Original Research

Phytochemical Screening and Pharmacological Activities of Entada Scandens seeds

Dey SK¹, Hira A¹, Ahmed A¹, Howlader Md. SI², Khatun A³, Rahman M⁴*, Siraj Md. A¹

¹Pharmacy Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh
²Department of Pharmacy, World University of Bangladesh, Dhaka-1205, Bangladesh
³Department of Pharmacy, Manarat International University, Dhaka-1216, Bangladesh
⁴Phytochemistry and Pharmacology Research Laboratory, Faculty of Health Sciences, Department of Pharmacy, Northern University Bangladesh, Dhaka-1205, Bangladesh

Summary. Entada scandens (E. scandens) (family. Mimosaceae) is a widely used medicinal plant has been traditionally used by the folklore medicinal practitioners of Bangladesh to treat pain, cancer, gastrointestinal disorders where antinociceptive, cytotoxic and anti-diarrheal medications are implicated. Therefore, phytochemical groups and antinociceptive, cytotoxic, and anti-diarrheal activities of ethanol extract of seed of E. scandens were investigated by using acetic acid induced writhing model in mice, brine shrimp lethality bioassay and castor oil induced diarrheal model in mice. Phytochemical study of the extract indicated the presence of alkaloids, glycosides, tannins, flavonoids and saponins. At the doses of 250 and 500 mg/kg body weight, the extract showed a significant antinociceptive activity showing 60.61 and 72.73% inhibition respectively (P<0.001) comparable to that produced by Diclofenac Na (80.30%) used as standard drug. The study tends to suggest the antinociceptive, cytotoxic and anti-diarrheal activities of the crude ethanol extract of the seed of E. scandens and justify its use in folkloric remedies.

Industrial relevance. Medicinal plants can form an excellent source for derivation of lead compounds or newer drugs. The knowledge base of folk medicinal practitioners can in this instance form an invaluable source on which further scientific studies may be based, for the folk medicinal practices of the Kavirajes date back to centuries ago. They also have proved to be a rich source of new active compounds which are less toxic and less costly when compared to the synthetic drugs. The present study will help the industry to produce herbal drug with less side effect, economically affordable and more effective in the treatment of pain, diarrhea and inflammation processes. Finally the phytochemical screening from the plant would be effective drug for the antinociceptive, cytotoxic and anti-diarrheal activities of the crude ethanol extract of the seed of E. scandens.

Keywords. Entada scandens; Phytochemical; Antinociceptive; Anti-diarrheal; Cytotoxic.

INTRODUCTION

Entada scandens auct. non. (L) Benth. (Synonym. E. monostachya DC., E. rheedii Spreng, Mimosa entada Linn.) belonging to Mimosaceae (or Mimosoidea), a subfamily of Leguminosae, locally known as Gila Lata (Bangla), Giley Ludi (Chakma) is a large woody climber (Joshi SG, 2000). The plant is found in Africa, Tropical Asia, Australia and in the small part of the Pacific Island. In Bangladesh, the species is commonly occurring in hilly tracts of Sylhet and Chittagong. The plant is also grown in the Asam in India where the seed of the plant is known as Mokori ghila. The plant has been used by the folkloric medicinal practitioners for long. The plant is used in the treatment of skin ulcer (cancer), snake bite, stomach disorders and ureterolithiasis (Uddin NS, 2006). The study of the plant is used to treat fever, dysentery and rheumatism (Barukial J and Sarmah JN, 2011). Seeds are used in pains of the loins, in debility and in glandular swelling. They are given internally as an emetic. The kernel of the seed is employed by the people living in the hills as febrifuge (Kirtikar KR and Basu BD, 2006).

In a previous study, a potent kunitz type trypsin inhibitor was reported by Lingaraju MH et al. (Lingaraju MH and Gowda LR, 2008) and the determination of xenopus index and haemolytic index in seeds of E. scandens Benth was reported by Marthe Blyberg (Blyberg M, 1960). Janardhanan et al. reported the chemical composition and antinutritional factors (Janardhanan K and
Nalini K, 1991). Gedeon J reported the presence of saponins and sapogenins (Gedeon J, 1954). Sasipriya G et al. reported the effect of different processing methods on antioxidant activity in the seed kernel (Sasipriya G and Siddhuraju P, 2012). Gautam B et al. reported the bioactive compounds, antioxidant activity and type II diabetes-related enzyme inhibition properties of the seed of the plant (Gautam B et al., 2012). Vadivel et al. reported the total free phenolic content of the wild legume of the plant (Vadivel et al., 2011). Dinesh Kumar et al. reported the potential antifertility agents in the plant (Dinesh Kumar et al., 2012).

Since no literature is currently available to substantiate phytochemical, antinociceptive, cytotoxic and antidiarrheal activities of the ethanol extract of *Entada scandens* seeds, therefore the present study is a part of our on-going pharmacological screening of selected Bangladeshi medicinal plants (Ahmed F et al., 2008a; Ahmed F et al., 2010a; Ahmed F et al., 2010b; Ahmed F et al., 2008b; Faisal KS et al., 2012; Naheed M et al., 2012; Nayem AA et al., 2011; Rahman M et al., 2010; Sadhu SK et al., 2007a; Sadhu SK et al., 2008; Sadhu SK et al., 2007b; Siraj MA et al., 2012a; Siraj MA et al., 2013b; Khatum A et al., 2013) and designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antinociceptive, cytotoxic and antidiarrheal activities that also confirm its use as pain killer, use against skin ulcer and cancer and other pathological conditions.

**Figure 1. Seeds of *Entada scandens***

**MATERIALS AND METHODS**

Collection and identification of plant materials. *Entada scandens* was collected from Rangamati in the month of December 2011. The plant was mounted on paper and the species was taxonomically confirmed by Sarder Nasir Uddin, Principle Scientific Officer, Bangladesh National Herbarium (BNH), Mirpur, Dhaka. The voucher specimen of the plant has been deposited and preserved in BNH library for further collection and reference and an accession no was provided as DACB-34179.

Preparation of ethanol extract. The seeds of *Entada scandens* were freed from any of the foreign materials. Then the seed were chopped and air-dried under shed temperature followed by air drying. The dried plant materials were then ground into powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). About 250g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1000ml of 80% ethanol. The container with its contents was coarse filtration through a piece of clean, white cotton material. Then it was filtered through Whatman filter paper (Bibby RE200, sealed and kept for a period of 15 days accompanying occasion al shaking and stirring. The whole mixture then underwent a acetic acid induced writhing model in mice (Nayeem AA et al., 2011). Dinesh Kumar et al. reported the potential antifertility agents in the plant (Dinesh Kumar et al., 2012).

Test for different chemical groups. The crude ethanolic extract was subjected for phytochemical study using standard methods (Rahman M et al., 2010) for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins. In each test 10% (w/v) solution of the extract in ethanol was taken.

Test Animals & Drug. Young Swiss-albino mice of either sex, 3-4 weeks of age, weighing 20 -25 g, were used for *in vivo* pharmacological screening. Mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B). They were housed in standard environmental conditions at animal house of Pharmacy Discipline, Khulna University and fed with rodent diet and water ad libitum. All experimental protocols were in compliance with Khulna University Ethics Committee on Research of Animals as well as internationally accepted principles for laboratory animal use and care. The standard drug diclofenac Na was used for this study and collected from Square Pharmaceuticals Ltd, Bangladesh.

Bacterial strains. Bacterial strains were collected from the Microbiology Laboratory of International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh. Test organisms include Gram negative bacteria- *Escherichia coli*, *Pseudomonas aeruginosa*, *Plesiomonas shigellosis*, *Salmonella typhi*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, *Shigella sonnei*, *Proteus vulgaris*; Gram positive bacteria- *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Staphylococcus aureus*, *Streptococcus pyogenes*; *Staphylococcus epidermidis*.

Antinociceptive activity. The antinociceptive activity of the crude ethanolic extract of *Entada scandens* was studied using acetic acid induced writhing model in mice (Nayeem AA et al., 2011). The animals were divided into control, positive control and test groups with five mice in each group. The animals of test groups received test substance at the doses of 250 and 500
mg/kg body weight. Positive control group was administered with diclofenac Na (standard drug) at the dose of 25 mg/kg body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed for writhing (constriction of abdomen, turning of trunk and extension of hind legs) for 5 min.

**Hatching of shrimp.** Artificial sea water was prepared by dissolving 20 g of NaCl and 18 g of table salt in one liter of distilled water and was filtered off to get a clear solution. A rectangular tank was divided in to two unequal compartments by a porous separator. The larger compartment was darkened while the smaller one was kept illuminated. The eggs of *Artemia salina* were hatched at room temperature (25-30 ºC) for 18-24 h. The larvae (nauplii) were attracted by the light and moved to the smaller compartment through the holes. They were then collected by a Pasteur pipette.

**Brine shrimp lethality bioassay.** The method of Meyer et al. was adopted to study the general toxicity of the extract (Meyer BN et al., 1982). The sample was dissolved in DMSO and then transferred to vials to get concentrations of 160, 80, 40, 20, 10 and 5µg/ml in 5 ml artificial sea water with ten nauplii in each vial. The concentration of DMSO did not exceed 0.01% in any of the vial. Control vials containing DMSO in artificial sea water at the same concentration as in test vials were also taken while anticancer drug 5-fluro uracil was used as positive control with the same concentration. After 24 h incubation at room temperature (25-30 ºC), the number of viable nauplii were counted using a magnifying glass.

**Antibacterial assay by disc diffusion assay.** Sterile blank discs (BBL, Cocksville, USA) were impregnated with test substances at the dose of 400 and 600 µg/disc. These discs, along with positive standard disc (30 µg/disc) (Kanamycin, Oxoid Ltd., UK) and negative control discs were placed in Petri dishes containing the Mueller-Hinton agar medium seeded with the test organisms using sterile transfer loop and kept at 4ºC to facilitate maximum diffusion. The plates were then kept in an incubator (37ºC) to allow the growth of the bacteria. The antibacterial activities of the test samples were determined by measuring the diameter of the zone of inhibition in terms of millimeter (Rahman M et al., 2010).

**Antidiarrheal activity.** Antidiarrheal activity was tested by using Castor oil induced diarrheal method in mice (Ahmed F et al., 2008; Shoba FG et al., 2001). Twenty Swiss albino mice were randomly divided in to four groups (n=5). Control group received only distilled water (2ml/mice), positive control group received loperamide (50mg/kg body weight) as standard and test groups received the extracts at the doses of 250mg and 500mg/kg body weight. Mice were housed in separate cages having paper placed below for collection of fecal matters. Diarrhea was induced in the mice by oral administration of castor oil (1.0ml/mice). Extract and drugs were given orally 1hr before the administration of castor oil. The time for first excretion of feces and the total number of fecal output by the animals were recorded for 4 hours. Normal stool was considered as numerical value 1 and watery stool as numerical value 2. Percent inhibition of defecation in mice was calculated by using the following equation.

\[
\% \text{ inhibition} = \frac{(Mo–M)}{Mo} \times 100; \text{ where, } Mo = \text{Mean defecation of control and } M = \text{Mean defecation of test sample.}
\]

**Statistical Analysis.** All the in vitro experimental results were given as mean±SEM of three parallel measurements and data were evaluated by using student’s t test. Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet’s multiple comparisons. The significant difference between the control group and experimental groups was determined. The results obtained from samples and control group were plotted in standard diagrams and good level of significance were found. P values<0.001 were regarded as significant.

**RESULTS**

**Chemical group test.** Results of different chemical tests on the ethanolic extract of *Entada scandens* seed showed the presence of alkaloid, glycoside, tannins, flavonoid and saponins and presented in Table 1.

**Table 1. Results of different group tests of ethanolic extract of Entada scandens seed.**

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Ethanol extract of Entada scandens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Gums</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>-</td>
</tr>
</tbody>
</table>

**Antinociceptive activity.** Table 2 showed the effect of the ethanol extract of *Entada scandens* on acetic acid induced writhing in mice. At the dose of 250 & 500 mg/kg of body weight, the extract produced 60.61 & 72.73% writhing inhibition in test animals respectively. The results were statistically significant (P <0.001) and was comparable to the standard drug Diclofenac Na, which showed 80.30% writhing inhibition at a dose of 25 mg/kg weight (Figure 2).
Pharmacological Activities of Entada Scandens seeds

Table 2. Effects of the ethanolic extract Entada scandens on acetic acid induced writhing of mice (n=5).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment and Dose</th>
<th>Number of writhes (% Writhing)</th>
<th>% Writhing Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1% tween 80 solution 10 ml/kg, p.o.</td>
<td>13.20±0.59</td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td>Diclofenac Na 25 mg/kg, p.o.</td>
<td>2.6 ± 0.51 * (100)</td>
<td>80.30</td>
</tr>
<tr>
<td>Test Group- 1</td>
<td>Et. Extract of E. scandens 250 mg/kg, p.o.</td>
<td>5.20 ± 0.38 * (39.39)</td>
<td>60.61</td>
</tr>
<tr>
<td>Test group- 2</td>
<td>Et. Extract of E. scandens 500 mg/kg, p.o.</td>
<td>3.6 ± 0.40 * (27.27)</td>
<td>72.73</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (Standard Error for Mean); Et. Ethanol; * indicates P < 0.001; one-way ANOVA followed by Dunnet’s test as compared to control; n = Number of mice; p.o. per oral.

Figure 2. Percent writhing inhibition of acetic acid induced writhing in mice by the extract of Entada scandens

Brine shrimp lethality bioassay. In brine shrimp lethality bioassay (Table 3), the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. For the extract, the number of nauplii died and percent mortality was counted. From the plot of percent mortality versus log concentration (Figure 3), LC50 and LC90 were deduced (LC50: 20 μg/mL; LC90: 80 μg/mL) while the LC50 and LC90 of the standard anticancer drug 5-fluoro uracil were 4.5 μg/mL and 6.5 μg/mL respectively. DMSO was used as a solvent. Control was used to see whether DMSO had any effect on brine shrimp lethality. The control group of brine shrimp nauplii without and without DMSO exhibited no mortality.

Table 3. Brine shrimp lethality bioassay of the ethanol extract of Entada scandens

<table>
<thead>
<tr>
<th>Test sample</th>
<th>Conc. (μg/ml)</th>
<th>Log of (Conc.)</th>
<th>No. of alive shrimp</th>
<th>% mortality</th>
<th>LC50 (μg/ml)</th>
<th>LC90 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract of Entada scandens</td>
<td>5</td>
<td>0.69</td>
<td>7</td>
<td>30</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1</td>
<td>6</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>1.3</td>
<td>5</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>1.7</td>
<td>3</td>
<td>70</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>1.9</td>
<td>1</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>2.2</td>
<td>0</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Antibacterial activity. The antibacterial property of the extract was assessed by conventional disc diffusion method using dried extracts of 500 µg/disc against a panel of 16 pathogenic bacterial strains and the results were compared with the activity of the positive control, kanamycin (30 µg/disc). But the extract did not show any significant zone of inhibition against the test organisms.

Antidiarrheal activity. Table 4 showed the effect of the ethanol extract of seed of *Entada scandens* on castor oil induced diarrheal method in mice. The results (Table 4) showed that the extract inhibited mean number of defeaction which were 13.21% (P<0.01) and 22.64 % (P<0.001) at the doses of 250 and 500mg/kg respectively. The latent periods (1.01 and 1.24 hr) for the extract treated group at the doses of 250 and 500mg/kg respectively were (p<0.01) increased (Figure 4) as compared to control group (0.65 hr).

**Table 4.** Antidiarrheal activity of *E. scandens* in castor oil induced diarrheal test method on mice (n=5).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose</th>
<th>Latent period</th>
<th>Defecation</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>2ml/mice, p.o.</td>
<td>0.65±0.06</td>
<td>10.6±0.25</td>
<td>--</td>
</tr>
<tr>
<td>Loperamide</td>
<td>50mg/kg, p.o.</td>
<td>3.5±0.16**</td>
<td>4±0.32**</td>
<td>62.26</td>
</tr>
<tr>
<td>Et. Extract <em>Entada scandens</em></td>
<td>250 mg/kg, p.o.</td>
<td>1.01±0.09*</td>
<td>9.2±0.37*</td>
<td>13.21</td>
</tr>
<tr>
<td>Et. Extract <em>Entada scandens</em></td>
<td>500 mg/kg, p.o.</td>
<td>1.24±0.18*</td>
<td>8.2±0.49**</td>
<td>22.64</td>
</tr>
</tbody>
</table>

*Values are expressed as mean±SEM (Standard Error for Mean); Et. Ethanol; *P<0.01 ; **P< 0.001; n = Number of mice; p.o. per oral*

**DISCUSSION**

The ethanol extract of seed of *Entada scandens* demonstrated the presence of alkaloids, tannins, saponins, flavonoids, and reducing sugar as secondary metabolites with cytotoxic, analgesic and antidiarrhoeal activities. The plant was reported for saponins and sapogenins, free phenolic content and of antioxidant activities (Gedeon J, 1954; Sasipriya G *et al.* 2012; Vadivel V *et al.*, 2011). Presence of saponins, sapogenins and phenolic contents may indicate the presence of tannins, saponins, flavonoids and other phenolic compounds (Gedeon J, 1954; Siddhuraju P, 2012; Gautam B *et al.*, 2012; Vadivel V *et al.*, 2011).
Analgesic activity of the extract was tested by acetic acid induced writhing model in mice. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings. The peripheral analgesic effect of the plant’s extract may be mediated via inhibition of cyclooxygenases and/or lipoxigenases (and other inflammatory mediators), while the central analgesic action of the extract may be mediated through inhibition of central pain receptors. This hypothesis is in consonance with those of Koster et al. and Williamson et al. who postulated that acetic acid-induced writhing method is useful technique for the evaluation of peripherally acting analgesic drug (Koster et al., 1959; Williamson et al., 1996). With respect to the writhing test, the research group of Derardt et al. described the quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of rats, obtained after intraperitoneal injection of acetic acid (Derardt et al., 1980). These authors found high levels of prostaglandins PGE2 and PGF2α during the first 30 min after acetic acid injection. The extract produced significant writhing inhibition (60.61% at dose 250 mg/kg and 72.73% at dose 500 mg/kg body weight) comparable to standard drug diclofenac sodium (80.3% at dose 25 mg/kg body weight). On the basis of the result of acetic acid induced writhing test, it can be concluded that the ethanol extract of *E. scandens* might possess a peripherally acting antinociceptive activity.

Brine shrimp lethality bioassay is an easy and straight forward bench top screening method for predicting important pharmacological activities like enzyme inhibition, ion channel interference, antimicrobial and cytotoxic activity (Nayeem AA et al., 2011; Rahman M et al., 2010; Meyer BN et al., 1982; Anderson JE et al., 1991). In the present study the extract showed LC50 at a low concentration indicating that the extract is significantly potent. Ideally, any agent useful in the treatment of cancer should not be toxic to normal cell. However, in reality, anticancer agents are often toxic to normal cells, particularly towards rapidly growing cells (Priestman T, 2008). It is necessary to test this extract against various cancer cell lines as well as normal cell lines to justify the potential to further investigate this plant for anticancer activity. Further investigation is required to find the responsible compound(s) for the cytotoxic activity observed for *E. scandens*.

Compared to control animals, the extract inhibited significantly the frequency of defecation and reduced greatly the wetness of faecal excretion. As with other laxatives, castor-oil changes the intestinal permeability and the histology (Mascolo N et al., 1993). The findings provide a support for the use of the plant as antidiarrhoeal remedies in Bangladeshi folk medicine.

**CONCLUSION**

Present study is based on the report of preliminary biological screening of *E. scandens* seed extract. The results are quite promising; support the use of the plant in traditional medicine and demands further investigation. Advanced studies including LC-MS can be carried out to get a bigger picture of the chemical constituents present in the plant. Screening methods applying various cell lines or bacterial enzymes can be carried out to find the underlying mechanism for the observed biological activities. On the basis of the results from above studies, bioassay guided approach can be undertaken to isolate and identify the active component(s).

**ACKNOWLEDGEMENT**

We are grateful to Khulna University Research Cell for providing us fund to carry out this work. We thank the Head of the Departments, Department of Pharmacy, Northern University Bangladesh and Manarat International University for allowing us to use their test procedures.

**REFERENCES**


