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Abstract: Objective: The present study was carried out to evaluate antioxidative potential of Cassia tora seeds. Method: The antioxidant activities of different concentrations of ethanol extracts of the seeds of Cassia tora were determined by the four assay techniques i.e. DPPH radical scavenging assay, reducing power ability, hydrogen peroxide scavenging assay and thiocyanate method. Results: Ethanol extract of Cassia tora seed has shown effective antioxidant activity in all assay techniques. The results obtained in the present study indicate that the seeds of Cassia tora are a potential source of natural antioxidants.

Keywords: Antioxidant activity, DPPH, Reducing power, Cassia tora.

Introduction

Cassia tora Linn. (Cassia obtusifolia Linn.), Caesalpiniaaceae, is a wild crop and grows in most parts of India as a weed. According to Ayurveda, the leaves and seeds are acrid, laxative, antiperiodic, anthelmintic, ophthalmic, liver tonic, cardiotonic and expectorant. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis and cardiac disorders. Literature survey revealed that previously two antioxidant assay techniques (ABTS cation radical-scavenging assay. Thiocyanate method) has been already studied for selected plant but present study is evaluating three more methods to confirm plant’s antioxidant potential along with phenolic content determination.

Material and Methods

Extraction of plant material

The fresh seeds of Cassia tora (CT) were collected in the month of July-August from the local market of Amaravati, Maharashtra state, India, and authenticated by the authority of the Botany Department, VMV, Amaravati. A voucher specimen was submitted at Institute’s Herbarium Department for future reference. Dried seeds were ground to coarse powder. Powder was first defatted with pet. ether and then extracted with ethanol.

DPPH radical scavenging assay

The free radical scavenging activity of the fractions was measured in vitro by 1, 1-diphenyl 2-picrylhydrazyl (DPPH) assay. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 ml of this solution was added to 3 ml of the fraction dissolved in ethanol at different concentrations. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a Shimandzu spectrophotometer. The percentage scavenging activity at different concentrations was determined and the IC50 value of the fractions was compared with that of ascorbic acid (vit. C), which was used as the standard.

Reducing power ability

Different concentrations of ethanolic extracts (1.0 ml) were mixed with 2.5 ml of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. After, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 1.25 ml from the supernatant was mixed with 1.25 ml of distilled water and
0.25 ml FeCl₃ solution (0.1%, w/v). The absorbance was measured at 700 nm. The assays were carried out in triplicate. Increased absorbance values indicate a higher reducing power.

Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentration of the extracts was determined.

Thiocyanate method (FTC assay)

The peroxy radical scavenging activity was determined by thiocyanate method using vit. C as standard. Increasing concentration of the fractions in 0.5 ml of distilled water was mixed with 2.5 ml of 0.02 M linoleic acid emulsion (in 0.04 M phosphate buffer pH 7.0) and 2 ml phosphate buffer (0.04M, pH 7) in a test tube and incubated in darkness at 37°C. At intervals during incubation, the amount of peroxide formed was determined by reading the absorbance of the red colour developed at 500 nm by the addition of 0.1 ml of 30% ammonium thiocyanate solution and 0.1 ml of 20 mM ferrous chloride in 3.5% hydrochloric acid to the reaction mixture. The percentages scavenging activity was calculated and were compared with the standard, vit. C.

Estimation of total phenolic content

The assay used for the determination of total phenolics content employs Folin and Ciocalteu’s phenol reagent which response depending on the chemical structure of phenolics (i.e. the higher the number of functional –OH group the higher the total phenolics content).

Total soluble phenolic compounds in the ethanolic extracts were measured and expressed as gallic acid equivalents. A sample of the ethanolic extract was added to distilled water for a final volume of 2 ml. After, it was mixed with 0.3 ml of a saturated sodium carbonate (Na₂CO₃) solution and 0.1 ml of 1 N Folin–Ciocalteu’s phenol reagent. The mixture was placed for 1 h at room temperature in the dark. The absorbance was measured at 725 nm against the blank. The total phenolic content was expressed as mg of gallic acid equivalents.

Calculation of percentage inhibition (I %)

Percentage inhibition (I %) was calculated using the formula,

\[ I\% = \frac{(Ac-As)}{Ac} \times 100 \]

where Ac is the absorbance of the control and As is the absorbance of the sample.

Results and Discussion

Phytochemical screening

Phytochemical screening of the crude ethanolic extract of the Cassia tora seeds revealed the presence of flavonoids, anthraquinone glycosides and phenolic compounds. In addition, we could suggest that although the reducing power of a substance may be an indicator of its potential antioxidant activity, there is not necessarily a linear correlation between all these activities. Total phenolic content of CT found to be 69.34%.

DPPH radical scavenging method

ROS produced in vivo include superoxide radical, hydrogen peroxide and hypochlorous acid. Hydrogen peroxide and superoxide can interact in the presence of certain transition metal ions to yield a highly-reactive oxidising species, the hydroxyl radical. The antioxidants react with the stable free radical DPPH (deep violet colour) and convert it to 1,1-diphenyl-2-picryl hydrazine with decoloration. The scavenging effects of extract increased with their concentrations to similar

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Absorbance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>20</td>
<td>0.351</td>
<td>64.23</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.232</td>
<td>79.59</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.279</td>
<td>82.79</td>
</tr>
<tr>
<td>AA</td>
<td>20</td>
<td>0.245</td>
<td>84.82</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.195</td>
<td>89.12</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.122</td>
<td>98.72</td>
</tr>
</tbody>
</table>
Antioxidant Activity of Cassia tora

Table 3. Results of ferric thiocyanate assay:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>20</td>
<td>0.08237</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.11338</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.20078</td>
</tr>
<tr>
<td>AA</td>
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<td>0.23508</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.28879</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.33213</td>
</tr>
</tbody>
</table>

The percentage inhibitions of concentration 20, 40, 60 mg/ml are about 64, 79 and 83 % respectively (Table 1). The standard AA presented a scavenging effect of 92% at the concentration of 60 mg/ml.

Reducing power method

Table 2 shows the reducing power of the CT ethanolic extracts as a function of their concentration. In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe$^{3+}$/ferricyanide complex used in this method to the ferrous form. By measuring the formation of Perl’s Prussian blue at 700 nm, it is possible to determine the Fe$^{2+}$ concentration. The reducing power of the CT ethanolic extracts increased with their concentrations. At 20, 40 and 60 mg/ml, reducing powers of both extracts were around 0.08, 0.1 and 0.2 respectively, while a solution of 40 mg/ml of vit. C, the positive control used in this test, had a reducing power value of 0.3.

Hydrogen peroxide

Extracts of Cassia tora scavenged hydrogen peroxide in a concentration-dependent manner. The ethanol extracts of Cassia tora showed strong H2O2 scavenging activity IC$_{50}$ 0.129 mg/ml whereas that of the standard, vit. C was 0.095 mg/ml.

Thiocyanate method

Results obtained from FTC assay (Table 3) revealed that extracts of Cassia tora carry the antioxidative potential for chain-breaking inhibition of lipid peroxidation and for free radical scavenging as extract has shown 59, 67 and 77% inhibition.

Conclusion

The present study suggests that the seeds of Cassia tora might be potential source of natural antioxidant.

References


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