



## Phytochemical and pharmacological investigation of *Xanthium indicum* Koen. (family-compositae)

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### Abstract

*Xanthium indicum* (Koen.) belongs to the family, Compositae, well known in traditional medicine systems in Bangladesh for the treatment of various ailments. The ethanol extract of the leaves of *Xanthium indicum* Koen. was subjected to pharmacological investigation to evaluate analgesic, antioxidant, and antibacterial activity. Phytochemical analysis of the extract indicated the presence of reducing sugars, tannins, carbohydrates, glycosides, alkaloids, and flavonoids. The ethanol extract showed statistically significant analgesic activity ( $P<0.001$ ) in acetic acid induced writhing test in Swiss-Albino mice at the doses of 250 and 500 mg/kg-body weight. The extract inhibited the acetic acid induced writhing in mice (47.5%  $P<0.001$  and 24.55%  $P<0.05$ ) at the dose of 500 and 250 mg/kg body weight respectively and it is comparable to the activity of the standard analgesic drug Diclofenac sodium (67.96%  $P<0.001$ ) at the dose of 25 mg/kg body weight. The extract showed free radical scavenging activity in DPPH (1,1-Diphenyl-2-picrylhydrazyl) assay. In quantitative assay, the extract exhibited DPPH radical scavenging activity with the  $IC_{50}$  value of 141.25  $\mu$ g/mL. In the castor oil-induced diarrhoeal mice, the ethanol extract at the dose of 500 mg/kg, reduced the total number of faeces as well as of diarrhoeic faeces, and the result was statistically significant. The results tend to suggest that the extract might possess some chemical constituents that are responsible for analgesic, antioxidant, and anti-diarrhoeal activity.

KEY WORDS: XANTHIUM INDICUM, WRITHING TEST, DICLOFENAC SODIUM, 1,1-DIPHENYL-2-PICRYLHYDRAZYL (DPPH), CASTOR OIL

## Introduction

*X. indicum* locally known as Ghagra, Banokra, Bichaphal is a coarse annual about a meter or more in height. Leaves are numerous, 5-7.5 cm long and almost as broad as long, broadly triangular-ovate or suborbicular, acute, often 3-lobed, rough with appressed hairs, irregularly inciso-serrate. Heads in terminal and axillary racemes. Fruits are ovoid; about 1.6 cm long, with 2 erect mucronate beaks, thickly clothed with usually hooked prickles. It grows as a gregarious weed in fallow paddy fields and by the canal or ditch banks in all areas of Bangladesh. The plant is reported to have diaphoretic, diuretic, sudorific, CNS depressant and styptic properties. Decoction of the plant is used in urinary and renal complaints, gleet, leucorrhoea and menorrhagia. Seeds are used to resolve inflammatory swellings while the root being useful against scrofulous tumours and cancer. The root extract of the plant is reported to be employed in cancer and scrofula, the fruits are rich in vitamin B and are utilized as demulcent and said to be effective in treating small pox, herpes and bladder affections. The plant is reported to contain alpha and gamma tocopherols, polyphenols, glucoside, xanthostrumarin and xanthonolides as the principal constituents(1).

Medicinal plants are still valuable source of safe, less toxic, lower price, available and reliable natural resources of drugs all over the world. Plants have been used as remedies and still they play an important role in health care for about 80% of the world's population from the beginning of civilization. The therapeutic basis of herbal medication has formed by the presence of diverse bioactive compounds like steroids, terpenoids, flavonoids, alkaloids, glycosides etc. in plants. Also for the treatment of diseases which are still incurable, medicinal plants can serve as a source of novel therapeutic agent. Considering the importance of this area and as a part of our ongoing investigation on local medicinal plants of Bangladesh(2).

In this project work, an attempt was made to justify the traditional uses as per scientific experi-

ments. In the present study, we therefore tried to evaluate the analgesic, antioxidant, and antidiarrhoeal activity of the ethanol extract of leaves of *X. indicum*.

## Materials and Methods

### Sample collection and extraction

The leaves of *X. indicum* were collected from Khulna University, Bangladesh, 2011 and identified by the experts at Bangladesh National Herbarium, Mirpur, Dhaka (Accession no.: DACB 35522). The leaves were shade dried. After sufficient drying, the leaves were cut into small pieces, and then slashed to coarse powder with the help of mechanical grinder. The powder was stored in a suitable container to avoid any possible fungal attack.

Cold extraction technique was applied for extracting leaves. About 120 mg of powder was extracted by maceration for 7 days with 500 mL of ethanol accompanying regular shaking and stirring. The extract was filtered off with clear cotton plug to remove plant debris. The solvent was evaporated at room temperature with an electric fan to get the dried crude extract (yield value 10.4%). After drying, the crude extract was stored in refrigerator at 4°C.

### Test Animals and Pathogens

Swiss-Albino mice of either sex (20-25 gm) were collected from animal resources department of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and were used for the current experiment. The animals were kept in the polypropylene cages and provided with standard rodent foods (ICDDR, B formulated). The animals were acclimatized in animal house, Pharmacy Discipline, Khulna University under the standard laboratory condition (relative humidity 55-60%, room temperature  $25\pm2^{\circ}$  C, and 12 hours light: dark cycle) for period of 7 days prior to the pharmacological experiment.

### Chemicals, Reagents and Standard Drugs

Acetic acid, Castor oil and ascorbic acid were

purchased from Merck, Germany. 1,1-Diphenyl-2-pycrylhydrazyl (DPPH) was obtained from Sigma-Aldrich, USA. Loba Chemie Pvt Ltd, India supplied Tween-80. Solvents were of analytical grade. Diclofenac sodium was obtained from Beximco Pharmaceuticals Ltd, Bangladesh.

### **Phytochemical Tests**

The crude extract was subjected to preliminary phytochemical screening for the detection of major functional groups present in the extract (3). The extract showed the presence of reducing sugars, tannins, carbohydrates, glycosides, alkaloids, and flavonoids.

### **Evaluation of Analgesic Activity**

The analgesic activity of the sample was studied using acetic acid induced writhing test in mice (4, 5). Experimental animals were randomly selected, and divided into four groups denoted as control, positive control, and test group I and II consisting of six mice in each group.

Control group received 1% Tween-80 in distilled water at the dose of 10 mL/kg body weight, and positive control group received diclofenac sodium at the dose of 25 mg/kg body weight. Test group I and II were treated with the test sample at the doses of 250 and 500 mg/kg body weight. All the treatments were provided in oral route. A thirty minutes interval was given to ensure proper absorption of the administered treatments. Then acetic acid solution (0.6%) was administered via intra-peritoneal route to each animal as writhing inducer. An interval of 5 minutes was given for absorption of acetic acid. Then number writhing was counted for 10 minutes. Percent inhibition of writhing was calculated and compared with the control group.

### **In Vitro Antioxidant Activity Test**

Antioxidant activity of the ethanol leaves extract was estimated by using stable free radical DPPH (1, 1-Diphenyl-2-pycrylhydrazyl) both qualitatively and quantitatively (6-9).

### **Qualitative Analysis**

Thin Layer Chromatographic (TLC) technique was

applied for qualitative assessment (7-9). TLC plates were developed with non-polar, medium polar, and polar solvent systems to resolve compounds of different polarities. The TLC plates were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH (yellow on purple background) radical was observed for the period of 30 min and noted.

### **Quantitative Analysis**

The anti-oxidant potential of the ethanol extract was estimated on the basis of their scavenging activity of the stable DPPH radical (7-9). At first 2.5 mg extract was mixed with 25 mL of ethanol to prepare 100 µg/mL solution of the extract as stock solution. Another six concentrations of sample were prepared by serial dilution method. These concentrations were 50, 25, 12.5, 6.25, 3.13, and 1.57 µg/mL. Then 1 mL of solution of each concentration was taken into test tubes designed for each concentration. 3 mL of 0.004% DPPH solution was then added to each test tube, and kept for 30 minutes at dark place to allow any reaction that is to be occurred. After 30 minutes, absorbance was measured by UV spectrophotometer at 517 nm. Ascorbic acid was used as standard. Percent inhibition was calculated using the following formula:

$$\% \text{ inhibition} = [( \text{Absorbance of control} - \text{Absorbance of sample or standard}) / \text{Absorbance of control}] \times 100$$

$IC_{50}$  value was determined from % inhibition vs. concentration graph.

### **In Vivo Anti-diarrhoeal Activity**

#### **Castor Oil Induced Diarrhoea**

The experiment was carried out according to the castor oil induced diarrhea method (10). Experimental mice were selected based on their sensitivity to castor oil-induced diarrhoea and divided into four groups ( $n = 5$ ). Test groups were treated with the bark extract (250 and 500 mg/kg, p.o.) and positive control group was provided with loperamide (3 mg/kg, p.o.) in suspension form. Control group was treated with 1% tween-80 in distilled water (10 mL/kg, p.o.). Each mouse was

provided with 0.5 mL of castor oil in oral route after the interval of 60 min for inducing diarrhoea. Each mouse was housed in individual plastic transparent cage and floor was lined with clean white blotting paper which was changed in every hour throughout the observation period of 4 h. Onset of diarrhoea and the number of stool for each mouse was counted. For the assessment of anti-diarrhoeal activity, onset of diarrhoea and percent inhibition of defecation were compared with the control group.

### Statistical Analysis

Student's t-test was used to determine significant differences between the control and test group. Results were considered as statistically significant when  $P<0.05$ .

## Results

### Phytochemical Tests

The ethanol extract was subjected to different qualitative phytochemical tests for detection of different biologically active chemical groups and the results are summarized in the Table 1.

Phytochemical groups	Results
Reducing sugars	+
Glycosides	-
Tannins	+
Saponins	+
Gums	+
Steroids	+
Alkaloids	+
Flavonoids	+

+ = Presence - = Absence

Table 1: Phytochemical tests of *X. indicum*

### Acetic Acid-Induced Writhing Test

The extract showed 29.67% ( $P<0.01$ ) and 59.86% ( $P<0.001$ ) inhibition of writhing in dose dependent manner at the doses of 250 and 500 mg/kg body weight respectively, which was highly comparable to diclofenac sodium 75.44% ( $P<0.001$ ) at the dose

of 25 mg/kg body weight.

see Table 2.

### DPPH Scavenging Activity

The extract exhibited DPPH radical scavenging activity with the  $IC_{50}$  value of 141.25  $\mu$ g/mL that is highly comparable to the ascorbic acid.

See Graph 1.

### Castor Oil Induced Diarrhoea Activity

In castor oil induced diarrhoea, the extract showed dose dependent and significant activity by considerable increasing in latent period of diarrhoea as well as decline in frequency of defecation. The percent inhibition of defecation is considered in various hours.

See Table 3.

## Discussion

The ethanolic extracts of the plant shows the presence of some promising phytochemicals, namely, reducing sugars, tannins, saponins, gums, steroids, alkaloids, and flavonoids.

For assessing *in vivo* analgesic activity in mice, acetic acid induced writhing model in mice is most extensively used. In this model, peripherally performing analgesic activity of the sample is assessed by inducing writhing through the sensitization of locally active peritoneal receptors by the release of endogenous substances. Generally, acetic acid liberates several endogenous substances like serotonin, bradykinins, histamine, prostaglandins (PGs), and substance P which are responsible for inducing pain by stimulating nerve endings. Locally active peritoneal receptors activate the abdominal constrictions response (11). The abdominal constriction response induced by acetic acid is a responsive procedure to estimate peripherally acting analgesics (12). The most credible pathway of peripherally acting analgesics may be the embarrassment of

prostaglandins (PGE<sub>2</sub> and PGE<sub>2α</sub>) synthesis.

The presence of saponin, tannins, reducing sugars, gums, flavonoids, and alkaloids in the ethanol extract of *X. indicum* may be accountable for the investigated activities, because it is well established that these phytochemicals may be responsible for a wide range of bioactivities (12-17).

*In vitro* antioxidant activity study illustrates that the extract must contains some promising antioxidant compounds. This activity was assessed by most widely used DPPH scavenging assay model. Here free radical, DPPH is converted to stable DPPH-H by accepting electron, or hydrogen radical, and deep violet colour of DPPH is converted to light yellow colour. The DPPH radical contains an odd electron, detected by UV spectrophotometer at 517 nm against blank and this absorption decreases due to the development of its non-radical form, DPPH-H due to the reduction with an antioxidant. DPPH radical scavenging activity of the extract was in concentration dependent manner that was comparable to the standard antioxidant ascorbic acid. Flavonoids may be responsible for this antioxidant activity (18- 20). Castor oil induced diarrhoeal model is very much rational for evaluating *in vivo* anti-diarrhoeal activity in mice. In this model there is the involvement of prostaglandins in causation of diarrhoea by castor oil through the release of ricinoleic acid. Ricinoleic acid causes irritation of the intestinal mucosa and subsequently increases bowel movement and poor absorption; ultimately watery diarrhoeal stools possess (10).

Numerous mechanisms are already reported in previous reports to explain the causes of castor oil induced diarrhoea such as inhibition of intestinal Na<sup>+</sup>, K<sup>+</sup>-ATPase activity, stimulation of prostaglandins formation through irritation of the intestinal mucosa, activation of adenylate cyclase mediated active secretion and involvement of nitric oxide.

## Conclusion

The extract of *X. indicum* demonstrated potential analgesic, antioxidant, and antibacterial activities.

The investigations were carried out using crude extract of the plant. Separation and isolation of pure compounds of the extract might be proved responsible for these pharmacological effects.

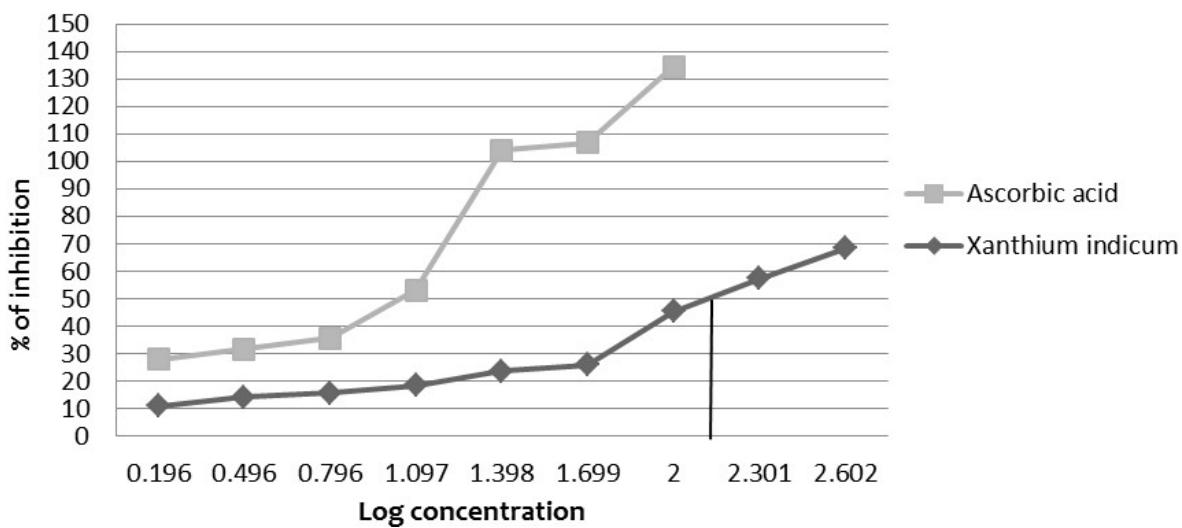
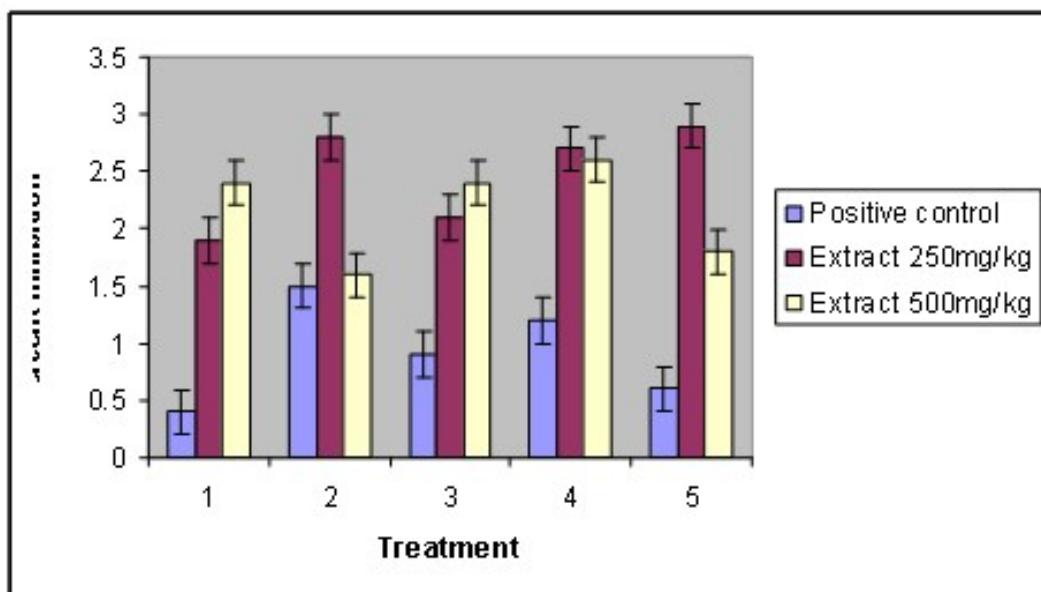
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Figure 1: DPPH scavenging activity of *X. indicum* leavesFigure 2: Effect of crude extract of *X. indicum* (Koen.) on castor oil induced diarrhoea of mice. Each bar represents percent of inhibition

Treatment	Dose (mg/kg, p.o.)	No of writhing (% writhing)	Inhibition (%)
Control (1% tween-80 solution in water, 10 mL/kg, p.o.)	-	44±3.312 (100)	0
Positive Control (Diclofenac sodium)	25	14.10±1.923 (32.04)	67.96**
Ethanol extract of <i>X. indicum</i>	250	33.20±1.475 75.45	24.55*
Ethanol extract of <i>X. indicum</i>	500	13.201±2.050 52.50	47.50**

Table 2: Effect of *X. indicum* leaves on acetic acid induced writhing in mice  
 Values are expressed as mean ± SEM (Standard Error of Mean); (n=5) n= number of mice;  
 \*\*P<0.001, \*P<0.05, vs. control, Student's t-test.p.o. = per oral.

group/treatment	(Percent of inhibition)				
	1 hr	2 hr	3 hr	4 hr	5 hr
I(control);0.7ml/Kg	5±0.8	5.6±0.32	2.4±0.15	3.2±0.62	3.0±0.25
Peroral, n=5					
II(Positive control) (Lopramide);50mg/Kg	0.4±0.25 <sup>b</sup>	1.5±0.52 <sup>a</sup>	0.9±0.117 <sup>a</sup>	1.2±0.32 <sup>c</sup>	0.6 ±021 <sup>a</sup>
Peroral, n=5	90	70	63	63	77
III (Ethanol extract) 250 mg/Kg	1.9±0.37 <sup>c</sup>	2.8±0.44 <sup>a</sup>	2.1±0.23	2.7±0.15	2.9±0.26
Peroral, n=5	62	50	13	16	12
IV(Ethanol extract) 500 mg/Kg	2.4±0.29 <sup>b</sup>	1.6±021 <sup>a</sup>	2.4±0.25	2.6±0.20	1.8±0.24 <sup>b</sup>
Peroral, n=5	52	71	0	19	31

Table 3: Effect of *X. indicum* (Koen.) on castor oil induced diarrhoea in mice  
 Values are t-test. ; cP<0.05; bP<0.02; aP<0.01 vs. control, Student's t-test. n=no. of mice