ANTIOXIDANT ACTIVITY AND PHENOLIC CONTENT IN METHANOLIC EXTRACT OF *S. melongena* and *S. lycopersicum* SEEDS

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ABSTRACT

The importance of polyphenolic compounds in seed of vegetables has been known for its biological activities. The seeds of S. melongena and S. lycopersicum are excluded and treated as waste materials during food processing. The seeds are innermost parts of these vegetables and need to access the importance of seeds with evaluation of various biological properties. In this study, the amount of total phenolics and their various antioxidant activities in methanolic extracts of S. melongena and S. lycopersicum seeds have been evaluated. The Folin-ciocalteau assay was used for estimation of total phenolics which found to be more in S. melongena than S. lycopersicum seeds. The seed extracts of both vegetables exhibited prominent DPPH free radical scavenging activity as compared to other antioxidant activities. The S. melongena seeds (EC_{50}25± 1.5 µg/ml) exhibited more DPPH free radical scavenging activity than S. lycopersicum seeds (EC_{50}55± 2.6 µg/ml). Similarly, seed extracts of both vegetables exhibited metal chelating, reducing power and hydrogen peroxide scavenging activities. All these activities available in seed extracts were comparatively lower than the activities exhibited by ascorbic acid. Antioxidant activities of seeds were positively correlated with amount of total phenolics. Therefore, it is necessary to avoid the removal of seeds from S. melongena and S. lycopersicum during food processing.

Keywords: *S. melongena, S. lycopersicum, Total phenolics, DPPH, Hydrogen peroxide*

Introduction

The oxygen is essential to survive for living things and during its utilization under normal physiological and metabolic processes, the approximately 5% of oxygen gets reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals (Yu, 1994; Halliwell and Gutteridge, 1985). The production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are responsible in the pathogenesis of several human diseases such as chronic inflammation, diabetes mellitus, atherosclerosis, neuro degenerative disorders and certain types of cancer (Boynes,1991). Generation of ROS collapse the antioxidant defence system of the cells, free radicals react with the cell proteins, lipids and carbohydrates and this promote the several physiological disorders (Yu et al., 1992). Generation of oxygen free radicals destroy cell membranes, disintegrate DNA and create disturbance among cell's basic enzymatic metabolic processes (Kerr et al., 1996). The necessity of compounds with antioxidant activity has been increasing for the preparation of therapeutic drug to control human degenerative diseases. The consumption of antioxidants containing diets such as legumes, fruits and vegetables have been gained the attention to prevent the causing of certain diseases. The fruits and vegetables contain considerable amounts of antioxidants including vitamin C and vitaminE, carotenoids, flavonoids and tannins etc., thus they are used to scavenge free radicals from human body (Pratt, 1992). The polyphenols are often considered the most abundant antioxidant in human diet and they possess the ability to neutralize or quench free radicals. Flavonoids and its derived compounds are the largest prominent group of polyphenols, exhibiting anticancer, anti-inflammatory, hepatoprotective, antioxidant activity and possessing the ability to inhibit oxidative

stress with scavenging reactive oxygen species (Kumar and Pandey, 2013).

Solanum melongena L.(eggplant) is a worldwide diffused vegetable and used as a common food; whole fruits contain antioxidant activity and is ranked among the top ten vegetables in terms of oxygen radical absorbance capacity (Cao et al., 1996). Besides antioxidant activity, the whole fruit contain other important activities such as anti-allergic (Lee et al., 2001), hepatoprotective (Akanitapichat et al., 2010), anti-inflammatory (Han et al., 2003), hypolipidemic (Sudheesh et al., 1997), and anticancer (Matsubara et al., 2005). The tomato (*Solanum lycopersicum* L.) is an important food crop, not only because of its economic importance, but also for its rich antioxidant content. The fresh whole tomato and its processed products such as sauce, juice, ketchup, canned tomato, stew and soup are consumed worldwide (Aguayo et al., 2010). It is a good source of vitamins (C and E), carotenoids, polyphenols, flavonoids, minerals, natural colour and several other compounds which show significant health benefit effects. Among these, carotenoids are present in large amounts, have anti-proliferative and antioxidant activities and associated with the inhibition of both heart diseases and prostate cancer (Campbell et al., 2004). The ant-oxidative property of *S.melongena* and *S.lycopersicum* has been studied but the similar activity from seeds of these vegetables has not been yet studied. The seeds from both vegetables are usually excluded during food processing at industrial and domestic level. Therefore, it is necessary to analyse various constituents and biological activity from excluded seeds. In the present study, the total phenolics and different antioxidant activities in seeds of both vegetables have been evaluated. The total phenolic content was estimated by the method of Folin-ciocalteau assay and antioxidant activities were determined by DPPH and other oxidative reagents.

Material and method

Chemicals and Reagents

DPPH (2, 2-diphenyl-1-picrylhydrazyl) was purchased from sigma Aldrich. Folin-ciocalteau, Gallic acid, Ferric chloride ($FeCl_3$), O-phenanthrolin, Potassium ferrocyanate, Trichloro acetic acid and Hydrogen peroxide were obtained from SRL and methanol was purchased from RANKEM. All chemicals and reagents used in this study were of analytical grade.

Collection and Preparation of sample

The matured *S. melongena* and *S. lycopersicum* were obtained from local market of Beed (M. S.) India. They were cut into small pieces by knife at room temperature and the seeds were removed from pieces in a tray containing distilled water. The removed seeds were washed thoroughly with distilled water and wiped by filter paper. The seeds were completely dried in an oven at 37^0C for 48 hrs. The dried seeds were crushed into fine powders grinder and powders were preserved at room temperature in asample storage tubes.

Preparation of extract

Extraction of total phenolics content from fine powders was performed by the earlier utilized procedure of Esmaeili et al. 2015. Fine powder of each sample (2 g) was soaked in 50ml of methanol and stirred at room temperature for 2 hrs by magnetic stirrer. The extracts were filtrated through Whatman filter paper and the methanol from extract was evaporated at room temperature. The obtained residues were preserved in refrigerator at 4^0C.

Estimation of Total phenolics

Amount of total phenolics in prepared extracts was determined by using Folin-ciocalteau assay with slight modification of method used by Ainsworth and Gillespie (2007). 10mg of each crude extract was individually dissolved in 1 mL of corresponding extracting solvent. Gallic acid was used as a reference standard for

plotting of calibration curve. Methanolic extract (10 µl) of each plant was diluted up to 1.5 ml with distilled water. To this extract, 0.5 ml Folin-Ciocalteau reagent was added and incubated at room temperature for 3 min. Thereafter, each aliquot was neutralized by adding 1 ml sodium carbonate (20% w/v). Reaction mixtures were incubated at room temperature for few min with intermittent shaking for color development. Absorbance of resulting blue color was measured at 650 nm using double beam UV-VIS spectrophotometer. Amount of total phenolics was estimated from standard graph and expressed as gallic acid equivalent GAE (mg/ml).

DPPH free radical scavenging assay

The free radical scavenging activity of each crude extract was measured by 2, 2-Diphenyl-1-picrylhydrazyl radical (DPPH) assay (Gordon et al., 2001) with slight modifications. Fresh methanolic solution containing different phenolics concentration (15, 30, 45, 60 and 75μg/mL) were treated with 2ml of DPPH (0.2mM) solution and incubated in the dark for 30 min, and the absorbance was read at 540 nm against the blank. Ascorbic standard as positive control was prepared in a similar manner, as for the test group except for the antioxidant solution's replacement. The inhibition of the DPPH radical by the sample was calculated based on the formula below.

$$\textbf{DPPH scavenging activity}(\%) = \left[\frac{\textbf{absorbance of control} - \textbf{absorbance of sample}}{\textbf{absorbance of control}}\right] \times \textbf{100}$$

Metal chelating activity

Metal chelating activities of crude extracts were determined according to the method Benzie and strain, 1996. In a typical reaction contains 0.5ml (0.5mg/ml) O-phenanthrolin, 1 ml $FeCl_3$(200M) and 2 ml of each extract with different concentrations (50, 100, 150, 200 and 250μg/mL) were incubated at ambient temperature for 15min. After incubation the optical density was recorded at 510nm. Blank was performed without crude extract. The percentage of iron chelating activity of each phenolic compound was calculated by using following formula.

$$\textbf{Percent of chelating activity} = \left[\frac{\textbf{Test absorbance} - \textbf{Control}}{\textbf{Test absorbance}}\right] \times \textbf{100}$$

Reducing Power Assay

The reducing powers of the extracts were determined by using the method of Atmani et

al., (2009). The one millilitres of extract containing different concentration of phenolics (30, 60, 90, 120 and 150 μg/ml) were mixed with 2ml of phosphate buffered saline (0.2 M, pH 6.6) and 2ml of potassium ferrocyanate (10mg/ml). The reaction mixtures were incubated at 50^0C for 20 min. After incubation 2ml of trichloro acetic acid (100 mg/l) was added to the each mixture. From each reaction mixture 2ml was mixed with 2ml of distilled water and 0.4 ml of 0.1% (w/v) ferric chloride and incubated at room temperature for 10min. 2ml of distilled waterand 0.4ml of 0.1% (w/v) ferric chloride. The absorbance was measured at 700 nm and the increased absorbance of the reaction mixture suggested that the reducing power was high.

Hydrogen Peroxide Scavenging Activity

For determination of scavenging hydrogen peroxide by extracts, the procedure of Ruchet al. (1989) was used. The hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate

buffer (pH 7.4). In a typical reaction containing 0.5 ml of extracted sample with different concentration of total phenolics (40, 80, 120, 160 and 200 μg/mL) were mixed with 0.5 ml of 50 mM phosphate buffer (pH 7.4) and followed by the addition of 1 ml prepared hydrogen peroxide solution. The solutions were mixed with shaking properly and absorbance of the hydrogen peroxide at 230 nm was recorded after 10 min against a blank. The ability to scavenge the hydrogen peroxide was calculated based on the following equation:

$$\textbf{Hydrogen Peroxide Scavenging Activity} = \left[1 - \frac{\textbf{Absorbance of Sample}}{\textbf{Absorbance of Control}}\right] \times 100$$

Results and Discussion

Total Phenolic content

Phenolic compounds are large group of secondary metabolites, synthesized in plants and serve different functions such as protection against pathogens and predators, mechanical

support, attraction of pollinating animals, and protection against ultraviolet radiation (Bravo, 2009). The total phenolics in *S. melongena* seeds were estimated as 225.45 μg/mg of extracted residue while in *S. lycopersicum* seeds it was found 98.50 μg/mg of extracted residue. The concentration total phenolics in both samples were found to be quite different from previously reported studies. Esteban et al. (1992), have evaluated the amounts of total phenolics are changed during development and ripening of three eggplant cultivars produced in hydroponic systems and level of phenolics at physiological maturation is around 130, 160 and 200 mg of gallic acid equivalents per 100 g. Peschel et al. (2006) have reported the level of total phenolics in tomato skin and seeds as 44.18 and 20.94 mg GAE/100 g respectively.

DPPH Radical Scavenging Activity

The antioxidants accumulated in plant parts significantly responsible for preventing oxidation of cellular components such as proteins, lipids, carbohydrates and DNA (Gupta and Sharma, 2006). 2, 2-Diphenyl-1-picrylhydrazyl radical is a stable organic free radical with showing an absorption band at 517 nm. It loses this absorption when it accepts an electron or a free radical species, resulting in a visually noticeable discoloration from purple to yellow. It is useful to analyse many samples as scavengers in a short span and is sensitive enough to distinguish active ingredients at low concentrations (Hseu et al., 2008). Figure 1 shows that phenolics extracted from *S. melongena* and *S. lycopersicum* seeds exhibited DPPH free radical scavenging activity with EC_{50} value 25± 1.5 and 55± 2.6 µg/ml respectively. The *S. melongena* exhibited more free radical scavenging activity than *S. lycopersicum.* The activity in both vegetables was found to be comparatively lower than the activity of standard ascorbic acid (EC_{50} 12± 0.5 µg/ml). DPPH free radical scavenging activity in both samples was found to be highest as compared to other anti-oxidant activities as shown in table 1.

Metal Chelating Activity

Metal chelating activity from seeds of both vegetables was studied in vitro assay. *S. melongena* seeds exhibited more chelating activity (EC_{50}130± 2.4 µg/ml) as compared to chelating activity (EC_{50}225± 3.1 µg/ml) of *S. lycopersicum* seeds (Figure 2). Both vegetables exhibited lower metal chelating activity as compared to chelating activity (EC_{50} 35± 0.09 µg/ml) of reference standard ascorbic acid. The metal chelating activity in both vegetables was detected as lowest as compared to other antioxidant activities as shown in table 1. The phenolics containing ortho substituted group may exerts prooxidant effects with interacting with iron. O-phenanthrolin form complexes with Fe2+, which could be disrupted in the presence of chelating compounds. The

extracts prominently interfered with the formation of ferrous-o-phenanthroline complex that indicates extracts have metal chelating activity. Iron stimulates lipid peroxidation by Fenton reaction and also brings about peroxidation by disintegrating lipid hydroper oxides into peroxyl and alkoxyl radicals which themselves abstract hydrogen and continue the chain reaction of lipid peroxidation. This activity is an important because since it decreases concentration of the catalyzing transition metal in lipid peroxidation (Mahakunakorn et al., 2004)

Reducing Power Activity

Reducing power of the samples could be determined by using a modified $Fe^{3+}$to Fe^{2+}reduction assay in which the colour of the test solution is yellow and it transforms to various hues of green and blue, based on reducing power of the samples. The reduction of $Fe^{3+/}$ferricyanide complex to the Fe^{2+}form is a formation of Perl's Prussian blue which indicates the presence of the antioxidants in the samples (Qingming et al., 2010). The presence of reductants (antioxidants) in the extract would bring about the reduction of Fe^{+3}/ferricyanide complex to the ferrous form by donating an electron. The increasing the absorbance of blue colour at 700 nm suggests an increase in its ability to reduce. Both vegetables exhibited reducing power, *S. Melongena* seeds showed more reducing power (EC_{50} 44 ± 1.45 µg/ml) than reducing power (EC_{50} 136 ± 2.09 µg/ml) of *S. Lycopersicum* seeds. Reducing power of both vegetables were significantly lower in comparison with the positive control (ascorbic acid) as shown in figure 3. The results of this study showed that increased the reducing ability of samples with increased concentration of phenolics and it was similar to that reported by Gulçin et al. (2003) who evaluated the antioxidative activity in *Pimpinellaanisum* seeds extracts and four types of Malaysian plants.

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide is a non-reactive, sometimes be poisonous to cells and may triggers the rise of hydroxyl radicals inside the cells (Halliwell, 1991).The hydrogen peroxide scavenging activity in *S. melongena* seeds (EC_{50} 75± 1.45 µg/ml) was found to be more as compared to *S. Lycopersicum*seeds(EC_{50} 174± 2.54 µg/ml) and both vegetables have been detected containing lower activity (EC_{50} 28± 0.71µg/ml) than standard ascorbic acid as shown in table 1. The hydrogen peroxide is an oxidising agent that interacts with enzymes and inactivates them directly, usually by oxidation of essential thiol (- SH) groups. It can come inside cell across the cell membrane and reacts with Fe^{2+} and Cu^{2+} ionsto form hydroxyl radicals and this could be production of many toxic effects(Kumaran et al., 2007).The reducing capacity of vegetable extracts can serve a significant indicator of its potential antioxidant activity.

Conclusion

The present study showed that*S. melongena* and *S. lycopersicum*seeds are good sourcesof polyphenols and antioxidants. Antioxidant activitiesof seeds extracts are lower than the standard ascorbic acid. The amount of polyphenolic compounds available in these seeds are positively correlated withantioxidant activities. The anti-oxidative properties of these vegetablesseeds suggest thattheinvolvement of seeds in foodduring food processing at industrial and domestic level could be beneficial to increase the functional property of processed foods and becoming wholesome food. Furthermore this study encourages the isolation and identification of active ingredients from these seeds so that the identified compounds could be utilized in concern foods as food additives.

Table 1: The concentration of totalphenolics extracted in methanol from *S. melongena* and *S. lycopersicum*seeds with EC_{50} values of different anti-oxidant activities.

Sr. No.	Name of sample (Seed Powder)	Concentration of total phenolics per mg of extracted residue (µg/mg)	EC_{50} of DPPH radical scavenging activity (µg/ml)	EC_{50} of metal chelating activity (µg/ml)	EC_{50} of reducing powers (µg/ml)	EC_{50} of hydrogen peroxide scavenging Activity (µg/ml)
1.	*S. melongena*	225.45	25± 1.5	130± 2.4	44± 1.45	75± 1.45
2.	*S. lycopersicum*	98.50	55± 2.6	225± 3.1	136± 2.09	174± 2.54
3	Ascorbic acid	---	12± 0.5	35± 0.09	20± 0.5	28± 0.71

Figures

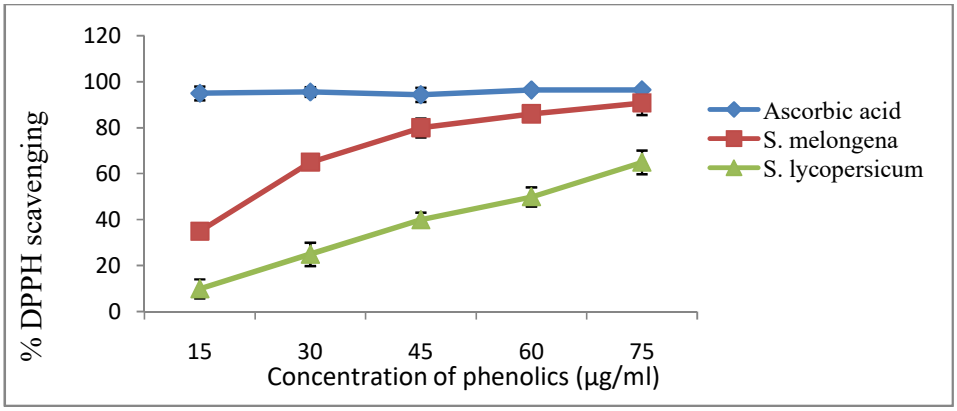

Figure 1: In-vitro DPPH radical scavenging activity of different concentration of total phenolics extracted from *S. melongena* and *S.lycopersicum*seeds.

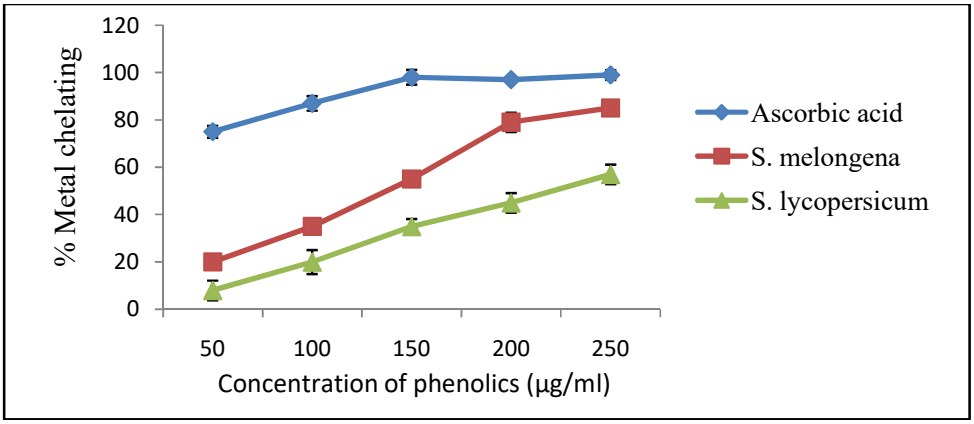

Figure 2: Graphical representation of % metal chelating activity exhibited by phenolics extracted from *S. melongena* and *S.lycopersicum*seeds by using methanol as solvent system.

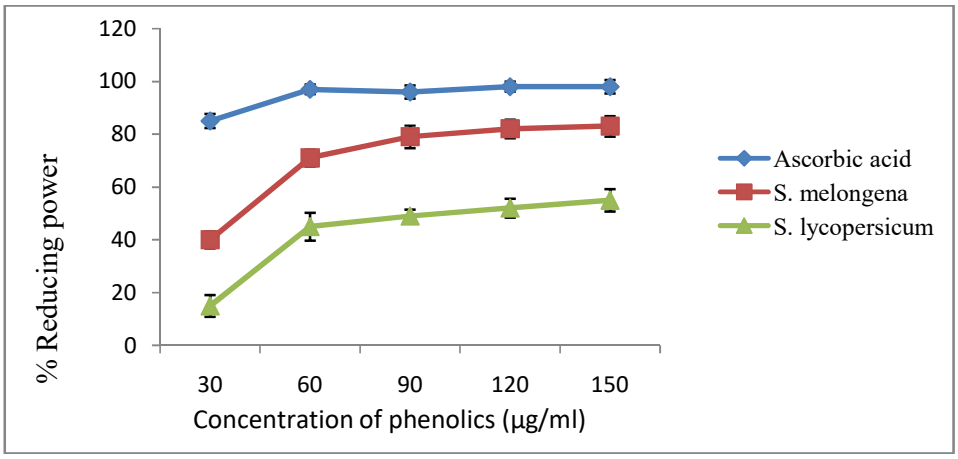

Figure 3: Dose dependent of reducing power of phenolicsextracted from *S. melongena* and *S.lycopersicum*seeds.

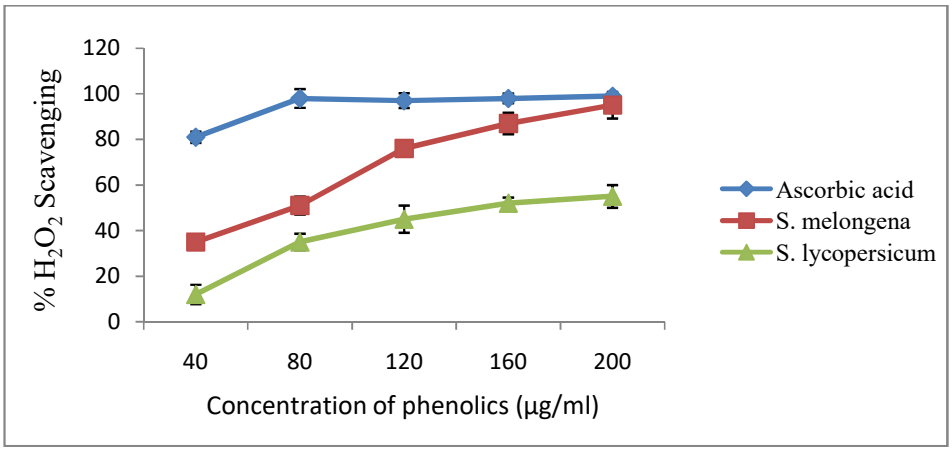

Figure 4: H_2O_2 scavenging activity of different phenolics extracted in methanol from *S. melongena* and *S.lycopersicum*seeds.

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