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Phytochemical and Pharmacological Screening of *Senna tora* Roxb.

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Abstract: Phytochemical analysis of the dried aerial part of *Senna tora* (L.) Roxb. (Family-Fabaceae) indicated the presence of reducing sugars, tannins, steroids, saponins and gums. The pharmacological interest of these compounds, coupled with the use of this plant in traditional medicine prompted the authors to check *Senna tora* (L.) Roxb. for its probable antibacterial and analgesic activities. The dried aerial part of the plant was subjected to successive extraction with ethanol and the extract was used to investigate the activities. The extract showed mild antibacterial activity against tested microorganisms in agar diffusion method. The extract, however exhibited significant ($p<0.001$) inhibition of writhing reflex at the dose of 500 mg kg^{-1} body weight compared with the standard drug Diclofenac Sodium at the dose of 25 mg kg^{-1} body weight. The obtained result provided a support for the use of this plant in traditional medicine and its further investigation.

Key words: *Senna tora*, phytochemical study, agar diffusion method, antioxidant and analgesic activity

INTRODUCTION

In Bangladesh medicinal plants are extensively used in both raw and semi-processed forms as medicines in various pharmaceutical dosage forms. While some of these medicinal plants are procured from indigenous sources, most of them are obtained through imports from other countries, although many of these imported plants grow naturally or cultivated in this country. The contributions of the plants are enormous in every sector of human life. It helps to growing up of the human body and also protects human being from sickness by being used as medicine. A large number of plants are used as medicinal agents in this world. Specifically in Bangladesh about two hundred fifty species are used as medicinal plants (Ghani, 1990). It has now been established that the plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins possesses medicinal properties (Ghani, 1998). Recent trend is to integrate the traditional medicine with modern medicine but the best therapeutic results are said to be obtained often with the traditional Chinese system (Bannerman, 1982). *Senna tora* (L.) Roxb. belonging to the family Fabaceae is an indigenous shrub of Bangladesh and is widely distributed throughout the country. Several studies have been conducted throughout the last decade to investigate chemical and biological properties of *Senna tora*. Antihepatotoxic naphtha-pyrine glycosides were reported to be isolated from the seeds of *S. tora* (Wong *et al*., 1989). Antioxidant properties (Yen and Chuang, 2000) and inhibitory effect (Wu *et al*., 2001) of the extract of *S. tora* have already been reported. A recent study was conducted by the scientists of the Department of Food Science and Nutrition, Catholic University of Daegu, Korea who concluded that *Cassia tora* supplements can help improve serum lipid status in type-II diabetic subjects without significant adverse effect (Cho *et al*., 2005). In the recent study,

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screening for antibacterial as well as analgesic activity of the extract of *Senna tora* was conducted to provide support for the use of this plant as traditional medicine. Phytochemical screening provides knowledge of the chemical constituents of this plant not only for the discovery of new therapeutic agents, but also for information in discovering new sources of other economic materials. Therefore, in present an attempt was made to detect the presence of reported compounds by using various standard qualitative chemical tests and to look for possible presence of other chemical constituents in the aerial part of the extracts.

MATERIALS AND METHODS

Collection and Identification

The plant was collected from Jessore Municipal Park, Dist-Jessore, Bangladesh during mid July, 2005 on the daytime. The plant (Accession No. DACB-30.216) was identified by the expertise of Bangladesh National Herberium, Mirpur, Dhaka by using standard taxonomical method and a voucher specimen was deposited there. Another voucher specimen was also preserved at the Pharmacy Discipline, Khulna University, Khulna. The identified aerial parts of the plant were cut into small pieces separately and then dried by shed drying for about one week. The plant parts were ground into coarse powder with the help of a suitable mechanical grinder and the powder was stored in a suitable container for extraction.

Preparation of the Extract

The powdered plant material (about 150 g) was taken in a clean, flat-bottled glass container and soaked in 650 mL of ethanol up to 2 inch. height above the sample surface as solvent can sufficiently cover the sample surface. The container with its contents was sealed and kept for a period of 2 weeks accompanying occasional shaking and stirring. The whole mixture was then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through filter paper and after filtration the remaining portion of the plant extract was given for re-extraction for 7 days with another 150 mL of ethanol. The mixture was again filtered by the same way as previous. The filtrate (ethanol extract) obtained was evaporated by air supplied from a continuously moving electric fan until dried. It rendered a greenish black type of residue of 3.2 g (yield: 2.13%). The greenish black type concentrate residue was designated as crude ethanolic extract of the aerial part of *Senna tora*. One gram of re-extracted residue was found followed by the evaporation of remaining part. The crude extract was then stored in a cool and dry place prepared for studies.

Phytochemical Study

Small amounts of dried extracts were appropriately treated to prepare sample solution and then subjected to the specific phytochemical tests (Evans, 1989).

Determination of *in vitro* Antibacterial Activities

Three types of discs were prepared for antibacterial screening: One gram sample extracts was dissolved in 10 mL of ethanol to prepare sample solution, 0.03 g/10 mL gentamicin standard disc used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by known antibacterial agent with that produced by test samples and third one was a blank sample (only ethanol) which was used as negative control to ensure that the residual solvents was not active. Specific organisms were inoculated into previously sterilized nutrient agar media, mixed thoroughly and transferred immediately to the sterile petri dish in an aseptic condition. It was stored in an incubator for about 24 h to allow the proper growth of microbes. Prepared sample solutions were applied to the corresponding cups or holes with the help of a

micropipette. The plates were then allowed to stand to diffuse the sample solution into the antibiotic medium at room temperature for 2 h. The plates were then incubated at 37°C for overnight. After proper incubation, clear zones of inhibition around the point of application of sample solution were formed. These inhibition zones were measured by slide calipers and expressed in millimeter (Bauer *et al*., 1966).

Screening of Analgesic Activity

Analgesic activity of the ethanolic extract of *Senna tora* was tested by using the model of acetic acid induced writhing in mice (Collier *et al*., 1968). Young Swiss-albino mice aged 4-5 weeks, averaged weight 20-30 g were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and research, Bangladesh (ICDDRB). Mice were kept under standard environmental condition for one week for adaptation and fed ICDDRB formulated rodent food and water. Experimental animals were randomly selected and divided into three groups denoted as group-I, group-II and group-III, consisting of 5 mice in each group. Each group received a particular treatment, i.e., control (1% Tween 80 solution in distilled water), positive control (Diclofenac Sodium in 25 mg kg^{-1} body weight) and test group (the ethanolic extract at the dose of 500 mg kg^{-1} body weight. Each mouse was weighed properly and the dose of the test sample and control materials was adjusted accordingly. Test sample, control and Diclofenac Sodium were given orally by means of feeding needle to the corresponding group of mice. A 30 min interval was given to ensure proper absorption of the administered substances. Then the writhing inducing chemical, acetic acid solution (0.7%, in 15 mL kg^{-1} body weight) was administered intraperitonially to each of the animals of a group. After an interval of 5 min, which was given for the absorption of acetic acid, number of squirms (writhing) was counted for 15 min. Each mouse of all groups was observed carefully to count number of writhing that they made in 15 min and from that data percentage of writhing inhibition as an indicator of intensity of analgesic activity for the sample was measured.

RESULTS AND DISCUSSION

Qualitative Phytochemical Test

Qualitative phytochemical tests were performed for the alcoholic extract of the aerial part of *Senna tora* and the result of various chemical tests for the detection and/or identification of chemical constituents are shown in Table 1.

As evident from the result, the alcoholic extract of aerial part of *Senna tora* has been found to contain carbohydrates, saponin, gum, steroid and tannin. However, the extract gave negative reaction to chemical constituents like flavonoid and alkaloid.

Antimicrobial Activity Test

An antimicrobial activity of the ethanolic extract of the aerial part of the plant was compared with the standard antibiotic gentamicin (control) by measuring the zone of inhibition diameter. The ethanolic extract of the plant *Senna tora* was tested for antibacterial activity against a number of both gram negative (coded as B-1 to B-6) and gram positive (B-7 and B-8) bacteria. The ethanolic extract for antibacterial activity was used in single concentration.

In this experiment, alcoholic extract of *Senna tora* showed mild sensitivity to the eight of the test organisms of both gram positive and gram negative type of bacteria. The highest zone of inhibition (9.0 mm) was recorded against Shigella flex. Again the experiment was conducted only with eight species of bacteria as test species. Therefore further research is essential to evaluate the sensitivity of the plant extract against other species of bacteria, fungi, virus of other microorganisms (Table 2).

Table 1: Chemical constituents identified in the crude alcoholic plant extract of the aerial part of *Senna tora*

	Saponin	Flavonoid	Carbohydrate	Gum	Alkaloid	Steroid	Tannin
Plant extract	+	-	+	+	-	+	+

+ = Presence, - = Absence

Table 2: *In vitro* antibacterial activity of the ethanolic extract of *Senna tora*

		Diameter of inhibition zones (mm)		
Code No.	Bacterial stains	Ethanolic extract of *Senna tora*	Blank	Control
B-01	*E.coli*	8.28	0	30.00
B-02	*Proteus* sp.	7.96	0	44.30
B-03	*Salmonelli typhi*	8.12	0	29.00
B-04	*Shigella boydii*	8.62	0	34.00
B-05	*Shigella dysenterie*	6.90	0	27.58
B-06	*Shigella flex*	9.00	0	28.34
B-07	*Staphylococcus epidermis*	8.00	0	33.00
B-08	*Staphylococcus saprophyticus*	6.40	0	25.36

Table 3: Evaluation of writhing effect of the ethanolic extract of *Senna tora*

Animal group	Mean writhing	% writhing	Inhibition of writhing	SD	SE for % writhing	p-value (t-test)
Group-I (Control) 1% Tween-80 at a dose of 15 mL kg^{-1},p.o	77±4.39	100.00	-	8.78	5.70	
Group-II (Positive Control) Diclofenac Sodium at a dose of 25 mg kg^{-1}, p.o	27±2.16	35.06	64.93	4.33	2.80	10.22 ($p<0.001$)
Group-III (Ethanolic extract of test sample)	18±0.705	23.38	76.62	1.41	0.92	13.26 ($p<0.001$)

Values are expressed as mean±SEM (SEM = standard error of mean), SD = Standard Deviation, Se = Standard Error

Analgesic Activity Test

The result of the test showed that the ethanolic extract of the aerial part of *Senna tora* (at the dose of 500 mg kg^{-1} body weight) exhibit potent writhing inhibition ($p<0.001$) as compared with the standard drug, Diclofenac Sodium (Table 3).

Though exhibited potent analgesic activity of the ethanolic extract of *Senna tora* provides support of the claim about aerial part being used as an analgesic in traditional practice, further study should be continued for its isolated, purified active principles.

CONCLUSIONS

As evident from the above discussion, *Senna tora* contains important chemical substances that confer upon it as a medicinal agent which has antimicrobial and analgesic activity. As apparent from our results and from other worker's reports, local use of this plant in various infectious diseases is not at much variance with their antimicrobial properties. Moreover, exhibited potent analgesic activity of the ethanolic extract of *Senna tora* provides support of the claim about aerial part being used as an analgesic in traditional practice. This fact also indicates that the traditional uses of the plant have some scientific basis and therefore, the plant should be thoroughly investigated to fully exploit their medicinal as well as pharmaceutical potentialities.

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