ABSTRACT

The aim of this work was to estimate the total phenolic and flavonoids content, and to evaluate in vitro antioxidant activity of ethanolic extract of Albizia procera. The ethanolic extract of Albizia procera was showed significant free radical scavenging activity than that of various standards. The greater amount of phenols and flavonoids were found in ethanolic extract of Albizia procera than that of other extracts. The radical scavenging activity was found to be concentration dependent manner. Further studies on isolation of constituents from the extract and their biological activities are under investigation.

Keywords: Albizia procera, antioxidant activities, phenolics, flavonoids.

INTRODUCTION

The role of free radicals and tissue damage in diseases such as atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus, hypertension and several other diseases are becoming increasingly recognized. Reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), are products of normal cellular metabolism and they are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. Antioxidant supplements or foods rich in medicinal plants may be used to help the human body in reducing oxidative damage by free radicals and active oxygen.

Therefore currently, the research interest is focused on the potential role of antioxidants in the treatment and prevention of above diseases. The most commonly used antioxidants at present are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-butyldihydroquinone (TBBQ). However, they are suspected of being responsible for liver damage and carcinogenesis in laboratory animals. Therefore, the development and utilization of more effective antioxidants of natural origin are desired.

Albizia procera is a medium-sized deciduous tree, sparingly grown in India. This plant is used traditionally in dropsy, pain, rheumatism, convulsions, delirium, and septicaemia. The bark of the plant is used as an astringent in the treatment of diarrhea. Its decoctions are recommended for ulcers as a useful was solution. They are reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc. Even though, traditionally, leaves of Albizia procera were extensively used for the treatment of variety of wounds, and no scientific data in its support is available. Our literature survey revealed that the antioxidant activity of various extracts from whole plant of Albizia procera was not investigated; hence these activities have been investigated in the present study.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The aerial parts of Albizia procera were collected from Tullarai, Thirunehvel District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai. The aerial parts of Albizia procera, were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powdered materials were successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus for 24 hrs. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of Antioxidant activity by in vitro Techniques:

Iron chelating activity

The method of Benzie and strain (1996) was adopted for the assay. The principle is based on the formation of O-Phenanthroline-Fe²⁺ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations ranging from 10 to 1000µg was incubated at room temperature for 10 min and the absorbance of the same was measured at 510 nm. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Total antioxidant activity (Phosphomolybdic acid method)

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999). An aliquot of 0.9 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 molar sodium phosphate and 4 molar ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expressed relative to that of ascorbic acid.

FRAP assay

A modified method of Benzie and Strain (1996) was adopted for the FRAP assay. The stock solutions included 300 molar acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM Fecl₃, 6H₂O. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml Fecl₃·6H₂O. The temperature of the solution was raised to 37°C before using. Plant extracts (0.15 ml) were allowed to react with 2.85 ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM FeSO₄. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Estimation of total phenol

0.5 ml of Folinis phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. The absorbance was measured at 650 nm in a spectrophotometer.

Estimation of total flavonoids

0.5 ml of extract and 4 ml of the vanillin reagent (1% vanillin in 70% conc. H₂SO₄) was added and kept in boiling water bath for 15 mins.
RESULTS AND DISCUSSION

Free radical is a molecule with an unpaired electron and is involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging and neoplastic diseases. They are also involved in autoimmune disorders like rheumatoid arthritis etc.

Iron chelating activity

Iron binding capacity of the ethanolic extract of Albizia procera and the metal chelator EDTA at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 3. The IC₅₀ value of plant extract and EDTA was recorded as 420µg/ml and 390µg/ml respectively.

Iron chelating activity of ethanolic extract of Albizia procera presented in Table 2. The ethanolic extract of Albizia procera exhibited a maximum total antioxidant activity of 63.78±0.03% at 1000 µg/ml whereas for ascorbate (standard) it was found to be 63.45 ± 0.97% at 1000 µg/ml and 60.64 ± 0.02% at 500 µg/ml respectively. The IC₅₀ values of methanolic extract and ascorbate were found to be 63.59±0.04% and 55.23 ± 0.01% respectively.

Table 1: Iron chelating activity of Ethanol extract of Albizia procera

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>% of activity(±SEM)*</th>
<th>Ethanol extract of Albizia procera</th>
<th>Standard (EDTA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>125</td>
<td>14.6±0.24</td>
<td>18.02 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>39.58±0.24</td>
<td>47.77 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>500</td>
<td>53.97±0.03</td>
<td>56.99 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>63.45±0.09</td>
<td>63.78±0.03</td>
<td></td>
</tr>
</tbody>
</table>

The total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of ethanolic extract of Albizia procera presented in Table 2. The ethanolic extract of Albizia procera exhibited a maximum total antioxidant activity of 59.36±0.01% at 1000 µg/ml whereas for ascorbate (standard) it was found to be 55.23 ± 0.01% at 1000 µg/ml. The IC₅₀ of the ethanolic extract of Albizia procera and ascorbate were found to be 237µg/ml and 410µg/ml respectively.

FRAP assay

The reducing ability of the ethanolic extract of Albizia procera and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 3. The maximum reducing ability at 1000 µg/ml for ethanolic extract and ascorbate were found to be 63.59±0.04% and 55.23 ± 0.01% respectively. The IC₅₀ values of methanolic extract and ascorbate were recorded as 253µg/ml and 410µg/ml respectively.

Total flavonoids

The total amount of flavonoids content of ethanolic extract of Albizia procera was summarized in Table 5. Flavonoids present in food of plant origin are also potential antioxidants. The higher content of flavonoids was found ethanolic extract of Albizia procera.

REFERENCES

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