Phytochemical Screening and Pharmacological Assessment of Justicia aurea Grown in Bangladesh

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ABSTRACT
Plants are the major source of therapeutic compounds and have huge applications in Pharma Industry. To identify new sources of therapeutic compounds, we studied acute toxicity, antihyperglycemic, anti-inflammatory, and anticoagulant activities of the polar and nonpolar fractions of Justicia aurea (J. aurea). Our initial phytochemical screening of J. aurea exhibited the presence of numerous secondary metabolites such as reducing sugars, glycosides, tannins, gums, alkaloids, phenolic compounds, and carbohydrates which might be primarily responsible for its medicinal properties. In the acute toxicity test, the result showed no toxicity up to 2000 mg/kg. In the antihyperglycemic activity test, administration of petroleum ether fraction and water fraction of J. aurea extract to glucose-loaded mice at 250 and 500 mg/kg body weight (BW) reduced the blood glucose levels compared to control mice. Between two fractions, the water fraction was found to exhibit better potentiality at 500 mg/kg BW. In the anti-inflammatory assay, at 250 and 500 mg/kg water fraction exhibited anti-inflammation by 20.00% and 31.67%, respectively and petroleum ether fraction by 13.33% and 18.00%, respectively. In anticoagulant activity, the test indicated that water fraction has minor anticoagulant potentiality compared to control. In conclusion, the polar water fraction shows better medicinal properties than the nonpolar petroleum ether fraction of J. aurea.

Keywords: Justicia aurea, Acanthaceae, acute toxicity, antihyperglycemic, anti-inflammatory, anticoagulant.

INTRODUCTION
Natural products play a key role in drug discovery and over one-third of drugs approved by the Food and Drug Administration (FDA) was developed from natural products and their derivatives1. Plants contain bioactive natural products and serve as raw materials to produce drugs2. The genus Justicia (Acanthaceae) contains 600 species that are primarily habitat to tropical and pantropical regions. These species have been reported with various biological activities where antidiabetic, anti-inflammatory, and antiviral activities are the most noteworthy3. J. aurea which is also known as Yellow Jacobinia is an evergreen suburb with ovate leaves (10-30 cm). It is almost 10 feet tall and 2 to 3 feet wide. It has bright yellow flowers which are blooming from midsummer to late summer and rains. This plant is best grown in well-drained soils and moist areas4. Upon literature survey, no work has been reported on the antihyperglycemic, anti-inflammatory, and anticoagulant activity of J. aurea. Therefore, this project aimed to evaluate the mentioned activities along with the phytochemical screening of polar and nonpolar fractions of crude extract and make a comparative feature.

MATERIALS AND METHODS
Plant collection and extraction
The whole plant of J. aurea was collected from Chalna, Khulna in March 2016. The collected plant was identified by the experts of Pharmacy Discipline, Khulna University, Bangladesh.

Plant extraction
Plant extraction is a method to extract certain chemical components present in the plants. The plant parts of J. aurea were isolated from unwanted materials and washed with fresh water. Next, the plant parts were air-dried which normally takes 7 days, and were powdered with a suitable grinder. The powder material of J. aurea was stored in an airtight glass container at room temperature in a dark, dry environment. Later, 460 g of powdered material was placed in a clean flat-bottomed glass container and soaked in 1600 ml 95.00% ethanol. Then the powdered materials were sealed for 14 days with continuous stirring. 14 days later, the whole mixture was filtered with clean white cotton followed by Whatman filter paper to remove unwanted debris. Finally, the solvent was evaporated to yield 2.83% w/w of the crude extract. Thereafter, the crude extract was separated into petroleum ether, and water fractions.

Phytochemical screening
The preliminary phytochemical studies revealed the existence of various chemical groups in test samples. Various tests on crude extracts were performed based on the established methods6-9.
Experimental animal
To experiment with the different fractions of plant extract, we chose Swiss-albino young mice aged 28-35 days (average weight of 20-30g). They were always kept in the animal house of Pharmacy Discipline, Khulna University, Khulna, Bangladesh at room temperature and relative humidity of 55-65% with 12 hours of daylight and 12 hours of darkness. These mice were fed with ICDDRBR formulated food and water. The mice were cared for and handled following internationally accepted standard laboratory protocols and guidelines. The animals were regularly checked before experimentation.

Acute toxicity test
Acute toxicity test of *J. aurea* extract was carried out using the method of Devnath et al\(^{10}\). At first, 500 mg/kg BW was given orally using a feeding needle to three animals which were initially weighed and marked, and then 1000, 1500, and 2000 mg/kg BW were used\(^{11}\). Then individual observations were started during the first half an hour and periodically during the first 24 hours. After 24 hours, if the animals were alive and does not show any adverse effects then the next dose was administered to another three animals.

Antihyperglycemic activity test
The antihyperglycemic activity was studied using the oral glucose tolerance test (OGTT)\(^{12}\). Forty overnight fasted mice were distributed into eight groups comprising five in each group. The control group was administered tween-80 (2%) in water at 10 ml/kg BW. The standard group was administered glibenclamide at 5 mg/kg BW. The rest of the groups were administered the petroleum ether ether and water fraction at 250 and 500 mg/kg BW. The tail tips of mice were dissected and the blood sample was taken to measure blood glucose levels using a glucometer. The povidone-iodine ointment was put on to the injured area to prevent infection/inflammation on the dissected tail end. The control, standard, and fractions of extracts were fed using a feeding needle in the respective test group\(^{13}\). Half an hour later, glucose was fed at the dose of 2 g/kg BW to all test groups. After feeding, the levels of blood glucose were recorded at 60 minutes intervals from 30-150 minutes.

Anti-inflammatory activity test
The anti-inflammatory potentiality of *J. aurea* fractions was carried out by the method of Jahan et al with slight modification\(^{14}\). In this experiment, 30 young Swiss albino mice were randomly allocated into 6 groups having 5 in each group. Group I (control group) was given 0.9% sodium chloride solution and Group II (standard) was given ibuprofen (100 mg/kg) orally. Group III and group IV were fed water fractions and Group V and VI were administered petroleum ether fractions at the doses of 250 and 500 mg/kg BW orally. One hour later, each mouse got 20 µl of xylene on the anterior and posterior surfaces of the right ear lobe. The left ear was accounted for as a control. Xylene-treated mice were sacrificed 60 minutes after administration. Circular sections were taken with the help of a cork borer having a 3 mm diameter and weighed. The level of percentage inhibition was measured following the equation:

\[
\text{Inhibition (%) = } (1 - \text{Et/Ec}) \times 100
\]

Where, Et = average edema of the experimental group and Ec = average edema of the control group

Anticoagulant test
0.2 ml of plasma, 0.1 ml of different crude fractions, and 0.3 ml of CaCl\(_2\) (25mM) were put together in fusion test tubes. For negative control, 0.1 ml of 0.9% saline, 0.2 ml plasma, and 0.3 ml of CaCl\(_2\) (25mM) were taken to the second test tube. For positive control, 0.1 ml warfarin, 0.2 ml plasma, and 0.3 ml CaCl\(_2\) (25mM) were taken to another test tube. All test tubes were heated in a water bath at 37°C. The clotting time (prothrombin time) was determined with a stopwatch by tilting the test tubes every 5 seconds. Each of the tests was performed thrice and the average scavenging time was noted\(^{15, 16}\).

RESULTS
Phytochemical tests
The phytochemical study of *J. aurea* crude extract revealed the existence of several natural compounds summarized in Table 1. These types of phytoconstituents might be contributors to different bioactivities\(^{17}\). Therefore, further biological studies were performed for assessing their antihyperglycemic, anti-inflammatory, and anticoagulant activities.

**Table 1:** Outcome of chemical group test of *J. aurea* crude extract

<table>
<thead>
<tr>
<th>Phytochemical groups</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing sugars</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>−</td>
</tr>
<tr>
<td>Gums</td>
<td>+</td>
</tr>
<tr>
<td>Protein-xanthoproteins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Acidic compounds</td>
<td>−</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic compounds</td>
<td>+</td>
</tr>
</tbody>
</table>

\(+ = \text{Presence}; − = \text{Absence}\)

Acute toxicity test
The result showed that no mice died at 500, 1000, 1500, and 2000 mg/kg BW). The doses used in different tests to evaluate the pharmacological profile of *J. aurea* are a
minimum of 10 times less than the toxic dose as observed. So, it ensures that the toxic effect of the crude extract does not interfere with the result during pharmacological investigations.

**Antihyperglycemic activity test**

Administration of water and petroleum ether fractions of *J. aurea* extract to glucose-loaded mice at 250 and 500 mg/kg BW reduced minor blood glucose levels compared to control and the effect was comparable with the standard glibenclamide at 150 min. Petroleum ether fraction was found to exhibit a significant effect at 250 mg/kg whereas water fraction showed a significant role at 500 mg/kg (Table 2).

**Anti-inflammatory activity test**

The results of the test (Table 3) showed that at 250 and 500 mg/kg water fraction exhibits inhibition of inflammation by 20% and 31.67%, respectively, and petroleum ether fraction shows inhibition of inflammation by 13.33% and 18%, respectively. Here, the standard drug ibuprofen inhibition was found to be 70% at 100 mg/kg BW.

So, it can be claimed that the anti-inflammatory activity of various fractions of *J. aurea* extract was significant compared to the negative control.

**Anticoagulant test**

Petroleum ether and water fractions of *J. aurea* were subjected at the dose of 350 and 175 mg/ml to carry out the anticoagulant potentialities. The petroleum ether did not exhibit any significant rise in the mean clotting time. However, the water fraction at 350 mg/ml significantly increased coagulation time by 4.98 minutes compared to the control (0.9% saline) clotting time of 3.36 minutes which indicates that the water fraction has lower anticoagulant activity (Table 4).

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**Table 2: Anti-hyperglycemic effect of fractions of *J. aurea* extract**

<table>
<thead>
<tr>
<th>Group</th>
<th>Fasting state</th>
<th>30 min</th>
<th>90 min</th>
<th>150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.93±.29</td>
<td>10.33±.17</td>
<td>9.13±.17</td>
<td>7.85±.13</td>
</tr>
<tr>
<td>Standard</td>
<td>5.70±.18</td>
<td>3.38±.17***</td>
<td>2.43±.17***</td>
<td>3.08±.18***</td>
</tr>
<tr>
<td>Petroleum ether fraction (250 mg/kg)</td>
<td>6.30±.37</td>
<td>10.63±.28</td>
<td>9.03±.67**</td>
<td>8.55±.30*</td>
</tr>
<tr>
<td>Petroleum ether fraction (500 mg/kg)</td>
<td>5.58±.25</td>
<td>10.23±.20</td>
<td>9.33±.58</td>
<td>7.60±.43</td>
</tr>
<tr>
<td>Water fraction (250 mg/kg)</td>
<td>5.57±.19</td>
<td>9.00±.18*</td>
<td>8.28±.26</td>
<td>7.10±.30</td>
</tr>
<tr>
<td>Water fraction (500 mg/kg)</td>
<td>5.63±.15</td>
<td>8.70±.40**</td>
<td>7.30±.51***</td>
<td>6.28±.21**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of the mean (n = 3); * indicates P<0.05, ** indicates P<0.01, and *** indicates P<0.001 when compared with control.

**Table 3: Anti-inflammatory effect of fractions of *J. aurea* extract**

<table>
<thead>
<tr>
<th>Test group</th>
<th>Dose</th>
<th>% Inhibition of inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (normal water)</td>
<td>10 ml/kg</td>
<td>0</td>
</tr>
<tr>
<td>Positive Control (ibuprofen)</td>
<td>100 mg/kg</td>
<td>70±0.029***</td>
</tr>
<tr>
<td>Water fraction</td>
<td>250 mg/kg</td>
<td>20±0.041**</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>500 mg/kg</td>
<td>31.67±0.063**</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>250 mg/kg</td>
<td>13.33±0.041*</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>500 mg/kg</td>
<td>18±0.075*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of the mean (n = 3); * indicates P<0.05, ** indicates P<0.01, and *** indicates P<0.001 when compared with control.

**Table 4: Anticoagulant effect of fractions of *J. aurea* extract**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentration of sample</th>
<th>Average time of coagulation (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.9% saline</td>
<td>3.36±0.09***</td>
</tr>
<tr>
<td>Warfarin</td>
<td>5 mg/ml</td>
<td>62.86±2.87***</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>175 mg/ml</td>
<td>3.11±0.02</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>350 mg/ml</td>
<td>3.44±0.03</td>
</tr>
<tr>
<td>Water fraction</td>
<td>175 mg/ml</td>
<td>3.74±0.34</td>
</tr>
<tr>
<td>Water fraction</td>
<td>350 mg/ml</td>
<td>4.98±0.22***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error of the mean (n = 3); * indicates P<0.05, ** indicates P<0.01, and *** indicates P<0.001 when compared with control.
DISCUSSION

To determine the toxicity and establish a safe dose of different fractions of crude plant extract, toxicity studies are carried out in various test animals. Since *J. aurea* has not been previously studied in mice model, the acute toxicity study was carried out following the Organisation for Economic Co-operation and Development (OECD) recommendations to evaluate its toxicity and identify the safe dose that might be used in further studies. In this study, the water and petroleum ether fractions appear to be non-toxic up to 2000 mg/kg.

A very little number of compounds that resemble antidiabetic drugs present in *J. aurea* may lower the blood glucose level in mice. The effect of the crude fractions was recorded at 30, 90, and 150 minutes after glucose administration. The observed reduction in blood glucose after administration of the crude fractions could be due to different mechanisms. As seen with *Mangifera indica* L., compound (s) found within the leaf may decrease glucose absorption in the gut. Alternately, any bio-active natural product (s) in the crude fractions may lower blood glucose by enhancing the pancreatic insulin release or enhancing glucose uptake, as has been shown in experiments using the extract from *Artemisia* and *Ageratum conyzoides*. Another probable mechanism can be the rise of peripheral glucose consumption induced by the extract, as has been exhibited by the extract of *Sapindus trifoliatus*. There have been reports of antidiabetic action of herbal extracts containing flavonoids and tannins. Based on this fact, it may be postulated that the flavonoids or tannins present in this plant may be the cause of the observed decrease in blood sugar levels.

Inflammation is a complicated series of reparative and protective reactions to tissue injury whatever the cause - infection, autoimmune response, or mechanical injury. Histopathologically, extreme vasodilation, edematous skin alterations, and infusion of inflammatory cells are recognized as indications of acute inflammation after topical xylene application. Xylene-induced ear edema model is partly related to substance P, an undecapeptide that is widely distributed in the central and peripheral nervous systems and serves as a neurotransmitter or a neuromodulator in a range of physiological conditions. When substance P is released from sensory neurons, it acts as a vasodilator, edematous skin, and inflammatory cells. Platelets, blood vessels, coagulation factors, plasma inhibitors, and the fibrinolytic system are only a few of the components that maintain physiology. Blood clotting, which is the main cause of heart attacks and strokes, is inhibited by anticoagulant medications. When there is a high risk of blood clots, anticoagulant medications may be taken. Since anticoagulants are prescribed for cardiac issues, hence, instead of depending on blood thinners, physicians can move to herbal remedies. According to certain reports, antioxidants can prevent oxidative stress, hepatocellular damage, and problems associated with blood coagulation and hematology. *J. aurea* is abundant with antioxidants, which could be the contributors to its anticoagulant action.

CONCLUSION

It can be concluded that both nonpolar and polar parts of *Justicia aurea* extract possess variable biological potentialities. However, the water fraction containing polar components showed better antihyperglycemic, anti-inflammatory, and anticoagulant effects than the petroleum ether fraction containing nonpolar components. Further studies are required to isolate and identify the secondary metabolite(s) liable for these biological activities.

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REFERENCES


