A novel approach and in-vitro evaluation of bioactive components for the development of nutraceuticals

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The nutritional ability of various bioactive components from Solanum melongena pulp (SMP) and Musa paradisiaca pseudo stem pulp (MSP) samples were investigated. The objective of the present investigation was the evaluation and determination of the nutraceutical properties of pulp extracts obtained from these plants. The antimicrobial activity was evaluated by the agar well diffusion method. The total phenolic contents were determined by the Folin-Ciocalteu colorimetric method. The highest total phenolic content was observed in S. melongena pulp extract (459.72 ± 6.3 mg GAE/g of sample on dry weight basis). The highest flavonoid content was observed in SMP (36.5 ± 6.7 mg QE/g of sample on dry weight basis). The antioxidant activity was evaluated by H₂O₂ radical scavenging methods. S. melongena showed the best antioxidant activity of 92 ± 0.1%. Among these two extracts the methonolic extracts of S. melongena had the best antimicrobial activity against Staphylococcus aureus. The extracts of both S. melongena and M. paradisiaca pseudo stem pulp showed best antimicrobial activity against Candida albicans. The high content of ascorbic acid was observed with M. paradisiaca pseudo stem pulp extract (35.6 ± 2.5 mg/g dry weight of the sample). Maximum catalase activity was found in M. paradisiaca pseudo stem pulp extract (3.099 ± 0.3 Units/mg of protein). The tocopherol content was also investigated. The formulated extracts of these plants may be given as the best nutritional health supplements in human diet without raising any controversial adverse effects.

Key words: Solanum melongena, Musa paradisiaca pseudo stem, antioxidant, ascorbic acid and antimicrobial activity.

INTRODUCTION

Plant products have fulfilled most of the human dietary requirements and also, they act as a protector against many diseases by means of its medicinal activity (Sonia et al., 2009). For example, the plant products such as neem, garlic and green vegetables provide us a vast source of fat-less, low calorie diet which is essential to lead a healthy life (Fatouma et al., 2010). Herbs are considered as medicinal plants and taken only for their medicinal or aromatic properties. It is estimated by the world health organization that approximately 75 to 80% of the world’s population uses plant derived medicines either in part or entirely. Growing number of American health care consumers are turning onto plant-originated medicines for reasons like, low cost, all time availability (Antonio et al., 2012) and seeking natural alternatives with fewer side effects.

The role of antioxidants is to detoxify the reactive oxygen intermediates (ROI) within the body (Edziri et al., 2012; Nisha et al., 2009). Over the past several years, antioxidants have attracted considerable interest in the
potential treatment for a wide variety of diseases including cancer (Masuda-Hollman et al., 1999), atherosclerosis, chronic inflammatory disease and aging (Paola Bontempo et al., 2013) and there is an increasing need to acquire them from our diet. In an attempt to study the antioxidant activity of vegetables, Solanum melongena (brinjal) and pseudo stem of Musa paradisiaca (banana) were selected (Cordeiro et al., 2004). The agricultural activity generates a large amount of residues because each plant produces only one bunch of bananas and after its harvesting, the bare pseudo stems are cut and usually left in the soil plantation to be used as organic material. These crops could and should find more rational way of utilization. These medicinal plants contain large amounts of antioxidants such as polyphenols (Djeridane et al., 2006) which can play an important role in adsorbing neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The extracts of both the plant pulps were used to study the antioxidant activity. The deep-purple skin gets its rich color and anti-aging power from nasunin like compounds which are present in S. melongena (Ma et al., 2012; Nadia, 2011).

Nasunin is high on the list of potent antioxidants. In this capacity, it helps to neutralize free radicals, making it an excellent anti-aging phytonutrient. Nasunin has many additional health benefits. It has antiangiogenic properties which help it to fight cancer by restricting the growth of new blood vessels (Salem et al., 2013). It also helps keep blood vessels clear and relaxed. It helps to fight the spread of cancerous cells by cutting off the blood supply they need to multiply (Matsubara et al., 2005). In this study, we have investigated the nutraceutical contents of the pulps from these two plants. Antimicrobial activities of these samples against a group of selected microorganisms were also investigated.

MATERIALS AND METHODS

Collection of samples

Fresh purple coloured moderate size Indian variety of S. melongena and the Indian variety of M. paradisiaca pseudo stem were purchased from a super market (Reliance fresh) in Chennai, Tamilnadu, India and prior to this study, these varieties were identified and authenticated by experts at Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India. The pulp of S. melongena and pseudo stem of M. paradisiaca was utilized for all the investigation.

Extraction procedure

About 200 g of S. melongena and pseudo stem of M. paradisiaca pseudo stems were cleaned and cut into pieces and ground by using mixer grinder (Preethi Chefro Model, Indian make 2013). Then the pulp was dried in a laboratory fluidized bed drier (Sherwood Model 501, England) at 45°C for 6 h (Lila et al., 2013). The dried powder sample was stored at 4°C for different studies. 50 g of S. melongena and pseudo stem of M. paradisiaca samples were extracted with methanol using Soxhlet apparatus at 55°C for 6 h. The extracts were filtered by Whatman filter paper No.1 and concentrated at 45°C using a rotary evaporator (Stuart RE 300 model, UK make 2012) and resultant residues were collected. Finally the samples were dissolved in methanol (1:10) for radical scavenging, total phenolic content, catalase assays and antimicrobial activity.

Hydroxyl radical scavenging

The reaction mixture contained 0.1 ml of deoxyribose, 0.1 ml of FeCl3, 0.1 ml of ethylenediaminetetraacetic acid (EDTA), 0.1 ml of H2O2, 0.1 ml of ascorbate, 400 µl of sample extracts in a final volume of 1 ml made up with KH2PO4-KOH buffer. The mixture was incubated at 37°C for 1 h. At the end of the incubation, 1 ml of 1% thiobarbituric acid (TBA) was added and heated at 95°C for 20 min to develop the color. After cooling, the thiobarbituric acid reactive substances (TBARS) formation was measured spectrophotometrically at 535 nm against an appropriate blank. The hydroxyl radical scavenging activity was determined by comparing the absorbance of the control with that of the sample (Aline et al., 2005). The percent TBARS production for positive control H2O2 was fixed at 100% and the relative percent TBARS was calculated for the extract treated groups (Marappan and Leela, 2007).

Estimation of ascorbic acid

Ascorbic acid (AA) content was determined using the method described by Omaye et al. (1979). The extract was prepared by grinding 20 g of fresh S. melongena skin pulp (SMP) and M. paradisiaca pseudo stem with 100 ml of 10% TCA. Then the mixture was centrifuged at 3500 rpm for 20 min and re-extracted twice. 0.5 ml of supernatant (clear solution without debris) was taken along with 1 ml of 2,4-dinitrophenyl hydrazine-thiourea-CuSO4 reagent (DTC reagent). Then the mixture was incubated at 37°C for 3 h and 0.75 ml of ice-cold 65% H2SO4 was added, allowed to stand at 30°C for 30 min and resulting color was read at 520 nm in spectrophotometer. The AA content was determined using a standard curve prepared with AA and the results were expressed in milligram per gram dry weight (DW) (Koh et al., 2009).

Estimation of total phenolic content (TPC)

Total phenolic content was determined with the Folin-Ciocalteu reagent using the method of (Lister et al., 2001). 100 µl of sample was dissolved in 500 µl (1:10 dilution) of Folin-Ciocalteu reagent and 1000 µl of distilled water. The solutions were mixed and incubated at room temperature (37°C) for 1 min. After 1 min, 1500 µl of 20% sodium carbonate (Na2CO3) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature (37°C). The absorbance of samples was measured at 760 nm using a Milton Roy 601 UV-Vis spectrophotometer and the results are expressed in mg of gallic acid per g (GEA) of dry weight of plant (Ephraim and Masaharu, 1997). A standard curve was plotted using gallic acid as standard. Different concentrations of gallic acid were prepared in 80% of methanol and their absorbances were recorded at 765 nm.

Estimation of tocopherol

Tocopherol content was assayed as described by Becker et al. (1980). Five hundred milligrams of sample was homogenized with 10 ml of a mixture containing petroleum ether and ethanol (2:1.6, v/v) and the extract was centrifuged at 10,000 rpm for 20 min then
the supernatant was used for estimation of tocopherol. To the 1 ml of extract, 0.2 ml of 2% 2,2-dipryridyl in ethanol was added and mixed thoroughly and kept in dark for 5 min. The resulting mixture was diluted with 4 ml of distilled water and mixed well. The resulting color in the aqueous layer was measured at 520 nm. The tocopherol content was calculated using a standard graph made with known amount of tocopherol (Filipa et al., 2011).

**Estimation of flavonoids**

The total flavonoid content was determined by aluminium chloride colorimetric method described by Chang et al. (2002). 0.5 ml of extract was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10% aluminium chloride hexahydrate (AlCl₃), 0.1 ml of 1 M potassium acetate (CH₃COOK) and 2.8 ml of deionized water. After incubation at room temperature (37°C) for 40 min, the absorbance of reaction mixture was measured at 415 nm against a deionized water blank on a spectrophotometer (Jenway 6305 model, UK make 2011). Quercetin was chosen as a standard. Using the seven point standard curve (0 to 50 mg/ml), the levels of total flavonoid contents in the sample were determined in triplicate, respectively. The data were expressed as milligram quercetin equivalents (QE)/g of DW.

**Estimation of catalase**

The activity of CAT was measured according to the method of Toshiki et al. (1995) with small modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH: 7.0), 0.4 ml of 15 Mm H₂O₂ and 0.04 ml of sample extract. The decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U mg⁻¹ protein (U = 1 mM of H₂O₂ reduction min⁻¹ mg⁻¹ protein). For all the enzymatic calculations protein was determined by the Bradford, using bovine serum albumin (BSA, Sigma, USA) as the standard.

**Antimicrobial activity of the samples**

The extracts were tested for antimicrobial activity against selected microorganisms such as *Bacillus subtilis* (MTCC 2391), *Staphylococcus aureus* (MTCC 9542), *Escherichia coli* (MTCC1677), *Candida albicans* (MTCC 7315) and *Mycobacterium smegmatis* (MTCC 991) microorganism using agar-well diffusion method (Igbinosa et al., 2009; Nabila et al., 2008). 20 ml of nutrient agar (Hi Media Pvt LTD) was poured into the Petri-dish and 8 mm well bored in the agar. 1 mg and 100 µg of each extract and pure compounds, respectively were dissolved in 200 µl of dimethylsulfoxide (DMSO) and poured into the wells. The plants were incubated for 24 h at 37°C and the zone of inhibition was measured in mm. DMSO was used as negative control, while Chloramphenicol was used as a positive control (Christian et al., 2005).  

**RESULTS AND DISCUSSION**

**Hydroxyl radical scavenging**

The present work was an attempt to study the phytochemical constituents present in fresh *S. melongena* pulp and *M. paradisiaca* pseudo stem pulp and to identify the various constituents present in their pulp extracts. Various solvents were used for extracting the samples. The antioxidant constituents present in the samples include ascorbic acid, phenolic compounds, catalase, tocopherol and flavonoids (Lister et al., 2001). Each of these has been estimated individually followed by a total hydroxy radical scavenging activity assay. Hydroxyl radicals are known to be the most reactive of all reduced forms of dioxygen and are thought to initiate cell damage *in vivo*. The effect of the extract on hydroxyl radicals generated by Fe³⁺ ions were measured by determining the degree of deoxyribose degradation, an indicator of TBA-MDA adduct formation. As shown in the Figure 1, the purified extract exhibited a dose dependent inhibition of the hydroxyl radical scavenging activity. A maximum of more than 90% inhibition was obtained at 400 µmol of SMP extracts. The inhibition was statistically significant when compared to samples without any inhibitor. The scavenging potential was compared with known antioxidants such as curcumin and butylated hydroxyanisole (BHA). Both extracts inhibited lipid peroxidation by 92 and 86% at 400 µl concentrations, respectively. Among the various concentrations tested, the extracts showed maximum hydroxyl radical scavenging activity at 800 µmoles, which was a much lower concentration than those of standard antioxidants such as curcumin and BHA. *S. Melongena* showed higher hydroxyl radical scavenging effect of 92 ± 0.1% when compared to the *M. paradisiaca* pseudo pulp extract. *M. paradisiaca* pseudo stem showed moderate inhibition of free radicals (86 ± 1.4 %) (Figure 1).

**Estimation of ascorbic acid**

Among the various samples the ascorbic acid content of *Musa* pseudo stem pulp was recorded higher when compared to other extracts (Table 1). This antioxidant contents play major roles in maintaining the balance between free radical production and elimination. *M. paradisiaca* pseudo stem sample was found to contain 48.7 ± 3.1 mg/g of dw which was higher than *S. melongena* sample (35.6 ± 2.5 mg/g of dw).

**Estimation of total phenolic content**

It has been recognized that the total phenolic compounds and flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. Highest total phenolic content was observed in the extract of *S. melongena* pulp was 459.72 ± 6.3 mg GAE/g dry weight when it was compared to *M. paradisiaca* pseudo stem pulp extract (290.91 ± 5.4 mg GAE/g dry weight) (Table 1).

**Estimation of tocopherol**

The standard reading was used to analyze the sample readings. From Table 1, it can be seen that *S. melongena* pulp extracts had 23.5 ± 3.6 mg/g of dw
tocopherol (Vitamin E) compared to *M. paradisiaca* pseudo stem pulp (12.7 ± 4.6 mg/g of dw).

**Estimation of flavonoids**

Based on this study (Margaret et al., 2009), we proposed that the potent free radical-scavenging and antioxidant activity of medicinal plant might result from its high contents of flavonoid type compounds. Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants (Nabila et al., 2008). The results are shown in Table 1. All the tested samples had high flavonoid content between the two. *S. melongena* pulp showed maximum flavonoid content (36.5 ± 6.7 mg QE/g dw) when it compared to *M. paradisiaca* pseudo stem pulp (26.5 ± 4.6 QE/g dw).

**Estimation of catalase**

The higher activity of catalase enzyme in *M. paradisiaca* pseudo stem sample was recorded (3.099 ± 0.3 U/mg of protein). The level of antioxidative response depends on the species, the development and the metabolic state of the plant, as well as the duration and intensity of the stress. CAT scavenges $H_2O_2$. The involvement of the antioxidative system in the regulation of free-radical metabolism was followed by measuring changes in the antioxidant enzyme activities when plant sample experiences stress from an abiotic factor, there should be an enhancement in the production of toxic free radicals of $H_2O_2$, $O_2^-$, $\cdot O_2$ or $\cdot OH$, which should be detoxified in terms of increased antioxidant enzyme activities (Table 2).

**Antimicrobial activity of samples**

The extracts of fresh *S. melongena* skin pulp and *M. paradisiaca* pseudo stem pulp were effective in inhibiting the growth of the tested strains. Both two extracts showed antifungal activity against *C. albicans*. Among the two extracts, *S. melongena* pulp extract has shown highest antibacterial activity against *S. aureus*. The observed antibacterial and antifungal activities of the extracts might also be attributed to the high quantity of poly phenols which are known to possess antimicrobial activity (Table 3).

**Conclusion**

Antioxidant and antimicrobial activities could be derived from compounds such as flavonoids, polyphenols and ascorbic acids. By coupling the results

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**Table 1.** Total Ascorbic acid (mg/gm DW), phenolic content (GAE/g DW), flavonoids (QE/g DW) and tocopherol content (mg/gm DW) of the extracts.

<table>
<thead>
<tr>
<th>Sample extracts</th>
<th>Ascorbic acid (mg/g dw)</th>
<th>Total phenolic content (mg GAE/g dw)</th>
<th>Total flavonoids (mg QE/g dw)</th>
<th>Tocopherol content (mg/gdw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solanum melongena pulp</td>
<td>35.6 ± 2.5</td>
<td>459.72 ± 6.3</td>
<td>36.5 ± 6.7</td>
<td>23.5 ± 3.6</td>
</tr>
<tr>
<td>Musa paradisiaca stem pulp</td>
<td>48.7 ± 3.1</td>
<td>290.91 ±5.4</td>
<td>26.5 ± 4.6</td>
<td>12.7 ± 4.6</td>
</tr>
</tbody>
</table>

![Graph showing the effect of hydroxyl radical scavenging by deoxyribose method.](image-url)
of different assays for evaluating antioxidant activity with H$_2$O$_2$ assay. Among these two extracts, *S. melongena* had the highest antimicrobial activity (Table 3). These results showed that they possess numerous nutraceutical properties as well. The present study evaluated and determined that the *in vitro* antioxidant and antimicrobial activities in terms of total phenolic content, flavonoids and H$_2$O$_2$ radical scavenging activity with the zone of inhibition. The extracts of *S. melongena* and *M. paradisiaca* pseudo stem pulp stem could be a significant source of important nutraceutical compounds for human diet without raising any side effects.

Concerning the antioxidant activity, high reducing power and inhibition of H$_2$O$_2$ activities of phenolic extract was observed. The present study confirm that the extracts of *S. melongena* and *M. paradisiaca* pseudo stem pulp stem could be a significant source of natural antioxidant with highest phenolic content that may have potent beneficial health effects. The formulated extracts of these *S. melongena* and *M. paradisiaca* pseudo stem pulp may be considered as a source of important nutraceutical compounds for human diet without raising any side effects.

**REFERENCES**


Table 2. Total catalase activity of the sample.

<table>
<thead>
<tr>
<th>Sample extracts (ml)</th>
<th>Total protein (mg)</th>
<th>Total catalase activity (Units)</th>
<th>Specific activity (Units/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Solanum melongena</em></td>
<td>5680</td>
<td>11700</td>
<td>2.06 ± 0.1</td>
</tr>
<tr>
<td><em>Musa pseudo</em> stemPulp</td>
<td>5028</td>
<td>15580</td>
<td>3.099 ± 0.3</td>
</tr>
</tbody>
</table>

Table 3. Results of antimicrobial activity of the extracts.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th><em>Solanum</em> extract</th>
<th><em>Musa pseudo</em> pulp</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Staphyloccocus aureus</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>Mycobacterium smegmatis</em></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Chloramphenanical was used as positive control at a concentration of 3 µg/mL. DMSO was used as negative control. + The size of inhibition is > 10 mm in diameter; - The size of inhibition is < 10 mm in diameter.