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Evaluation of Antioxidant Activity of *Solanum melongena* Fruit Peel Extract

J. Sarkar*, A. Garg and P. Gupta
K.R. Mangalam University, School of Medical and Allied Sciences, Dept. of Pharmacy, Sohna Road, Gurgaon, Pin. - 122103, Haryana, India.

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***Corresponding Author Email:** sombhu89@gmail.com

Abstract

Aim:This study was planned to evaluate *in-vitro* anti-oxidant activity of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract (PHAE). ***Methods:*** Antioxidant activity of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract (PHAE) was first subjected to preliminary phytochemical screening and then various *in-vitro* anti-oxidant studies were conducted namely; 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Scavenging activity, Hydrogen Peroxide (H_2O_2) Scavenging activity, Nitric Oxide (NO) Scavenging activity, Superoxide (O^-_2) Scavenging activity, Total Reducing-Power assay (TRP) and Total Phenol content (TPC). ***Results:*** The percent (%) inhibition against DPPH radical, H_2O_2 radical, NO radical, O^-_2 radical by PHAE was found to be 62.17, 64, 69.65, 74.77 respectively. Effective Concentration (EC_{50}) in TRP assay was found to be 59.31 µg/ml for PHAE. TPC was found to be 782 mg of gallic acid equivalent per gram of PHAE. ***Conclusion:*** Under the testing conditions, the results from these *in-vitro* antioxidant tests evince that PHAE has significantly high antioxidant activity. So, this merits further investigation for hepatoprotective activity *in-vivo*/ humans too.

Keywords
antioxidant, free radical scavenging, hydro-alcoholic extract, *in-vitro*, *Solanum melongena*.

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INTRODUCTION:

India has been a rich repository of medicinal plants since ages and herbs continue to be main stay for the treatment of various disease and ailments due to several factors especially side effects that are associated with allopathic drugs. Also, drug resistance phenomenon is on rise now a days, so plants and herbs seem the best alternative.[1]

Purple coloured *Solanum melongena* fruits are known to have more healing and health improving properties than other varieties due to polyphenols/ flavonoids/ anthocyanin.[2] Therefore the present study was designed to systematically screen the phytochemicals and its antioxidant activity using contemporary technologies and well-established experimental models.

MATERIALS AND METHODS:

Chemicals and reagents:

All the chemicals were purchased from Nice Chemicals, India. Ascorbic acid, DPPH, Greiss reagent, Folin-Ciocalteau reagent and other standards were purchased from Sigma Aldrich. The purple colour fruits of *Solanum melongena* were purchased from local market and authenticated by plant taxonomist Dr. K. Madhava Chetty, Sri Venketeswara University, Tirupati, Andhara Pradesh, India and a voucher specimen number of 1894 has

been retained in Sri Venketeswara University. Green coloured crown were removed from the fruits and then air dried under shade and finally powdered.[3]

Extraction

To perform isolation from peel of *Solanum melongena* fruits, powdered peels was subjected to Soxhlet extraction with petroleum ether at 30-40°C and isolation with hydro-alcohol (ethanol: water-1:1) and done with temperature not exceeding up to 55 °C. Both extractions was carried out till solvent got discoloured in the siphon tube with time not exceeding up to 24 hours. Both extracts were vacuum dried in rotary evaporator manufactured by Perfit India; at 100 RPM, 40°C. Both extracts obtained were dried, weighed and percentage yield were calculated. The percentage yield of hydro-alcoholic extracts was found to be higher than that of aqueous extracts which shows hydro-alcoholic extracts contain much more constituents than aqueous extract.[4]

Phytochemical screening

The hydro-alcoholic and aqueous extracts of the peels of *Solanum melongena* fruits were separately subjected to preliminary phytochemical tests using standard methods.[5] Test with results are mentioned in Table 1.

***Antioxidant activity of PHAE*:**

Solanum melongena fruit peels contain many phenols, anthocyanins and ascorbic acid which are also a powerful antioxidant. [4, 6, 2] Herein are the conducted *in-vitro* tests:

1. ***DPPH - scavenging activity***

   The scavenging activity of DPPH free radical was evaluated for the estimation of antioxidant property of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract following procedure of Gupta *et al*, 2012.[4] DPPH solution (0.1 mM) was prepared in ethanol and 1.0 ml of this solution was added to 3.0 ml of extract solution prepared in water at different concentrations (1-5 µg/ml). Mixture was incubated in dark for 30 minutes and the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. The result of this method was expressed in the form of % DPPH scavenging effect and was calculated by the following formula.[7]

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

   Where A_0 was absorbance of blank and A_t absorbance in presence of extract. Test was carried out in triplicate.

   IC_{50} value was used to represent the antioxidant activity of the *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract. The IC_{50} value is the concentration (µg/mL) of the extract required to inhibit DPPH radical formation by 50%. Test was carried out in triplicate.

2. ***Hydrogen Peroxide - scavenging activity***

   The H_2O_2 scavenging activity was determined in *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract according to the method of Ruch *et al.* (1989)[8] with some modifications.[9, 6] The mixture containing sample (1 ml; 10-320 µg/ml), phosphate buffer solution (PBS) (2.4 ml; 0.1 M, pH 7.4) and H_2O_2 solution (0.6 ml; 40 mM) was shaken vigorously and incubated at room temperature for 10 minutes. Absorbance of the reaction mixture was determined at 230 nm. Ascorbic acid was used as positive control.

   The percentage of hydrogen peroxide scavenging of both *Solanum melongena* peel extract and standard was calculated with:

$$\% \text{ Inhibition} = 1 - \left(\frac{A_1 - A_2}{A_0}\right) \times 100$$

   Where, A_0 is the absorbance of the control (water instead of sample), A_1 is the absorbance of the sample, and A_2 is the absorbance of the sample only (phosphate buffer instead of H_2O_2 solution). The IC_{50} value represented the concentration of the compounds that caused 50% inhibition of H_2O_2.[10]

3. ***Nitric Oxide - scavenging activity***

   5 mM of Sodium nitroprusside in Phosphate buffered saline (PBS) was added to 3.0 ml of different concentrations (10-320 µg/ml) of the *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract. For 2.5 hours, the mixture was kept away at 25˚C for incubation. Further Greiss reagent which is a mixture of sulphanilamide (1%), Phosphoric acid (H_3PO_4 (2%)) and napthylethylenediamine dihydrochloride (0.1%); was added into sample solution. Diazotization of nitrite with sulphanilamide and subsequent coupling with napthyl ethylenediamine generates a chromophore. The absorbance of the chromophore formed was noted at 546 nm.[11] Ascorbic acid was used as the reference compound. Calculation was done by using the following formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where Ao was the absorbance of the control (blank, without extract) and At was the absorbance in the presence of the test (extract).

4. ***Superoxide - scavenging activity***

The superoxide radical scavenging activity in *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract was estimated using nitro blue tetrazolium (NBT) as given by Sabu *et al.*, 2002.[12] Different concentrations of extract (10-320 µg/ml) were taken in a test tube. To the test tube; 1 ml of (50 mM) sodium carbonate, 0.4 ml of (24 mM) NBT and 0.2 ml of 0.1 mM EDTA solutions were added and absorbance of the mixture was measured immediately at 560 nm. Then to initiate the reaction 0.4 ml of (1 mM) of hydroxylamine hydrochloride was added. Finally, for 15 minutes reaction mixture was incubated at 25˚C and NBT reduction was measured at 560 nm. Increased superoxide anion scavenging activity is indicator of decreased absorbance of the reaction mixture. Ascorbic acid was used as the standard compound. All the extracts were treated similarly, and percentage inhibition was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_0 was absorbance of blank and A_t absorbance of samples. Test was performed in triplicate.

5. ***Total Reducing Power assay***

Reducing power was evaluated using 2.5 ml volume of various concentrations of extracts (10-320 µg/ml).[13] To the extracts, sodium phosphate buffer (2.5 ml, 200 mM) and potassium ferricyanide (2.5 ml, 1%) was added at pH 6.6. The reaction mixtures were incubated for 20 minutes at 50 ˚C. Then trichloroacetic acid (2.5 ml of 10% w/v) was added into the solution. Then the solutions were stirred properly and then centrifuged (8 minutes at 1000 rpm) to separate into layers. After centrifugation for 8 minutes, upper layer was separated and subjected to estimation. 5 ml of upper layer was added into deionized water (5 ml) and ferric chloride (1 ml, 0.1%). After mixing properly, absorbance was measured at 700 nm by using double beam spectrophotometer. [10, 14] This procedure was repeated three times. EC_{50} value was calculated from concentration-absorbance graph and ascorbic acid was used as standard analytical agent.

6. ***Total Phenol Content***

Total phenolic (soluble) content was estimated following Folin–Ciocalteau method, by using the Folin–Ciocalteau reagent.[15] This method is based on the oxidation reaction. Gallic acid was used as standard reagent in this procedure. *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract (1.0 g/ml) was diluted with 46 m distilled water in conical flask. Post dilution, Folin–Ciocalteau reagent (1 ml) was added and mixed properly. Solution was kept standing for 3 minutes. To this, sodium carbonate was added and allowed to stand for 180 minutes with occasional shaking. Blue color was developed, and its absorbance was measured at 760 nm which was calculated as gallic acid equivalents (GAE: mg/100 g) of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract. [7, 16, 17]

Statistics

All the data are presented as mean ± SEM and calculations were performed using MS Office- Excel 2016 software.

RESULTS AND DISCUSSION:

Phytochemical screening

Phytochemical screening test results of aqueous and PHAE are mentioned in Table 1.

1. ***DPPH - scavenging activity***

Efficient concentration (EC_{50}) was determined by plotting a graph between percent radical scavenging activity against final extract concentration. EC_{50} is the concentration of extract required to scavenge free radical by 50% and defined as 50% effective concentration (EC_{50}).

The % inhibition against DPPH radical was found to be 62.17 and 100 for *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract and ascorbic acid respectively (Table 2; Figure 1). The % inhibition of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract were found statistically significant ($p<0.01$) in comparison to control.

- The results are depicted in individual graphical form in attached file names as: 1. DPPH-Scavenging Activity.

2. ***Hydrogen Peroxide - scavenging activity***

The % inhibition against hydrogen peroxide radical was found to be 56.64 and 100 for *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract and ascorbic acid respectively which is expressed in percentage scavenging (Table 3; Figure 2). The % inhibition of

Solanum melongena purple coloured fruit peel hydro-alcoholic extract were found statistically significant ($p<0.01$) in comparison to control.

- The results are depicted in individual graphical form in attached file names as: 2. Hydrogen Peroxide–Scavenging Activity.

3. ***Nitric Oxide - scavenging activity***

In a dose-dependent manner at various concentrations of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract (10–320 µg/ml) inhibited NO. The % inhibition against nitric oxide radical was found to be 69.65 and 100 for *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract and ascorbic acid respectively (Table 4; Figure 3). The % inhibition of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract were found statistically significant ($p<0.01$) in comparison to control.

- The results are depicted in individual graphical form in attached file names as: 3. Nitric Oxide–Scavenging Activity.

4. ***Superoxide - scavenging activity***

In a dose-dependent manner, *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract has shown significant activity against superoxide radicals (Table 5; Figure 4). Inhibitory concentration (IC_{50}) of ascorbic acid was found to be 27.96 µg/ml and that of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract was found to be 32.65 µg/ml. The % inhibition against superoxide radical was found to be 74.77 and 100 for *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract and ascorbic acid respectively. The % inhibition of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract were found statistically significant ($p<0.01$) in comparison to control.

- The results are depicted in individual graphical form in attached file names as: 4. Superoxide-scavenging activity.

5. ***Total Reducing Power assay***

Total Reducing Power was concluded in terms of absorbance at 700 nm for different concentrations (10 - 320 µg/ml) by the formation of ferrous ion complex and EC_{50} (effective concentration at which the absorbance is 0.5) was calculated (Table 6; Figure 5). EC_{50} was calculated to be 59.31 µg/ml for *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract and 32.42 µg/ml for ascorbic acid. The % inhibition of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract were found statistically significant ($p<0.01$) in comparison to control.

- The results are depicted in individual graphical form in attached file names as: 5. TRP assay.

6. ***Total Phenol Content***

The total phenol content was found to be 782 mg of GAE/gram of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract.

The present investigations were conducted to isolate the main chemical ingredient responsible for the overall said effect. Taxonomical identification was carried out to verify the species. From farm fresh fruits were collected. Since colour matter is extremely delicate, so extra care was taken to handle the fruits. By using qualitative tests, it was confirmed that the colouring matter is of anthocyanin group.

Discussion:

The Health Authority of Indian Government is trying to promote ethnobotanical uses of plants and vegetables to treat metabolic ailments with natural resources and by asking public to adopt healthy living lifestyle. So, search and research for healthy and low-cost alternatives; that are easily assessible to all classes of socio-economic strata, becomes imperative to improve health from medicinal and prophylactic point of life. *Solanum melongena* (purple peeled fruit) is a vegetable plant that has been reported for various medicinal uses like anti-asthmatic, proliferative diseases, cardiovascular diseases etc.[18]

The previous studies of *Solanum melongena* reported antioxidant activities on whole fruits. However, this is the first report on the antioxidant activity on purple colour *Solanum melongena* fruit peels.

In this present study, *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract was studied for its antioxidant activities by employing DPPH scavenging activity, Hydrogen Peroxide–scavenging activity, Reducing-Power assay, Nitric Oxide–scavenging activity, Superoxide-scavenging activity and Total Phenolic content activities.

An assay based on the use of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals is the most popular spectrophotometric method for determination of the antioxidant capacity of vegetable extracts because the radical compounds can directly react with antioxidants [6, 19]. The effect of antioxidant activity on DPPH radical scavenging is thought to be due to their hydrogen donating ability: $DPPH^{\bullet} + AH \rightarrow DPPH{-}H + A^{\bullet}$

EC_{50} value is presented in Table 1. A lower value of EC_{50} of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract co-relates to a higher antioxidant activity. Out of five concentrations of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract used (10, 20, 30, 40 and 50 µg/ml), 50 µg/ml showed highest activity (62.17%) and was comparable to ascorbic acid that was used as standard antioxidant.

Under normal conditions, cells by both enzymatic and non-enzymatic cellular pathways, produce a highly reactive species called Hydrogen peroxide (H_2O_2). Due to its high lipid solubility, it easily diffuses across the membranes. Hence anti-oxidant potential of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract is represented by scavenging of H_2O_2.[6, 8, 9]

Out of six different concentration of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract (10, 20, 40, 80, 160 and 320 µg/ml); 320 µg/ml showed highest inhibition potential against hydrogen peroxide radical at 56.64 %.

Nitric oxide (NO) plays an important role in various types of inflammatory processes in the body. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitric oxide.[11] Scavenging of free radicals generated from Nitric oxide metabolism was seen to be highest among all six different concentration of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract (10, 20, 40, 80, 160 and 320 µg/ml). *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract at 320 µg/ml showed strong activity at 69.65 % against ascorbic acid.

Superoxides are the major source of free radical production in-vivo that are produced when a free electron leaks during its transport in mitochondria. Since it's a reactive oxygen species, so it can cause damage to the cells and DNA that may lead to various diseases. It has strong biological importance because its decomposition can lead to the formation of a stronger oxidative species such as singlet oxygen and hydroxyl radicals. [7, 10]

In Table 4, IC_{50} values are presented. A lower value of IC_{50} of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract co-relates to a higher antioxidant activity. IC_{50} for *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract was found to be 32.65 µg/ml which proves that *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract has very high superoxide scavenging activity that may be due to its high polyphenols/flavonoids/anthocyanins content. Out of six different concentration of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract (10, 20, 40, 80, 160 and 320 µg/ml); 320 µg/ml showed highest activity (74.77%) and was closely comparable to ascorbic acid when used as a standard antioxidant.

In TRP assay, if antioxidants such as polyphenols are in higher concentrations; then reduction of oxidation form of Fe^3+/ferricyanide complex to ferrous (Fe^2+) will be higher. Eventually, absorbance will increase.

Among many decolorization assay (including DPPH assay), TRP is also an assay of this category, which quantifies the relative antioxidant abilities of extracts from natural sources to scavenge free radicals that are produced in the assay by measuring the amount of formation of ferrous ion complex, depicted by EC_{50}. Comparing all six concentrations of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract (10, 20, 40, 80, 160 and 320 µg/ml) with ascorbic acid, EC_{50} was slightly higher for *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract at 59.31 µg/ml than ascorbic acid 32.42 µg/ml. But significantly there was not very high difference in reducing power capacity of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract which was 74.77% and was closely comparable to ascorbic acid.

In *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract, Folin–ciocalteu reagent was used to determine total phenolic contents and was expressed as gallic acid equivalents (GAE). Metals like polytungston is present in Folin–ciocalteu reagent. Metal of reagent is reduced by phenols of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract and colour of reagent changes to Prussian blue from yellow. [7, 16, 17] It suggests that concentration of phenol is directly proportional to the colour intensity.

After exploring literatures, few relevant studies showed an approximate of 62.5 mg gallic acid equivalent/100 g in the fresh pulp. While in this study under testing conditions, 782 mg of GAE/gram of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract was obtained. So there is a quite significant difference.

Solanum melongena fruits have been evaluated for their phytoconstituents where the reports show that they are rich in polyphenols. The observed antioxidant action with the fruits of *Solanum melongena* can be attributed to the presence of polyphenols/flavonoids/anthocyanins which may be responsible for scavenging of free radicals. However, the latter need further studies for justification.[20]

The purple peeled fruits of *Solanum melongena* eggplant, is claimed to treat human body from various metabolic disorders, proliferative damages and degenerative changes due to aging and senesce. Cells in human body suffer damages that are caused by reactive species generated from various metabolic processes in body. Such chemically unstable species are called free radicals and body can be protected and repaired from damage antioxidants. They work by slowing or preventing formation of free radicals. Out of many, one of the antioxidants contained in *Solanum melongena* fruit is flavonoids/polyphenols/anthocyanins (exceptional in brinjal to possess anti-oxidant activity).[21] Chemically speaking, all anti-oxidants donate free electrons to free radicals (like carbon tetrachloride free radical is CCl_3^-, thus prevent the formation of $CCl_3O_2^-$) and prevent formation of covalent bonds with fat. In this way, lipid peroxidation is inhibited, thereby diminishing/preventing/inhibiting hepatic cellular tissue.[18]

The widespread use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), chemicals exposure, contaminated food and water, alcohol drinking habits among population is expected to raise the disorders of liver due to cellular and molecular mechanisms that leads to the pathogenesis of hepato-toxicity and lipid peroxidation, signals the involvement of advanced glycation end products and oxidative stress.[1] The commercially available therapeutic agents capable of treating the damages are either very costly or exhibit side effects whose risk-benefit ratio is questionable. Considering all such factors and after observing results from all these assays, it can be inferred that increase in concentrations, exhibited beneficial effects of *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract. This study proved that *Solanum melongena* fruit is a good choice of addition in food general population, due to its high fiber, minerals, phenolic compounds, saponins, ascorbic acid, tyrosine, and phenolic acids, additionally showing antioxidant activity by in-vitro assays. However, on close literature survey, none of study was identified that has truly isolated any particular chemical constituent and individually assessed its beneficial effects (reduce oxidative stress and hepatoprotective effects) in conjunction to each other both in humans as well as *in-vivo*. These questions may be answered by *in-vivo* experiments on animals with models that mimic the damages caused in humans.

CONCLUSION:

The *Solanum melongena* purple coloured fruit peel hydro-alcoholic extract was observed for noticeable antioxidant potential. The fruits of *Solanum melongena* have been reported for higher level of polyphenols, and the observer antioxidant activity can be attribute to the same. However, the same may require further studies for concise conclusions.

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