IN VITRO ANTIOXIDANT ACTIVITY AND HPTLC ANALYSIS OF ROOT OF PANDANUS ODORATISSIMUS L.

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ABSTRACT

The aim of the present study was to screen extracts of Pandanus odoratissimus L. root with respect to the contents of phenolics and flavonoids and their in vitro antioxidant activities to find new sources of antioxidants. Total phenolics and total flavonoids of P. odoratissimus were measured by Folin- Gocalte reagent and aluminium chloride methods respectively. HPTLC analysis was performed in methanol extract to examine the polyphenols. Antioxidant property of the extracts was determined using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical quenching assay and reductive capacity assay. The methanol root extract yielded 56.24 ± 0.02 mg/g GAE of phenolic content and 81.25 ± 0.046 mg/g QE of flavonoid content. Higher antioxidant potential was observed in both DPPH scavenging assay (EC50 = 48.3 ± 0.002 µg mL−1) and reducing capacity (OD at 1000 µg mL−1 = 0.797) by the methanolic root extract than in the aqueous extract. A positive correlation was found between phenolics and flavonoid contents and antioxidant properties of the extracts. These findings indicate that polyphenolic components may have antioxidant potential, as reported in other medicinal plants. The root of P. odoratissimus might be useful in the development of raw materials of medicine.

Keywords: Antioxidant activity, flavonoids, HPTLC, phenolics, Pandanus odoratissimus L.

INTRODUCTION

Reactive oxygen species (ROS) mediated oxidative stress is mainly involved in the pathogenesis of several chronic ailments. Antioxidants from natural sources play a paramount role in helping endogenous antioxidants to neutralize oxidative stress. Several epidemiological, clinical and experimental data suggested that plant based antioxidants have beneficial effects on prevention on chronic diseases. As a result, there has been a keen interest in evaluating the role bioactive constituents from medicinal plants in reducing the risk of the aforesaid diseases. Recent studies have demonstrated the antioxidant activities and health benefits of the phenolic compounds occurring in plants. In the view of the aforesaid, in the present study, the contents of phenolics, flavonoids and antioxidant activities of methanol and aqueous extracts of root of P. odoratissimus were assessed using established assay procedures. We have used DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical quenching and reducing capacity assays to determine the antioxidant activities.

Pandanus odoratissimus L., a shrub, belongs to the family Pandanaceae. The root is used to treat skin diseases, ulcers, dyspepsia, diabetes, fever and leprosy. Root juice is administrated for ‘Prameha’, i.e. extreme heat constitution. Four strong antioxidative (thioscyanate method) lignan type compounds and a new benzofuran derivative activities were isolated from the root parts of P. odoratissimus. The methanolic leaf extract of P. odoratissimus were evaluated for its free radical scavenging activities by DPPH, super oxide, nitric oxide and hydroxyl scavenging assays. Our present work encompasses chemical profile and antioxidant activity to authenticate the medicinal properties of P. odoratissimus root.

MATERIALS AND METHODS

Preparation of extract

Root parts of P. odoratissimus were collected from Kerala State, India. The taxonomic identification was done by Dr. R. Gopalan, Karpagam University. A voucher specimen has been deposited in Karpagam University, Coimbatore. The plant part collected were shade dried and then pulverized. The powder (100 g) was exhaustively extracted with methanol in the ratio of 1:5 (w/v) for 24 h using soxhlet apparatus. The extract was completely evaporated to dryness using rotary flash evaporator (Buchi type). Different concentrations of extracts were prepared from the resultant crude methanol and aqueous extracts to determine in vitro antioxidant activity.

Chemicals

All chemicals used including the solvents, were of analytical grade. 1, 1-diphenyl-2-picryl hydrazyl (DPPH), butylated hydroxyl toluene (BHT) and butylated hydroxy anisole (BHA) were purchased from Merck Co (Mumbai, India). Folin ciocalteu reagent, gallic acid, trichlorocetic acid (TCA), ferric chloride and ascorbic acid were purchased from SD fine Chemicals.

Estimation of total phenolics

Total phenolic content of root was measured based on Folin - Ciocucleau assay. Briefly, 1.2 ml of sodium carbonate (7.5% w/v) was added to the 5 gm of methanolic extract of root. After 30 min, absorbance was measured at 765 nm with UV/Vis spectrophotometer (Elico, India). Total phenolic content was expressed as mg gallic acid equivalents (GAE)/g fresh weight.

Estimation of total flavonoids

The content of total flavonoid in P. odoratissimus root extract was estimated based on the method previously described. A volume of 0.5 ml of 2% AlCl3 ethanol solution was added to 0.5 ml of sample solution. After one hour at room temperature, the absorbance was measured at 420 nm with UV-Visible spectrophotometer (Elico, India) Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content were calculated as quercetin equivalent (mg QE/g) fresh weight.

HPTLC analysis for polyphenols

A densitometric HPTLC analysis was performed for the development of characteristic finger printing profile. The P. odoratissimus methanolic extract of root was dissolved with HPLC grade methanol 100 mg/0.5 ml. The solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis. Then, 2 µl of the samples were loaded as 7 mm band length in the 10 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINO MAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapor) with respective mobile phase (polyphenolic compound) and the plate was developed in the respective mobile phase (Toluene-Acetone-Formic acid 4.5:4.5:1) up to 90 mm. The developed plate was dried using hot air to evaporate solvents from the plate and sprayed with stannic chloride reagent. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at UV366 nm. Finally, the plate was fixed in scanner stage and scanned at 254 nm. The Peak table, Peak display and Peak densitogram was identified.
Determination of antioxidant activity

DPPH radical quenching activity

DPPH radical scavenging activity was determined by a standard method. Briefly, 5 ml of methanol solution of DPPH (0.1 mM) was added to 1 ml of the sample solutions at different concentrations (1000-25 μg mL⁻¹) and vortexed. The mixtures were allowed to stand at room temperature for 20 minutes. Changes in the absorbance of the samples were measured at 517 nm. The scavenging activity of the extract was expressed as 50% effective concentration (EC₅₀). BHT and ascorbic acid were used as reference compounds. The inhibition percentage was calculated using the following formula:

\[
\text{DPPH scavenging activity (\%) = 1 - \frac{\text{Absorbance sample}}{\text{Absorbance control}} \times 100.}
\]

Butyl hydroxy anisole (BHA), butylated hydroxyl toluene (BHT) and ascorbic acid were taken as reference standards.

Reducing capacity

The reducing power of the methanol and aqueous extracts was assayed as described by Oyaizu. Different volumes of the test samples were mixed with phosphate buffer (5 ml, 0.2 M, pH = 6.6) and 5 ml of 1% potassium ferric cyanide solution [K₃Fe(CN)₆]. The mixtures were incubated in a water bath at 50°C for 20 minutes. After the incubation period, 5 ml of 10% trichloro acetic acid was added to the mixture and the contents were centrifuged at 1000 rpm for 10 minutes. 5 ml aliquots were mixed with 5 ml of distilled water and 1 ml ferric chloride (0.1%) and the absorbency of the reaction mixtures was measured spectrophotometrically at 700 nm after reaction for 10 min. The higher the absorbency represents the stronger the reducing power. Butyl Hydroxy Anisole (BHA) and Butyl Hydroxy Toluene (BHT) were taken as reference standards.

RESULTS AND DISCUSSION

The identification and investigation on antioxidants from medicinal plants is a fast expanding field of research and several antioxidants have been investigated such as flavonoids and other phenolic compounds. In the view of the upsurging interest in the health benefits of the medicinal plants, we examined total phenolics and flavonoids and evaluated the antioxidant effects of crude methanolic and aqueous extracts of root of P. odoratissimus. In the present study, total phenolics was examined using gallic acid as standard (R² = 0.9968) and total flavonoids using quercetin as standard (R² = 0.9965) and the contents of methanol and aqueous extracts of P. odoratissimus root and the results are reported. The content of total phenolics and flavonoids in methanol extract of P. odoratissimus root amounted to 56.24 ± 0.02 mg/g GAE and 81.25 ± 0.046 mg/g CE respectively which is higher than that of the aqueous extract.

HPTLC profile

HPTLC profile of methanol extract of P. odoratissimus root was recorded in table 2. Blue, brown color zone was detected in UV after derivatization in the chromatogram. (Fig. 1 & 2.) confirms the presence of polyphenols. The extracts were run along with the standard polyphenols compounds. The root extract which shows the presence of polyphenols in the chromatogram as well as in UV after derivatization. The Rf value of the root extract was found to be 0.24, 0.3, 0.52, 0.58, and 0.7 of peak 1, 2, 3, 4, 5 respectively. Among them peaks 3, 4, 5 were found as polyphenols. The peak height of the respective polyphenols was also given in the table 1.
The reductive capability of the methanol extract was also higher when compared with the reference standard Butyl hydroxyl toluene (0.683 ± 0.008 at 1000 µg mL⁻¹). The reducing power of the extract might be due to the di and mono hydroxyl substitutions in the aromatic ring which possess potent hydrogen donating abilities. The result revealed that the methanolic extract of *P. odoratissimus* possess a tendency to donate electrons and could react with free radicals to convert them into more stable products and to terminate radical chain reactions.

**Table 1: Shows peak table with Rf values, height and area of polyphenols and unknown compounds in methanol extract of *P. odoratissimus* root**

<table>
<thead>
<tr>
<th>Track</th>
<th>Peak</th>
<th>Rf</th>
<th>Height</th>
<th>Area</th>
<th>Assigned Substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1</td>
<td>0.24</td>
<td>86.8</td>
<td>3242</td>
<td>unknown</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>0.3</td>
<td>91.9</td>
<td>3010.5</td>
<td>unknown</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
<td>0.52</td>
<td>178.9</td>
<td>5266.6</td>
<td>Polyphenol</td>
</tr>
<tr>
<td>C</td>
<td>4</td>
<td>0.58</td>
<td>167.4</td>
<td>3998.1</td>
<td>Polyphenol</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>0.7</td>
<td>318.1</td>
<td>12169</td>
<td>Polyphenol</td>
</tr>
</tbody>
</table>

**DPPH scavenging activity**

The DPPH assay constitutes a quick and low cost method, which has frequently been used for the evaluation of the antioxidative potential of various natural products. As illustrated in table 2, the methanol extract from root of *P. odoratissimus* exhibited a dose dependent radical quenching activity with an EC₅₀ value of 48.3 ± 0.002 µg mL⁻¹. It is higher than the aqueous extract (>1000 µg mL⁻¹) and also higher than that of BHA (EC₅₀ = 53.27 ± 0.005 µg mL⁻¹). However, the activity was lesser than that of BHT (26.22 ± 0.005 µg mL⁻¹) and L-ascorbic acid (11.24 ± 0.022 µg mL⁻¹). The results of this study infer that the methanol extract reduces the radical to the corresponding hydrazine when it reacts with the hydrogen ions released from the samples which contain antioxidant principles.

**Reducing power**

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reduction of ferrous ion (Fe²⁺) to ferric ion (Fe³⁺) was measured by the intensity of the resultant Prussian blue colour complex which absorbs at 700 nm. As it can be seen in Table 2 the extracts caused significant elevation of reducing power. The reducing power of methanol extract (0.787 ± 0.042 at 1000 µg mL⁻¹) was relatively more pronounced than that of aqueous extract (0.324 ± 0.008 at 1000 µg mL⁻¹). The higher absorbance at high concentration indicates the strong reducing power potential. The reductive capability of the methanol extract was also higher when compared with the reference standard Butyl hydroxyl toluene (0.683 ± 0.008 at 1000 µg mL⁻¹). The reducing power of the extract might be due to the di and mono hydroxyl substitutions in the aromatic ring which possess potent hydrogen donating abilities. The result revealed that the methanolic extract of *P. odoratissimus* possess a tendency to donate electrons and could react with free radicals to convert them into more stable products and to terminate radical chain reactions.

**Table 2: Shows the effects of methanol and aqueous extracts of *P. odoratissimus* root on DPPH radical and Fe²⁺ reduction**

<table>
<thead>
<tr>
<th>Concentration (µg mL⁻¹)</th>
<th>Inhibition of DPPH (%)</th>
<th>Fe²⁺ reduction Absorbance (700 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>ME AE</td>
<td>ME AE</td>
</tr>
<tr>
<td>500</td>
<td>90.3 47.86 0.787 ± 0.042</td>
<td>324 0.324 ± 0.008</td>
</tr>
<tr>
<td>125</td>
<td>59.2 35.7 0.387 ± 0.002</td>
<td>166 0.166 ± 0.044</td>
</tr>
<tr>
<td>50</td>
<td>52.2 28.9 0.243 ± 0.002</td>
<td>109 0.093 ± 0.022</td>
</tr>
<tr>
<td>25</td>
<td>46.1 23.4 0.182 ± 0.042</td>
<td>ND - Not determined.</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>48.3 ± 0.002</td>
<td></td>
</tr>
<tr>
<td>BHA (IC₅₀)</td>
<td>53.27 ±0.727</td>
<td>0.683 ± 0.008</td>
</tr>
<tr>
<td>BHT (IC₅₀)</td>
<td>26.22 ±0.005</td>
<td>1.022 ± 0.022</td>
</tr>
<tr>
<td>AA (IC₅₀)</td>
<td>11.24 ±0.022</td>
<td>ND - Not determined.</td>
</tr>
</tbody>
</table>

**Multiple correlation analysis**

All the findings from the experiments were used to find an association between TP, TF and antioxidant activities based on multiple regression analysis (Table 3). The correlation matrix between DPPH radical scavenging activity (R² = 0.897) and TP was positive. A linear correlation was also observed between DPPH activity (R² = 0.988) and TF. Significant correlations were observed between DPPH radical scavenging and reducing power, TP and TF. Many supportive reports emphasize the positive correlation between phenolic content and antioxidant efficacy. A positive correlation between antioxidant activity and polyphenol content was found, suggesting that the antioxidant capacity of the plant extracts is due to a great extent to their polyphenols. High positive correlations between antioxidant properties evaluated by all the assays and TP and TF indicate that the polyphenolic compounds are important contributors of antioxidant potential of *P. odoratissimus* root extract.

**Table 3: Linear correlation coefficients among assays of *P. odoratissimus* root**

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>TF</th>
<th>DPPH</th>
<th>RC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF</td>
<td>0.947</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPPH</td>
<td>0.897</td>
<td>0.968</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>RC</td>
<td>0.994</td>
<td>0.959</td>
<td>0.965</td>
<td>1</td>
</tr>
</tbody>
</table>

TP - Total phenol content; TF - Total flavonoid content; DPPH - diphenyl picryl hydrazyl; RC - reducing capacity
CONCLUSION

Our study demonstrated that *P. odoratissimus* has high phenolics content and strong antioxidant activity. It would be interesting to investigate further its antioxidant activity in vivo. Recent years have seen an exponential increase in research antioxidant properties of medicinal plants. If it is accepted that higher intakes of natural antioxidants containing phenolics are associated with long term health benefits, then the results presented in this paper offer possible avenues toward health promotion by identifying those compounds. The health promoting properties of *P. odoratissimus* root may be due to its antioxidant properties and is also attributed to its multitherapeutic characteristics. Thus, *P. odoratissimus* might be useful in the development of raw materials of medicine. In vivo antioxidant study and toxicological tests on root of *P. odoratissimus* are warranted.

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REFERENCES