Evaluation of anti-oxidant, anti-bacterial and in-vitro anti-inflammatory activity of *Albizia richardiana* seed extract

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ABSTRACT

The current study is to evaluate antioxidant, antibacterial and in-vitro anti-inflammatory potential of *Albizia richardiana* seed extract. Aqueous seed extract of *A. richardiana* was screened for the presence of phytochemicals and the results obtained indicated the presence of bioactive compounds like carbohydrate, saponin, alkaloids, glucoside and glycoside. On performing antioxidant assays, the IC$_{50}$ for FRAP assay was found to be promising with a value of 0.16 ± 0.05 mg/ml. The IC$_{50}$ for DPPH assay and TBARS assay was found to be 0.29 ± 0.02 and 0.46 ± 0.02 mg/ml, respectively. Thus, antioxidant study demonstrated that the seed extract has better reducing property making it a good source for stabilizing the free radicals. The antibacterial analysis was done through well-diffusion method where the results elucidated that the extract was more effective against gram positive bacteria than on the gram negative. The extract also showed to possess significant anti-inflammatory activity which was accessed through in-vitro membrane stabilization (47.6%) and inhibition of protein denaturation (59.6%) at a concentration of 500 μg/ml. From the aforementioned results it was evident that the plant *A. richardiana* has notable medicinal property and can be considered as a medicinal plant.

Key words: *Albizia richardiana*, anti-oxidant, anti-inflammatory, antibacterial, well-diffusion method, medicinal plant.

INTRODUCTION

Today, mankind is encountered by the wide spread of various diseases and ailments that pose threat to the continuance of life (Rang et al., 2007). To overcome this concern, humans utilize every piece of material he believes confer usefulness to their survival. Allopathic medicines are the modern drug that is incorporated by the back of science in medicine. Even though they are effective, these drugs have side effects because of their harmful chemical nature (Agarwal, 2005); thus, the use of traditional therapies is increasing gradually.

Over the years, people have used locally grown herbal plants as an antidote to treat illness and infections (Qureshi and Ghufran, 2005). However, the use of these plants still needs evidences to support its claims and actions, before being used in major therapy (Patwardhan et al., 2005). Plants produce secondary metabolites for protecting the plant against stress, pathogens and to provide structural support (Amita et al., 2010). These bioactive compounds are not essential for the growth of the plant but rather for the survival and longevity of the organism (Karuppusamy, 2009). Identification of these polyphenols are gaining attention as they are said to have effective clinical properties like antimicrobial, anti-inflammatory, antiviral property and also antioxidant capacity (Ignat et al., 2011). Understanding the chemical composition of a plant is important and also to learn and observe the mechanism by which it exerts a therapeutic response to the human body (Agbafor and Nwachukwu, 2011).

*Albizia richardiana* locally known as Raj koroi (Common name: Hatamiuki) belonging to the family Fabaceae is
distributed in West Bengal, Assam and Peninsular India (Allan and Porter, 2000). In folk medicine, it is considered
to increase appetite, promoting blood circulation, chest
tightness, blurred vision, back pain and injuries from falls
(Xinrong et al., 2003). The plants of this genus have been
reported to contain various significant medicinal
properties, for instance, they are recognized to treat
asthma, arthritis, antiseptic, burns, anti-dysenteric and
allergic rhinitis etc. Considering these various and distinct
uses of the Albizia family, the current study evaluated A.
richardiana’s antioxidant, anti-microbial and anti-
inflammatory activities with a view to reveal its other
medicinal properties so that it can be given the recognition
of medicinal plant. The extract was tested for antioxidant
properties through DPPH, FRAP and TBARS assays.
Antibacterial and anti-inflammatory was also determined
for the extract by in-vitro methods.

MATERIALS AND METHODS

Collection of Albizia richardiana seeds

The seeds were collected and washed with distilled water.
It was then made to a coarse powder by grinding. The
powder was then stored in an airtight container till use
(Figure 1).

Preparation of seed extract

The plant extract was prepared through maceration that
was previously explained by Monica et al. (2015). In three
amber bottles, 100 g of seed powder was soaked in 240 ml
of water each. It was then sealed and left in a dry place for
few days with occasional shaking and stirring. The mixture
was then filtered and reduced using water bath. The filtrate
was thereafter kept for drying to obtain the crude dry
extract.

Phytochemical screening of the seed extract

The phytochemical constituents of the plant extract was
determined by the previously reported methods of Banu

Test for carbohydrates (Fehlings test)

To 1 ml of a solution mixture of Fehling’s solutions A and B
(equal in volume), small quantity of aqueous plant extract
was added. The mixture was boiled for few minutes.

Tests for glycosides

A small quantity of an alcoholic extract of the plant extract
was dissolved in about 1 ml of water. Few drops of aqueous
sodium hydroxide solution (NaOH) was then added.

Test for glucosides

A small quantity of plant’s alcoholic extract was dissolved
using both water and alcohol. The solution was divided into
two portions and treated in the following ways:

1) First portion was boiled with solution containing equal
volumes of Fehling’s solutions A and B;
2) Other portions were boiled for about 5 min with few
drops of dilute sulphuric acid. Few drops of sodium
hydroxide solution were added. Thereafter, a solution
containing equal quantity of Fehings’s solutions A and B
was added to it followed by boiling the mixture.

Test for resins

A small quantity of extract was dissolved in about 5mL of
acetic anhydride by using gentle heat. The solution was
cooled and then about 0.05 ml of sulphuric acid was added
to it.

Tests for proteins

About 5 to 8 drops of 10% sodium hydroxide solution
(NaOH) and 1 to 2 drops of 3% copper sulphate solutions were added to 1 ml of a hot aqueous plant extract.

**Test for saponins**

A small quantity of the extract was taken and diluted with 20 ml of distilled water. The test tube was then shaken for 15 min by hand.

**Test for steroids**

A small quantity of the extract was taken and 1 ml of concentrated H₂SO₄ was added to the side walls of the test tube.

**Test for tannins**

A small quantity of the extract was taken and dissolved in 45% of the ethanol. It was then boiled for 5 min and 1 ml of 15% ferric chloride solution was added.

**Test for alkaloids**

A small quantity of the extract was taken and dissolved in 2 ml of the Wagner’s reagent.

**Test for flavonoids**

A small quantity of the extract was taken and few drops of diluted NaOH were added.

**Determination of anti-oxidant activity of extract**

**Estimation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity**

Radical scavenging activity using DPPH was determined using the methods as reported by Veeru (2009). Various concentrations of seed extracts (50 to 250 μg/ml) were taken. To that, various volumes (950 to 750 μl) of methanol were added. 1 ml of DPPH was added to each of the test tubes and allowed to incubate for 60 min in the dark. Thereafter, absorbance was read at 517 nm.

The percentage inhibition of the DPPH radical by the samples was calculated using the equation:

\[
\% \text{Inhibition} = \frac{(A_1 - A_0)}{A_0} \times 100
\]

Where \(A_0\) is the absorbance of control sample and \(A_1\) is the absorbance of the test sample.

**Estimation of Fe³⁺ reducing power (FRAP) assay**

Reducing power of the seed extract was determined by the reported methods of Quy et al. (2014). Various concentrations of seed extract (50 to 250 μg/ml) were taken. To that, various volumes (950 to 750 μl) of methanol were added. To each of the test tubes, 1 ml of 1% Potassium ferricyanide and 1 ml of Phosphate buffer was added. It was incubated in water bath for 20 min at 50°C. Then, 500 μl of 10% of TCA was added along with 500 μl of 0.01% of FeCl₃. Absorbance was read at 700 nm.

The inhibition (%) of radical scavenging activity was calculated using the equation:

\[
\% \text{Inhibition} = \frac{(A_1 - A_0)}{A_0} \times 100
\]

Where \(A_0\) is the absorbance of control sample and \(A_1\) is the absorbance of the test sample.

**Estimation of thiobarbituric acid reactive substances (TBARS) assay**

Lipid peroxidation activity of the solvent extract was determined by the reported methods of Kikuzaki and Nakatani (1993). Egg homogenate was used as the lipid source for the assay. In a test tube, 200 μl of various concentrations of seed extract were taken. To this 500 μl of egg homogenate was added. The whole reaction mixture was made up to 1 ml using distilled water. 50 μl of FeSO₄ and 20 μl of ascorbic acid were added to induce lipid peroxidation by incubating it for 1 h at 37°C. 1 ml of EDTA and 1.5 ml TBA solution was added which was then placed in a water bath for 15 min at 100°C. The tubes were collected and centrifuged at 3000 rpm. The absorbance of the supernatant was read at 532 nm.

The percentage inhibition of lipid peroxidation by the samples was calculated using the equation:

\[
\% \text{Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100
\]

Where \(A_0\) is the absorbance of control sample and \(A_1\) is the absorbance of the test sample.

**Determination of anti-bacterial activity of extract**

Antibacterial activity of the extract was determined by the
The seed extracts anti-inflammatory property was determined in-vitro using the methods reported by Leelaprakash and Dass (2011).

**Membrane stabilization technique**

**Preparation of human red blood cells (HRBC) suspension**

2 ml of human blood was collected. This was then centrifuged at 3000 rpm for 10 min followed by washing the cells with isosaline (0.85%, pH 7.2) thrice. The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline.

**Heat induced hemolysis**

A reaction mixture (2 ml) containing 1 ml of extract at different concentrations (50 to 250 μg/ml), 1 ml of 10% RBCs suspension was taken. This was then incubated for 30 min in water bath at 56°C. The tubes were then allowed to cool down to room temperature and centrifuged for 5 min at 2500 rpm. The supernatant was separated and its absorbance taken at 560 nm.

The percentage inhibition of membrane stabilization technique was calculated using the formula:

\[
\text{% inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Where \(A_0\) is the absorbance of control sample and \(A_1\) is the absorbance of the test sample.

**Inhibition of protein denaturation technique**

Reaction mixture (5 ml) consisting of 0.2 ml BSA, 2.8 ml of phosphate buffered saline and 2 ml of varying concentrations (50 to 250 μg/ml) of seed extract was taken. Thereafter, the mixtures were incubated at 37°C for 15 min and then heated at 70°C for 20 min. It was then cooled and absorbance read at 660 nm.

The percentage inhibition of the protein denaturation technique was calculated using the formula:

\[
\text{% Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100
\]

Where \(A_0\) is the absorbance of control sample and \(A_1\) is the absorbance of the test sample.

**Statistical analysis**

The statistical analysis was done using One-way ANOVA with post hoc turkey HSD test where \(p<0.001\) was considered as extremely significant.

**RESULTS AND DISCUSSION**

After extraction through maceration, the yield was found to be 1.76, 1.86 and 2.02 g that were obtained after 6, 10 and 14 days, respectively. The crude dry extract was completely soluble in water and hence, water was chosen as the desired solvent.

Phytochemical analysis provides a guide for determining the biological action of the plant (Kennedy and Thorley, 2000). Phytochemical screening of *A. richardiana* seed extract reported the presence of major phytochemicals like saponins and alkaloids along with other bioactive compounds like carbohydrates, glucosides and glycosides (Table 1). These screened results were consistent with the previously conducted studies on the bark extract of *A. richardiana* (Rahman et al., 2015).

The antioxidant profile of *A. richardiana* was studied by performing three assays. In DPPH assay, the seed extract was seen to possess moderate quantity of scavenging activity. The IC\(_{50}\) for DPPH assay was calculated and found to be 0.29±0.02 mg/ml, mean ± SD (Figure 2). These results suggest that the plant extract contains compounds which have fair share of scavenging activity towards a stable radical.

To analyze the plants reducing property, FRAP assay was performed. Here, the extract was confirmed to possess better reducing capacity with an IC\(_{50}\) value of 0.16 ± 0.05 mg/ml, mean ± SD (Figure 3).

Analyzing lipid peroxidation inhibition activity of a plant extract is essential as all biological specimens contain a mixture of thiobarbituric acid reactive substances (TBARS), including lipid hydroperoxides and aldehydes, which increase as a result of oxidative stress. TBARS return to
Table 1: Phytochemical analysis of *Albizia richardiana* seed extract.

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Glucosides</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) Positive result; (-) Negative result.

---

**Figure 2:** Percentage inhibition of DPPH assay. IC\(_{50}\) for the extract was calculated using the formula \( y = 0.213x - 13.11 \).  

**Figure 3:** Percentage inhibition of FRAP assay. IC\(_{50}\) for the extract was calculated using the formula \( y = 0.296x + 0.82 \).
normal levels over time, depending on the presence of antioxidants (Schinella et al., 2000). The plant extract was reported to possess only partial lipid peroxidation inhibition activity with an IC<sub>50</sub> of 0.46 ± 0.07 mg/ml, mean ± SD (Figure 4). Thus, at the end of antioxidant studies it was evident that the seed extract has significant metal reducing capacity than radical scavenging and lipid peroxidation inhibition.

Banerjee and Bonde (2011) studied the total phenolic component and antioxidant activity on Bridelia retusa bark extract and reported that phytochemical constituents were responsible for the plants antioxidant activity. According to the findings, the polyphenolic contents of the A. richardiana seed extract such as alkaloids and saponins may have aided for the extracts antioxidant potential.

The antibacterial efficiency of the seed extract was checked on two bacterial strains; Gram-positive: L. plantarum and Gram-negative: E. coli. Agar well-diffusion is one of the widely used methods to access plant extracts efficiency on microbes (Magaldi et al., 2004). The seed extract had notable antibacterial activity against gram-positive L. plantarum with a zone of inhibition of 7 mm (Table 2). This result was in accordance with the previous findings of Joshi et al. (2011). The outcome is in line with the statement provided by Elisha et al. (2017) where difference in activity of the seed extract is due to the alteration in the cell wall framework of the bacterium. The absence in outer membrane and thin peptidoglycan layer in gram-positive species makes it susceptible to antibiotics and other organic solvents.

Anti-inflammatory activity of the extract was studied through RBC Membrane stabilization technique and Protein denaturation technique. An RBC membrane is similar to a lysosomal membrane. A plant extract that can stabilize a RBC can stabilize a disrupted lysosome thereby preventing inflammation (Leelaprakash and Dass, 2011). On performing the membrane stabilization technique on RBC, it was seen that the extract had a maximum of 47.6% of inhibition at 500 μg/ml, while the standard drug aspirin had an activity of 49.32% at 100 μg/ml (Figure 5). The level of significance of the extract was determined by comparing the results with the control where the seed extract concentration of 300 to 500 μg/ml was found to be moderately significant (p<0.01) which exhibited a protective effect against RBC cells from hemolysis. Leelaprakash and Dass (2011) stated that the outcome for their anti-inflammatory study was dose dependent which was in accordance with the current study.

External factors like stress and heat cause protein denaturation. Protein denaturation results in inflammation and as such through the analysis of protein denaturation technique it was observed that, the findings exhibited a concentration dependent inhibition of protein denaturation. At 500 μg/ml, the percentage inhibition of the extract was observed to be 59.6%, whereas, aspirin had an activity of 59% at 100 μg/ml (Figure 6). On analyzing the level of significance, it was found that 500 μg/ml of the extract concentration was found to be extremely significant.

Table 2: Zone of inhibition of the extract and the standards.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Zone of inhibition</th>
<th>Standard (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>4</td>
<td>10 (Tetracycline)</td>
</tr>
<tr>
<td>L. plantarum</td>
<td>7</td>
<td>8 (Streptomycin)</td>
</tr>
</tbody>
</table>

Figure 4: Percentage inhibition of TBARS assay. IC<sub>50</sub> for the extract was calculated using the formula y = 0.095x + 5.5.
Mandal et al. (2005) and Gepdiremen et al. (2005) concluded that plant saponins have been reported to possess anti-bacterial and anti-inflammatory activity respectively. Given that the current plant under study, *Albizia richardiana* was reported to possess these phytochemical and hence, the Antibacterial and Anti-inflammatory activity of the seed extract might be due to the presence of this bioactive compound.

**Conclusion**

The current study reveals that the plant materials have moderate to significant antioxidant activity. This suggests that the selected plant could be used as a source of antioxidants for pharmacological preparations which is very well evidenced by the present work. The aqueous extract possessed the highest efficacy as free radical scavengers and had the lowest capacity as lipid peroxidation inhibitor. It was evident that the antibacterial activities are present in the seed extract of this plant on comparing it with the standard antibiotics and the activity was found to be more efficient on Gram positive bacteria compared to gram negative bacteria. The anti-inflammatory studies on the extract shows that the plant extract has noticeable anti-inflammatory property. This ability seems to be related with phytochemicals present in the aqueous
extract. Thus, this study provided support for the use of *A. richardiana* (Fam. Fabaceae, subfam. Mimosoideae), as a medicinal plant due to their anti-oxidant, anti-bacterial and anti-inflammatory activities and deserves further extensive studies to find out bioactive constituents responsible for these activities.

**REFERENCES**


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