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In Vitro Antioxidant and *In Vivo* Antidiarrhoeal Activity of Hydromethanolic Extract of *Xanthium Indicum* Koenig. Leaves

Raushanara Akter
Assistant Professor, Department of Pharmacy, Stamford University Bangladesh
51 Siddeswari Road, Ramna, Dhaka-1217, Bangladesh
E-mail: raushanarabd@yahoo.com
Tel: +8801674899392

S. M. Raquibul Hasan
Lecturer, Department of Pharmacy, Stamford University Bangladesh
51 Siddeswari Road, Ramna, Dhaka-1217, Bangladesh
E-mail: smraquib_1114@yahoo.com
Tel: +8801716487173

Md. Mokarram Hossain
Lecturer, Department of Pharmacy, Stamford University Bangladesh
51 Siddeswari Road, Ramna, Dhaka-1217, Bangladesh
E-mail: smmh1123@yahoo.com
Tel: +8801727348428

Mariam Jamila
Lecturer, Department of Pharmacy,Bangladesh University, 15/1 Iqbal Road
Mohammadpur, Dhaka-1207, Bangladesh
E-mail: shimupharmacy@yahoo.com
Tel: +8801715029114

Md. Ehsanul Hoque Mazumder
Corresponding Author Assistant Professor, Department of Pharmacy
Jahangirnagar University, Savar, Dhaka-1342, Bangladesh
E-mail: munnapharmacy@yahoo.com
Tel: +88028913836

Shafiqur Rahman
Associate Professor, Department of Pharmaceutical Sciences
College of Pharmacy, South Dakota State University, USA
E-mail: Shafiqur.Rahman@sdstate.edu
Tel: 605-688-4239

Abstract

Xanthium indicum Koenig. is a medicinal herb belonging to the family Compositae, which has been reported to possess strong diaphoretic, antimalarial, anticancer and demulcent properties according to indigenous systems of medicine in Indian Sub-

Continent. But its antidiarrhoeal and antioxidant potential is still unknown. Therefore, the present study was designed to investigate antioxidant and antidiarrhoeal properties of hydromethanol extract of *X. indicum* leaves. The extract was examined for its antioxidant action using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and nitric oxide (NO) scavenging assays. The extract displayed a dose dependent scavenging of DPPH radical and NO. The extract was also studied for antidiarrhoeal property using castor oil and $MgSO_4$-induced diarrhoeal model and charcoal induced gastrointestinal motility test in mice. At the doses of 200 and 400 mg/kg body weight, the extract reduced the frequency and severity of diarrhoea in test animals throughout the study period. At the same doses, the extract delayed the intestinal transit of charcoal meal in test animals as compared to the control and the results were statistically significant (p <0.05). The results of the present study confirm antioxidant and antidiarrhoeal potential of the leaves of *X. indicum*, thus may provide the scientific basis for the traditional uses of this plant as the modality for diarrhoea and other pathological conditions where free radicals are implicated.

Keywords: *Xanthium indicum*, antioxidant, DPPH, NO, antidiarrhoeal property.

1. Introduction

Xanthium indicum Koenig. *locally* known as Ghagra or Bichphal (Beng.), is a coarse unarmed annual herb with alternate lobed leaves, bur-like flower-heads, and small oblong fruits covered with curved hooks. 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 (Ghani, 2003).

Moreover, the untapped wealth of plant kingdom is a major target for the search of new lead compounds in drug discovery. In Bangladesh, huge number of plants still remains unexplored. So well designed, systematic and objective research in this area might benefit our people who have been deluged with superfluity of disease, and who lack technological and economic resources to cope up with them with orthodox medicine. Considering the importance of this area and as a part of our ongoing investigation on local medicinal plants of Bangladesh (Hasan et al., 2009; Alam et al., 2008; Mazumder & Rahman 2008), in this paper, we reported a study of the antioxidant and antidiarrhoeal activity of the leaves of *X. indicum*.

2. Materials and methods

2.1. Chemicals and drugs

DPPH (1, 1-diphenyl, 2-picryl hydrazyl) was obtained from Sigma chemical co. USA, Ascorbic acid from SD Fine chem. Ltd., Biosar, India, Naphthyl ethylene diamine dihydrochloride from Roch-light Ltd., Suffolk, England and Sodium nitro prusside was obtained from Ranbaxy Lab., Mohali, India. Loperamide and Atropine were purchased from local market.

2.2. Plant material

For the present investigation, the *X. indicum* was collected from Boteshahor, Khadim Nagar, Sylhet, Bangladesh in February, 2008 at day time and was identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka where the Voucher specimen no:32785 has been deposited for reference. Then the leaves were dried in hot air woven at 55ºC for 5 days, coarsely powdered and extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus at 65ºC. The solvent was completely removed and the dried crude extract thus obtained was used for investigation.

2.3 Animal

For the present study, Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 gm, were collected from the animal research branch of the International Center for Diarrheal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: (24.0±1.0°C), relative humidity: 55-65% and 12hrs light/12 hrs dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for one week prior to performing the experiment.

2.4 Phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. These were identified by characteristic color changes using standard procedures (Ghani, 2003).

2.5 Tests for antioxidant activity

2.5.1 DPPH radical scavenging activity

The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1, 1-diphenyl-2- picrylhydrazyl (DPPH) free radical, was determined by the method described by Braca et al. (2001). Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from $[(A_0–A_1)/A_0]$ x100, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/ standard. The inhibition curves were prepared and IC_{50} values were calculated.

2.5.2 Nitric oxide scavenging assay

Nitric oxide radical scavenging was estimated on the basis of Griess Illosvoy reaction using method followed by Govindarajan et al (2003). In this investigation, Griess-Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *Xanthium indicum* extract (5 to 250µg/ml) or standard solution (ascorbic acid, 0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions.

2.6. Tests for antidiarrhoeal activity

2.6.1 Castor oil-induced diarrhoea

The experiment was performed according to the method described by Shoba and Thomas (2001). Briefly, mice fasted for 24 h were randomly allocated to four groups of five animals each. The animals were all screened initially by giving 0.5 ml of castor oil. Only those showing diarrhoea were selected for the final experiment. Group I received 1% CMC (10 ml/kg, p.o.), groups III and IV received orally the drug extract (200 and 400 mg/kg), respectively. Group II was given Loperamide (3 mg/ kg, p.o.) in suspension. After 60 min, each animal was given 0.5 ml of castor oil, each animal was placed in an individual cage, the floor of which was lined with blotting paper which was changed every hour, observed for 4 h and the characteristic diarrhoeal droppings were recorded.

2.6.2 Magnesium sulphate-induced diarrhoea

Diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals 30 min after pre-treatment with vehicle (1% Tween 80 in water, 10 ml/kg, p.o.) to the control group, loperamide (3 mg/kg) to the positive control group, and the methanol extract at the doses of 200 and 400 mg/kg to the test groups (Doherty, 1981).

2.6.3 Effect on gastrointestinal motility

Animals were divided into four groups of five mice each and each animal was given 1 ml of charcoal meal orally (5% activated charcoal suspended in 1% CMC) 60 min after an oral dose of the test drugs and vehicle. Group I was administered 1% CMC (10 ml/kg) and animals in groups III and IV received extract at the dose of 200 mg/kg and 400 mg/kg body weight respectively. Group II received atropine sulfate (0.1 mg/kg,) as the standard drug. After 30 min, animals were killed by light ether anaesthesia and the intestine was removed without stretching and placed lengthwise on moist filter paper. The intestinal transit was calculated as a percentage of the distance travelled by the charcoal meal compared to the length of the small intestine (Lutterodt, 1989).

2.7. Statistical Analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the control group. p values < 0.001 were considered to be statistically significant.

3. Results

Phytochemical analyses of the crude extract revealed the presence of alkaloid, tannin and flavonoid as represented below in Table 1.

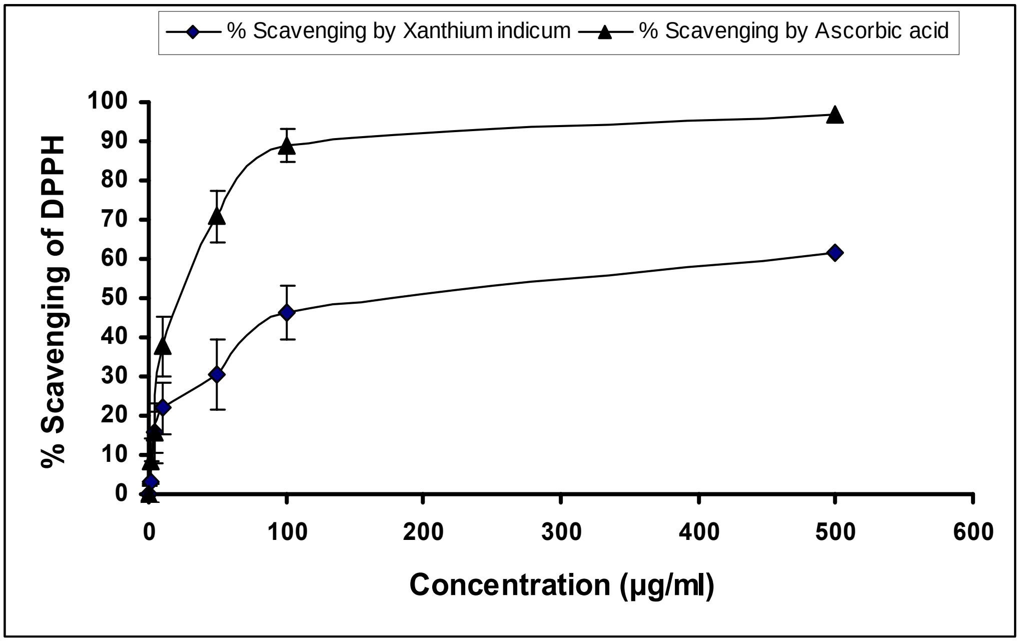
Table 1: Result of chemical group test of the crude extract of *X. indicum* leaves.

Extract	Steroid	Alkaloid	Reducing sugar	Tannin	Gum	Flavonoid	Saponin
HME of *X. indicum*	-	++	++	++	+	++	-

HME: Hydromethanolic extract; (+): Present; (-): Absent

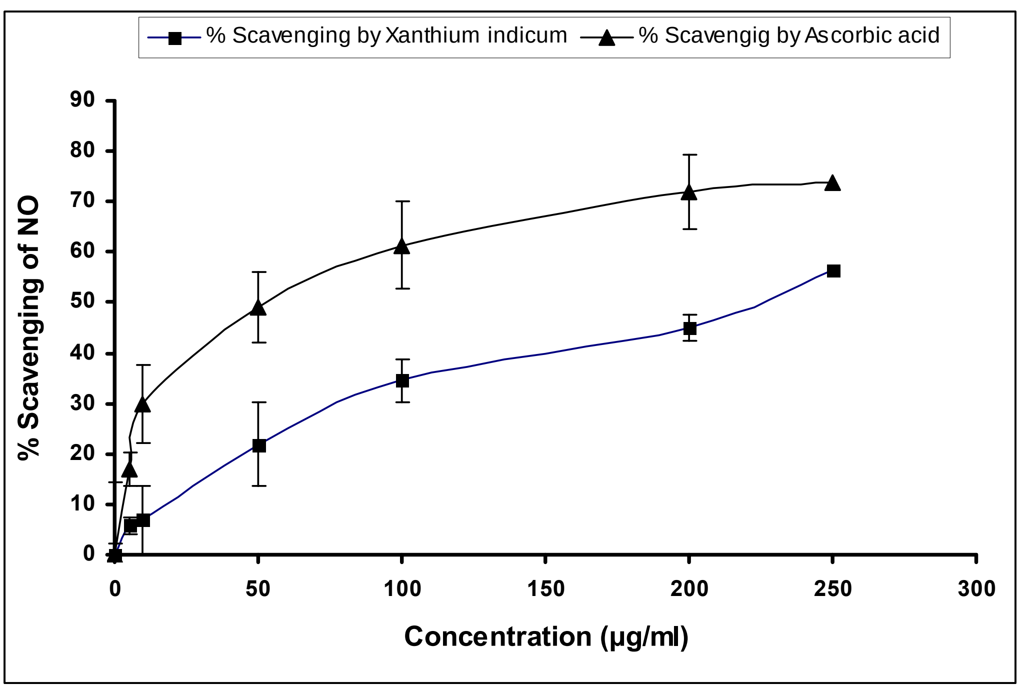
In DPPH radical scavenging assay, the extract showed dose dependent scavenging of DPPH radical as was with the reference ascorbic acid (Figure 1); the IC_{50} value of the extract was 33.54 µg/ml while the IC_{50} value for the reference ascorbic acid was 12.46µg/ml.

Figure 1: DPPH radical scavenging activity of the hydromethanol extract of *X. indium*. Values are the average of duplicate experiments and represented as mean ± SD.



Scavenging of nitric oxide was also found to rise with increasing concentration of the extract and the result was comparable to ascorbic acid which was used as the reference (Figure 2). The IC_{50} values of the extract and ascorbic acid were 11.5 µg/ml and 21.0 µg/ml respectively.

Figure 2: Nitric oxide scavenging activity of the hydromethanol extract of *X. indium*. Values are the average of duplicate experiments and represented as mean ± SD.



In castor oil-induced diarrhoeal model, the methanol extract of *X. indicum*, at the doses of 200 and 400 mg/kg, reduced the total number of faeces in a dose dependent manner (Table 2).

Table 2: Effect of *X. indicum* leaf extract on castor oil-induced diarrhoea in mice.

Groups	Treatment	Dose (p.o.)	No. of faeces in 4 h	% Inhibition of defaecation
Group-I	1% Tween 80 in water	0.4 ml/mouse	23.4±3.235	-
Group-II	Loperamide	10 mg/kg	6.6±2.391**	71.79**
Group-III	HME of *X. indicum*	200 mg/kg	17.4±3.117	25.64
Group-IV		400 mg/kg	12±1.826**	48.72**

Values are presented as mean ± SEM, (n = 5); ** $p < 0.05$, Dunnet test as compared to control. HME: Hydromethanolic extract.

The maximum inhibition of characteristic diarrhoeal feces was observed at 400 mg/kg dose of the extract, which was found to be statistically significant ($p < 0.05$). Similarly, the extract at 400 mg/kg dose level significantly ($p < 0.05$) reduced the extent of diarrhoea in test animals in magnesium sulphate-induced diarrhoeal experiment (Table 3).

Table 3: Effect of *X. indicum* leaf extract on $MgSO_4$-induced diarrhoea in mice.

Groups	Treatment	Dose (p.o.)	No. of faeces in 4 h	% Inhibition of defaecation
Group-I	1% Tween 80 in water	0.4 ml/mouse	19.4±1.245	-
Group-II	Loperamide	3 mg/kg	4.6±1.597**	76.29**
Group-III	HME of *X. indicum*	200 mg/kg	13.8±2.244	28.87
Group-IV		400 mg/kg.	8.8±2.070**	54.64**

Values are presented as mean ± SEM, (n = 5); ** $p < 0.05$, Dunnet test as compared to control. HME: Hydromethanolic extract.

However, both the doses were shown to reduce the total number of faeces when compared to control. In the gastrointestinal motility test, the methanol extract, at the doses of 200 and 400 mg/kg, retarded the intestinal transit of charcoal meal in mice when compared to the control (Table 4).

Table 4: Effect of *X. indicum* extract on charcoal meal-stimulated gastrointestinal transit

Treatment	Dose (p.o.)	Mean Intestinal length (cm)	Mean distance traveled by charcoal (cm)	% GI transit
1% Tween 80 in water	0.4 ml/mouse	66.4±3.104	51.2±2.070	77.54±3.250
Atropine	0.1 mg/kg	64.2±2.507	22±3.329**	33.70±3.928**
HME of *X. indicum*	200 mg/kg	61.6±2.852	37.6±37.6**	61.28±3.235**
	400 mg/kg.	64.6±2.778	30.2±2.935**	47.41±5.544**

Values are presented as mean ± SEM, (n = 5); ** $p < 0.05$, Dunnet test as compared to control. HME: Hydromethanolic extract.

3. Discussion

In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease (Liao and Yin, 2000). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals. Preliminary phytochemical screening of the extract revealed the presence of flavonoid, alkaloid and tannin. Polyphenolic compounds, like flavonoids, tannins and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity. Flavonoids and tannins present in the plant extract, as evident from phytochemical screening, may be responsible for the antioxidant action in the tested models. Moreover, nitric oxide is implicated for inflammation, cancer and other pathological conditions. Hence, nitric oxide scavenging capacity of the extract may help to arrest the chain of reactions initiated by excess generation of nitric oxide that are detrimental to the human health (Moncada et al., 1991).

Several mechanisms have been previously proposed to explain the diarrhoeal effect of castor oil including inhibition of intestinal Na^+,K^+-ATPase activity to reduce normal fluid absorption (Gaginella and Bass, 1978), activation of adenylate cyclase or mucosal cAMP mediated active secretion (Capasso et al., 1994), stimulation of prostaglandin formation (Galvez et al., 1993), platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil (Mascolo et al., 1996). However, it is well evident that castor oil produces diarrhoea due to its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion (Gaginella et al., 1975). Since the methanol extract of *Xanthium indicum* successfully inhibited the castor oil-induced diarrhoea, the extract might have exerted its antidiarrhoeal action via antisecretory mechanism which was also evident from the reduction of total number of wet faeces (not shown separately) in the test groups in the experiment. Again, flavonoids present in the plant extract are reported to inhibit release of autacoids and prostaglandins, thereby inhibit motility and secretion induced by castor oil (Veiga et al., 2001). The antidiarrhoeal activity of the extract may also be due to denature proteins forming protein tannates which make intestinal mucosa more resistant and reduce secretion.

On the other hand, magnesium sulphate has been reported to induce diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been reported that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water (Galvez et al., 1993; Zavala et al., 1998). The methanol extract was found to improve the diarrhoeic condition in this model. The extract may have increased the absorption of water and electrolyte from the gastrointestinal tract, since it delayed the gastrointestinal transit in mice as compared to the control. The delay in the gastrointestinal transit prompted by the extract might have contributed, at least to some extent, to their antidiarrhoeal activity by allowing a greater time for absorption.

4. Conclusion

The results of the present study led us to the inference that the plant extract possess modest antidiarrhoeal and antioxidant properties. Since the extract is reported to contain a range of compounds, it is difficult to ascribe these observed activities to any specific group of compounds. Hence, further studies are suggested to be undertaken to pin point the exact compound(s) and to better understand the mechanism of such actions of *X. indicum* scientifically.

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