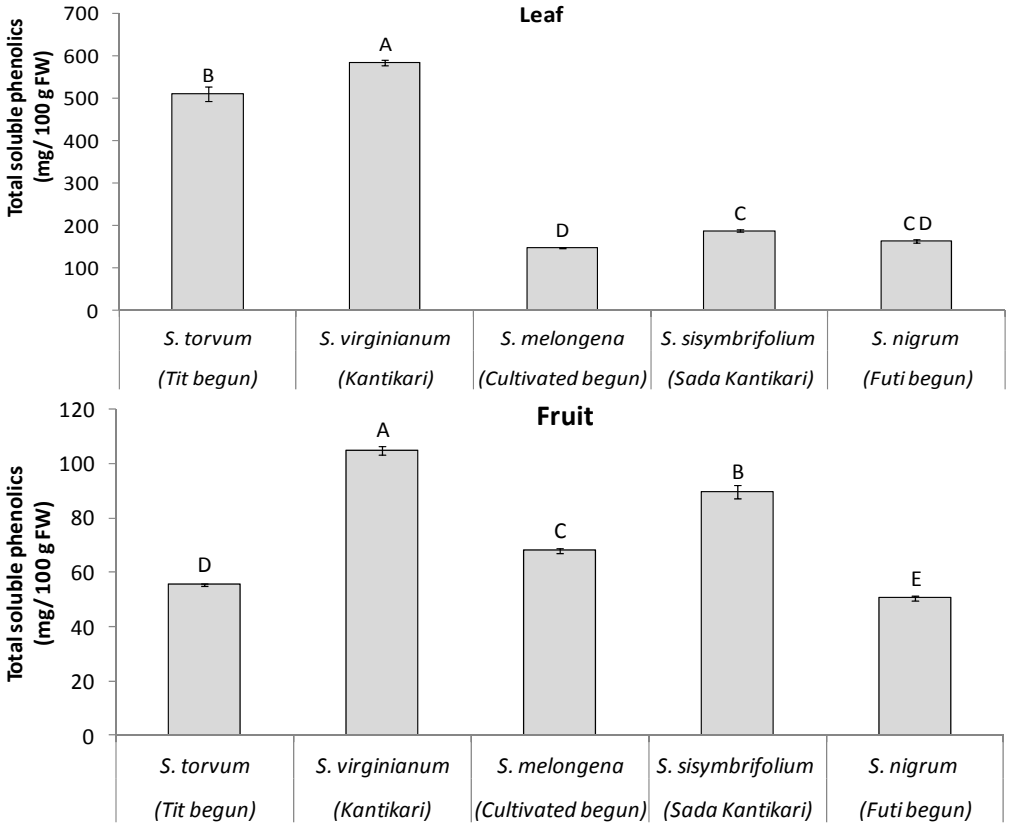

Fig. 1. Phenolics content in the leaf (above) and fruit (below) of eggplant and its four wild relatives. The amount of total phenolics content is expressed as mg gallic acid equivalent (GAE)/ 100 g of leaf fresh weight. Each data point is the average of three replicates ± SEM. Bars sharing different letters are significantly different from each other at $P \leq 0.05$.

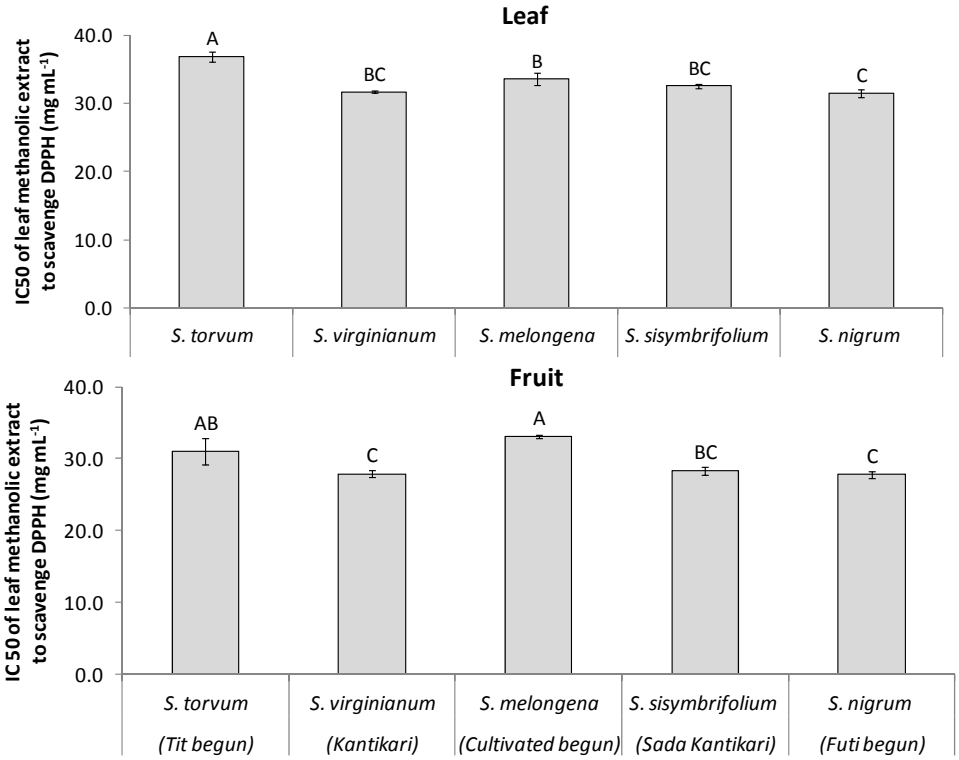

Fig. 2. IC_{50} values of the methanolic extracts of leaf (above) and fruit (below) of eggplant and its four wild relatives to scavenge DPPH radical. Each data point is the average of three replicates ± SEM. Bars sharing different letters are significantly different from each other at $P \leq 0.05$.

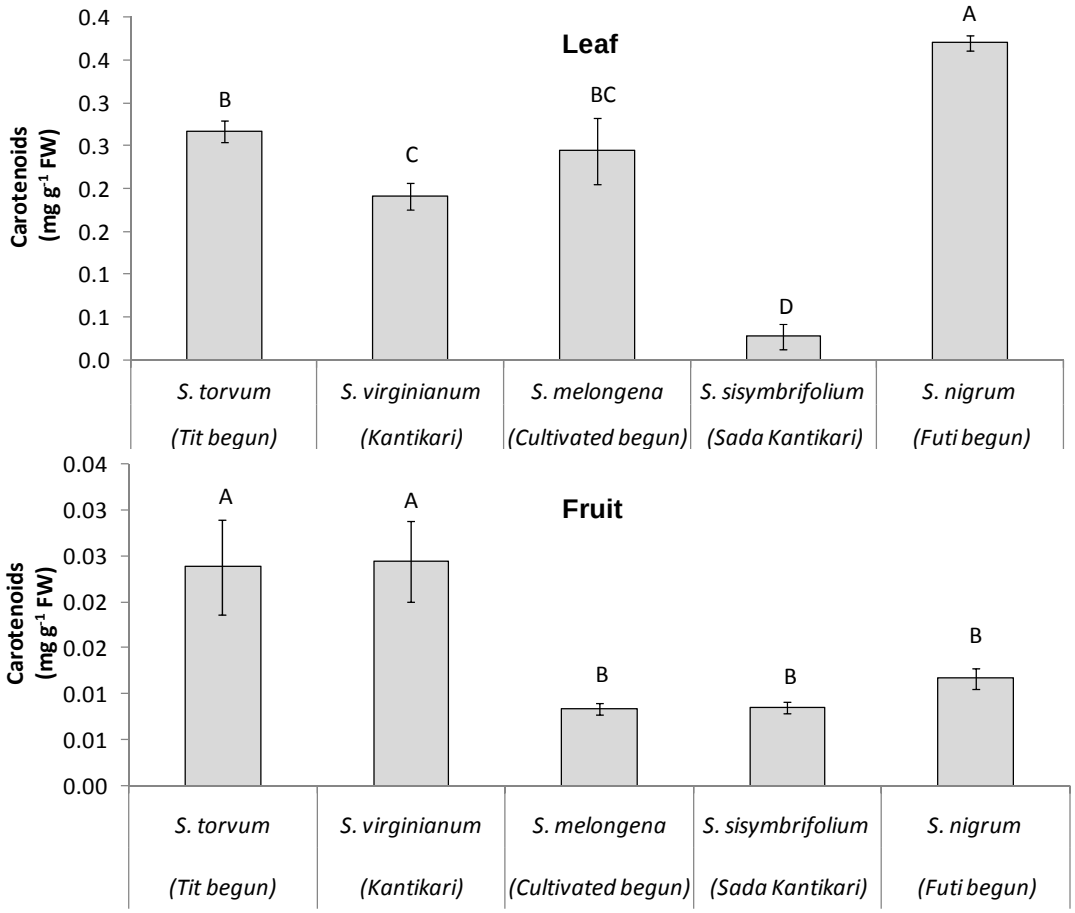

Fig. 3. Amount of total carotenoids content in leaf (above) and fruit (below) of eggplants and its four different wild relatives. Each data point is the average of three replicates ± SEM. Bars sharing different letters are significantly different from each other at $P \leq 0.05$

Discussion

Irrespective of genotypes leaves contained maximum phenolics compared to their fruits. Unique structure and a high tendency of phenolic compounds for metal chelation and their redox properties allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers which could lead to anti-oxidant activity (Khoddami *et al*., 2013). 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 (Nisha *et al*., 2009). Phenolics in eggplant have been identified as major bioactive chemicals responsible for their antioxidative properties (Kwon *et al*., 2008). In this study, *S. virginianum* had been diagnosed to have highest amount of phenolics in both fruit and leaves compared to the other four genotypes. While *S. torvum* fruit has been reported to contain higher amount of phenolics as compared to that in *S. menongena* fruit (Kaur *et al*., 2014), our study showed higher amount of phenolics in *S. melongena* fruit as compared to the *S. torvum.* This may be due to the differences of varieties of *S. melongena* used in these two different experiments. For example, total phenolics of *S. melongena* was 271 mg GAE/100 g of FW in green variety and 394 mg GAE/100 g in violet one (Sharmin *et al*., 2011). However, our results of leaf phenolics content confirm the result of Kaur *et al*., 2014. Overall, cultivated genotypes had significantly lower leaf phenolics content than wild types. It is a well-established fact that presence of appreciable content of phenolics in food confers health promoting effects due to their antioxidant action. Natural phenolic compounds have been shown to possess antimicrobial, anti-cancerous, neuroprotective activities and help improve insulin secretion and manage obesity (Del Rio *et al*., 2010). This may adequately explain why certain landraces and wild relatives (e.g. *S. torvum*) are more popular than cultivated types in Southern and North-eastern states of India. *S. torvum* is a popular delicacy in Tamil Nadu too.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The antioxidants in the sample scavenge this free radical. This method is widely used to evaluate the

free radical scavenging capacity of natural antioxidants (Maharana *et al*., 2010). In this study IC_{50} of plant extracts to scavenge DPPH were assayed for the studied medicinal plants. The IC_{50} values are considered to be inversely proportional to the antioxidant activity of plant extract.

An eggplant genotype was compared with its four wild relatives (Fig. 2). DPPH radical scavenging activity was found highest in both leaf and fruit of *S. nigrum, S. sisymbrifolium* and *S. virginianum*. In contrast, *S. torvum* leaf and fruit showed the lowest capacity to scavenge DPPH. Free radicals, produced as a result of normal biochemical reactions in the body, are implicated in contributing to cancer, atherosclerosis, aging, immune-suppression, inflammation, ischemic heart disease, diabetes, hair loss, and neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Beal, 1995).

Based on the absorbency value calculations were made using (Lichtenthaler, 1987) equation and the amount of carotenoids (sum of carotene and xanthophlyll) were estimated. In this study the highest carotenoids contents were detected in *S. nigrum* leaf as well as in *S. torvum* and *S. virginianum* fruits. Carotenoids are best recognized for their antioxidant capacity, especially in the membranes, since they are pigments located within membranes. Hydrogen peroxide, singlet oxygen, nitrogen oxides, superoxide anion, and other reactive oxygen species insulting the body from either endogenous or exogenous routes (and thus aggravating to a plethora of chronic human disease conditions) can be inactivated by carotenoids. Carotenoids are considered the most potent of biological quenchers of singlet oxygen (Paiva and Russell, 1999).

Conclusion

The leaf of *S. virginianum* acts as a good source of phenolics as it has the highest amount of phenolics than that in the fruits. *S. virginianum, S. sisymbrifolium* and *S. nigrum* leaf and fruit extracts showed the lowest IC_{50} values, and thus had the highest radical scavenging capacity while *S. torvum* and *S. melongena* fruit extracts had the lowest radical scavenging capacity. Coarotenoids contents were maximum in *S. nigrum* leaf extracts as well as in *S. torvum* and *S. virginianum* fruit extracts. Thus, *S. virginianum* leaves and fruits appear to be superior for their health beneficial biochemical constituents.

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