IN-VITRO ANTI-OXIDANT ACTIVITY OF DESMODIUM OOJEINENSE
(ROXB.) H. OHASHI

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Summary

The present study was aimed at evaluating the in vitro anti-oxidant activity of ethanolic extract of the stem bark of Desmodium oojeinense by various methods namely Total Reducing power, Nitric oxide radical scavenging activity and 1,1-diphenyl 2 picryl hydrazyl(DPPH) radical scavenging activity. The extract showed significant percentage of inhibition in dose dependent manner compared to standard antioxidant sodium metabisulphate. Our findings provide evidence that the crude ethanolic extract of stem bark of Desmodium oojeinense is a potential source of natural antioxidant, and this justified its uses in folkloric medicines.

Key words: Desmodium oojeinense, Anti-oxidant, DPPH Radical scavenging, Reducing power, nitric oxide scavenging.

Introduction

Humans are impacted by many free radicals both from inside our body and surrounding environments, particularly reactive oxygen species (ROS) is generated in living organisms during metabolism[1]. It is produced in the forms of superoxide anion (O₂⁻), hydroxyl radical (OH), hydrogen peroxide (H₂O₂) and nitric oxide (NO). In addition, oxidative stress may cause inadvertent enzyme activation and oxidative damage to cellular systems[2, 3]. The negative effects of oxidative stress may be mitigated by antioxidants [4, 5].

Antioxidant activity has become a hot topic and the subject of intensive investigations due to the ever increasing demand by the food and pharmaceutical industries to develop natural bioactive anti-aging and anticarcinogenic compounds that demonstrate measurable health benefits.
Antioxidative substances obtained from natural sources, such as seed oil, grains, beans, vegetables, fruits, leaf waxes, bark, roots, spices and hulls have already been investigated [6-8].

The discovery of medicinal drugs from natural products is not new. Some of the best drugs we have today are from natural products. Herbal or natural products are becoming popular which can be attributed to the belief that they are safe.

Desmodium Oojeinense (Roxb.), belonging to the family Fabaceae. It is found distributed in the sub and outer Himalayan valleys and slopes up to altitude of 5000 ft, from Punjab to Bhutan, Chota Nagpur, Central India, Orissa, Bombay, Marvar of Rajputan, forest of Ganjam, and Vizag.

The bark is acrid and hot, anthelmintic, astringent to the bowels, cures “kapha” and “vata”, dysentery, leucoderma, urinary discharges, ulcers, blood diseases, skin diseases, burning sensations and anemia (Ayurveda). In the central prominence the bark is used as a febrifuge. The bark when incised furnishes a Kino-like exudation, which is used in cases of dysentery and diarrhoea[9].

Based on ancient practices and traditional uses of this plant, an effort has been made to establish the anti-oxidant activity of ethanolic extract of stem bark of D.oojeinense.

Materials and Methods

Collection of plant material

Bark was collected from the medicinal garden of Sri.Ragavendra Ayurvedic Medical College Malladihalli, Karnataka, India .The plant was authenticated by Prof. Gopal Krishna Bhatt, Department of Botany, Poornapragna College, Udupi, Karnataka, India. A voucher specimen(No.106a) was deposited in NGSM Institute of Pharmaceutical Sciences, Paneer, Mangalore-5, Karnataka.

Preparation of the ethanolic extract

The dried powder material of stem bark of D.oojeinense was extracted with ethanol(95%) for 72 hours in a soxhlet extractor. The process was repeated for six times. The solvent from the total extract was distilled off and the concentrate was evaporated on a water bath to a syrupy consistency and then evaporated to dryness.

Preliminary phytochemical screening:

The ethanolic extract of stem bark of D.Oojeinense was subjected to systematic qualitative analysis for the identification of various plant phytoconstituents.

Evaluation of antioxidant activity:

1. Reducing power:

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this method antioxidant compound forms a colored complex with potassium ferricyanide,
trichloro acetic acid and ferric chloride, which is measured at 700nm. Increase in absorbance of the reaction mixture indicates the reducing power of the Samples. The reducing power of ethanolic extract of stem bark of \textit{D.Oojeinense} was determined according to the method of Oyaizu[10].

Different doses of ethanolic extract of stem bark of \textit{D.ojojeinense} were mixed in 1 ml of distilled water so as to get 30µg, 60µg, 90µg, 120µg and 150µg, concentration. This was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes if precipitate occurs. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl$_3$ (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700nm.

Increased absorbance of the reaction mixture indicates increase in reducing power. The % reducing power was calculated by using following formula.

\[
\frac{\text{Test OD} - \text{Control OD}}{\text{Control OD}} \times 100
\]

The results are compiled in Table 2 and graphically shown in Figure 1


Nitric oxide (NO) radical were generated from sodium nitroprusside solution at physiological pH. Sodium nitroprusside (1ml of 10mM) were mixed with 1ml of ethanolic extract of stem bark of \textit{D.ojojeinense} different concentration (25-150 µg/ml) in phosphate buffer (pH 7.4). The mixture was incubated at 25°C for 150 min. To 1 ml of the incubated solution, 1ml of Griess’s reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546 nm. % inhibition of OD was calculated by using the formula.

\[
\frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100
\]

The results are compiled in the Table 3 and graphically shown in Figure 2

3. Estimation of 1, 1-Diphenyl 2-Picryl Hydrazyl (DPPH) radical scavenging activity:

DPPH free radical scavenging activity[12-14]:
Free scavenging activity was measured by a decrease in absorbance at 516 nm of a methanol solution of colored DPPH brought about by the sample. A stock solution of DPPH (1.3 mg/ml in methanol) was prepared such that 75 µl of it in 3 ml methanol gave an initial absorbance of 0.9. Decrease in the absorbance in the presence of ethanolic extract at different concentrations was noted after 15 min. EC$_{50}$ (i.e. the concentration of the test solution required to give a 50% decrease in the absorbance compared to that of blank solution) was calculated from percent inhibition. A blank reading was obtained using methanol instead of the extract. Ascorbic acid was used as standard. The percentage inhibition of antiradical activity was calculated using the formula,

\[
\text{Absorbance of blank} - \text{Absorbance of test sample} \times 100
\]

The results are complied in the Table 4 and graphically shown in Figure 3.

**Results and discussion**

**Preliminary phytochemical screening:**

Qualitative test for phytoconstituents revealed the presence of alkaloids, carbohydrates, steroids, triterpenoids, flavonoids. Results are shown in Table 1.

**Table 1: Preliminary Phytochemical investigation**

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Phytoconstituents</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>+ve</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrates</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>Triterpenoids</td>
<td>+ve</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>+ve</td>
</tr>
</tbody>
</table>

**Reducing power activity :**

In Reducing power activity it is observed that ethanolic extract of stem bark of plant *D. oogeinense* have demonstrated concentration dependent increase in the reducing property. Sodium met bisulphate (std. 100 µg) has 545.07 % reducing property. The test extract showed concentration dependent increase reducing property. However, 150 mcg of ethanolic extract showed comparable reducing power i.e. 431.69%.
Table 2: Reducing power activity of ethanolic extract of stem bark of *D. oojeinense*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Absorbance Mean ± SEM</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.142±0.006</td>
<td>--</td>
</tr>
<tr>
<td>Control + standard 100 µg (Sodium metabisulphate)</td>
<td>0.916±0.008***</td>
<td>545.07</td>
</tr>
<tr>
<td>Control + Ethanol Extract 30 µg</td>
<td>0.151±0.008ns</td>
<td>6.34</td>
</tr>
<tr>
<td>Control + Ethanol Extract 60 µg</td>
<td>0.357±0.006***</td>
<td>151.41</td>
</tr>
<tr>
<td>Control + Ethanol Extract 90 µg</td>
<td>0.532±0.010***</td>
<td>274.65</td>
</tr>
<tr>
<td>Control + Ethanol Extract 120 µg</td>
<td>0.676±0.007***</td>
<td>376.06</td>
</tr>
<tr>
<td>Control + Ethanol Extract 150 µg</td>
<td>0.755±0.013***</td>
<td>431.69</td>
</tr>
</tbody>
</table>

**Figure 1: Reducing power activity of ethanolic extract of stem bark of *D. oojeinense.***
Nitric oxide scavenging activity:

In Nitric oxide anion scavenging activity it is observed that the ethanolic extract of stem bark of plant *D. oojeinense* have demonstrated concentration dependent inhibition. Whereas 25µg sodium meta bisulphate (std.25 µg) has 49.28 % nitric oxide radical scavenging activity. However, test extract even at 150µg showed lesser inhibition than standard in the antioxidant model. The results are summarized in Table 3 and graphically depicted in Figure 2.

**Table 3: Nitric oxide scavenging activity of ethanolic extract of stem bark of *D. oojeinense***

<table>
<thead>
<tr>
<th>Groups</th>
<th>Absorbance</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.487 ±0.032</td>
<td>--</td>
</tr>
<tr>
<td>Control + standard 25 µg</td>
<td>0.247±0.012***</td>
<td>49.28</td>
</tr>
<tr>
<td>Control + Ethanol extract 30 µg</td>
<td>0.349 ± 0.008***</td>
<td>28.34</td>
</tr>
<tr>
<td>Control + Ethanol extract 60 µg</td>
<td>0.345 ± 0.010***</td>
<td>29.16</td>
</tr>
<tr>
<td>Control + Ethanol extract 90 µg</td>
<td>0.342 ± 0.016***</td>
<td>29.77</td>
</tr>
<tr>
<td>Control + Ethanol Extract 120 µg</td>
<td>0.325 ± 0.020***</td>
<td>33.27</td>
</tr>
<tr>
<td>Control + Ethanol extract 150 µg</td>
<td>0.312 ± 0.007***</td>
<td>35.93</td>
</tr>
</tbody>
</table>

**Figure 2: Nitric oxide scavenging activity of ethanolic extract of stem bark of *D. oojeinense*.**
DPPH free radical scavenging activity:

In DPPH method, the free radical scavenging activity of ethanolic extract of stem bark of *D. oojeinense* is expressed in terms of percentage inhibition. The decrease in percentage of inhibition shows increased absorbance. The decrease in optical absorbance at 517 nm after addition of the test compounds is measured. The percentage of DPPH radical scavenged for ethanol extract maximum of 84.88%. The ethanolic extract exhibited a significant dose dependent inhibition of DPPH activity, with a 50% inhibition (IC 50) at a concentration of 50 µg. The results of extract are given in the Table 4. The IC 50 value of ethanol extract was found to be nearer to the IC50 value of standard Ascorbic acid which is given in Table 4 and Figure 3.

Table 4: DPPH free radical scavenging activity of ethanolic extract of stem bark of *D. oojeinense*

<table>
<thead>
<tr>
<th>Conc. of extract/std (µg/ml)</th>
<th>Ascorbic acid</th>
<th>Ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>25.58±1.44</td>
<td>17.82±0.69</td>
</tr>
<tr>
<td>20</td>
<td>41.38±1.27</td>
<td>35.09±1.11</td>
</tr>
<tr>
<td>40</td>
<td>62.10±1.77</td>
<td>44.93±1.09</td>
</tr>
<tr>
<td>60</td>
<td>79.83±1.68</td>
<td>64.81±2.05</td>
</tr>
<tr>
<td>80</td>
<td>91.21±2.32</td>
<td>77.86±2.77</td>
</tr>
<tr>
<td>100</td>
<td>92.68±2.88</td>
<td>84.88±1.94</td>
</tr>
</tbody>
</table>

Fig-3: DPPH free radical scavenging activity of ethanolic extract of stem bark of *D. oojeinense*
Conclusions

The ethanolic extract of the stem bark of *D. oojeinense* was subjected to invitro anti-oxidant activity. The present research concluded that the ethanolic extract of stem bark of *D. oojeinense* was endowed with significant anti-oxidant properties, thereby justifying its use in indigenous system of medicine.

Acknowledgement

The authors are thankful to Dr. Shivismurthy Murugha Sharanaru, President, SJM college of Pharmacy, Chitradurga for providing all necessary facilities through the Principal. The authors are also thankful to the Principal, NGSMIPS, Mangalore and NITTE educational trust.

References