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(Research Article)

Evaluation of Antidiarrhoeal Potential of the Ethanolic Extract of Three Bangladeshi Medicinal Plants

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ABSTRACT

The present study was designed to investigate the antidiarrhoeal activity as well as phytochemical screening of ethanolic extract of three Bangladeshi medicinal plants namely Diospyros blancoi A. DC (leaf), Acacia nilotica Linn. (bark) and Hibiscus sabdariffa Linn. (seed and calyxes). The ethanol extracts at graded doses (250 and 500 mg/kg body weight) was investigated for antidiarrhoeal activity in acetic acid induced writhing model in mice. Diospyros blancoi leaf extract significantly ($p<0.001$) inhibited the mean number of defecation which were 33.19% and 45.28% at the doses of 250 and 500mg/kg respectively. The latent period was increased and number of stools for the extract treated group was significantly ($p<0.001$) decreased as compared to control group. The result was found comparable to the effect of standard antidiarrhoeal drug loperamide (62.26% at the dose of 3mg/kg). The bark extract of Acacia nilotica at the dose of 500mg/kg body weight produced 32.64% inhibition of defecation. The percent inhibition of the ethanol extract of Hibiscuss sabdariffa calyxes and seed extracts were found to be (16.98% and 11.32%) at the dose of 250mg/kg and (24.53% and 20.75%) at the doses of 500mg/kg body weight respectively. Phytochemical analysis of the extract of these three Bangladeshi medicinal plants indicated the presence of reducing sugar, gum, tannin and alkaloid types of compounds. Therefore, the obtained results tend to suggest the antidiarrhoeal activities of the crude ethanolic extract of the leaves of Diospyros blancoi A. DC and that of bark of the Acacia nilotica Linn. thus provide the scientific basis for the traditional uses of these plants as modality for diarrhea.

Key Words: *Antidiarrhoeal activity, Acacia nilotica, Diospyros blancoi, Hibiscus sabdariffa*

INTRODUCTION

According to World Health Organization more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance[1]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds[2].

Diarrheal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people each year[3]. Despite immense technological advancement in modern medicine, many people in the developing countries still rely on the healing practices and medicinal plants for their daily health care needs[4]. Therefore, the World Health Organization encouraged studies for the treatment and prevention of diarrheal diseases depending on traditional medical practices[5].

The people of developing countries depend on plant resources mainly for herbal medicines, food, forage, construction of dwellings, making household implements, sleeping mats, and for fire and shade. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries[6,7].

Diospyros blancoi (Family: Ebenaceae), *Acacia nilotica* (Family: Fabaceae), *Hibiscuss sabdariffa* (Family: Malvaceae) were selected for this project. The plants possess a wide range of medicinal properties. *A. nilotica* is used for the treatment of gonorrhoea, leucorrhoea, diarrhea, dysentery and diabetes[8,9]. It also has styptic and astringent

property. In Siddha medicine, the gum is used to consolidate otherwise watery semen. The water extracts of *Hibiscus* flowers were reported to have a relaxation effect on the uterus and to lower the blood pressure[9]. Studies in both animal[10,11,12] and human models have demonstrated that extracts or infusions affects atherosclerosis mechanisms, blood sugar, lipids and blood pressure [13].

MATERIALS AND METHODS

Plant Materials

The plants and their parts to be used for this study were selected based on their traditional uses. The fresh plant parts were collected from different areas of Bangladesh. Then the plant parts were kept under a shed till dried. The plant was identified by experts in Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh.

Preparation of Ethanolic Extract

The different plant parts of three medicinal plants were freed from any of the foreign materials. Then the plant materials were chopped and air-dried under shed temperature followed by drying in an electric oven at 40º C. The dried plant materials were then ground into powder. About 100g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 400 ml of 96% ethanol. The container with its contents was sealed and kept for a period of 4 days accompanying occasional shaking and stirring. The ethanolic extract was filtered by Buchner funnel and the filtrate was concentrated with rotary evaporator at bath temperature not exceeding 40˚ to have gummy concentrate of deep greenish black extract (yield: *D. blancoi* 5.92%; *A. nilotica* 12.01%; *Hibiscuss sabdariffa* seed 4.68% and *Hibiscuss sabdariffa* calyxes 6.35%).

Test for Different Chemical Groups

The crude ethanolic extract was tested for its different chemical groups as alkaloids, flavonoids; reducing sugars, saponins, steroids and tannins.[14] In each test 10% (w/v) solution of the extract in ethanol was taken.

Test Animals and Drugs

Young Swiss-albino mice 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 Pharmacology Laboratories, Pharmacy discipline, Khulna University, Khulna. 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 cages were cleaned once daily. These studies were carried out following approval from the ethical committee comprising pharmacologist and toxicologist expert on the use and care of animals of Pharmacy discipline, Khulna University, Khulna.

The standard drug loperamide was used for antidiarrhoeal activity testing and the drug was purchased from Square Pharmaceuticals Ltd, Bangladesh.

Antidiarrhoeal Activity

Antidiarrhoeal activity was tested by using Castor oil induced method in mice[15, 16]. 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 3mg/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. 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:

% inhibition = {(Mo–M)/Mo}x100; where, Mo = Mean defecation of control and M = Mean defecation of test sample

Statistical Analysis

Data were presented as mean ± Standard Error Mean (S. E. M). 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 < 0.01 were considered to be statistically significant (p indicates probability).

RESULTS AND DISCUSSION

Chemical Group Test

Results of different chemical tests on the ethanolic extract of *D. blancoi* leaves showed the presence of reducing sugars, gums, tannins and alkaloids. The ethanolic extract of *A. nilotica* bark showed the presence of reducing sugars, gums, tannins and saponins. *H. sabdariffa* seed showed the presence of reducing sugars, gums, and alkaloids and *H. sabdariffa* calyxes showed the presence of reducing sugars, gums, tannins, saponins and alkaloids (Table 1).

Test for Antidiarrhoeal Activity

Diospyros blancoi leaf extract significantly ($p<0.001$) inhibited the mean number of defecation which were 33.19% and 45.28% at the doses of 250mg/kg and 500mg/kg respectively. Protection of the severity of diarrhea induced by castor oil was also observed. The latent period was increased and number of stools at 1st, 2nd, 3rd, 4th, 5th and 6th hours for the extract treated group was significantly ($p<0.001$) decreased as compared to control group. The result was found comparable to the effect of standard anti diarrheal drug loperamide (62.26% at the dose of 3mg/kg) (Table 2; Figure 1). The ethanolic extract of *Acacia nilotica* at the dose of 250mg/kg body weight produced 13.21% inhibition of defecation and that of at the dose of 500mg/kg body weight was 32.64%. Similarly *Hibiscuss sabdariffa* calyxes extract showed significant ($p<0.01$, $p<0.001$) inhibition of diarrheal episodes. Percent inhibition by the extract was found to be 16.98% at the dose of 250mg/kg and 24.53% at the doses of 500mg/kg body weight. Again *Hibiscuss sabdariffa* seed extract produced significant ($p<0.01$, $p<0.001$) inhibition of defecation at the both doses and the results were 11.32% and 20.75% at the doses of 250mg/kg and 500mg/kg body weight of mice respectively.

From the table 2 it was also revealed that the latent periods were increased and number of stools in 4hr for all the plants' extract were significantly ($p<0.01$, $p<0.001$) decreased as compared to control group. The result was also found to be comparable with the effect of standard antidiarrhoeal drug loperamide.

Several mechanisms have been proposed to explain the diarrheal effect of castor oil including inhibition of intestinal Na^+,K^+-ATPase activity to reduce normal fluid absorption [17], activation of adenylate cyclase or mucosal cAMP mediated active secretion [18] , stimulation of prostaglandin formation [19], platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil [20]. 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 [21]. Since the ethanol extract of *B. tersa* 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 [22]. 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.

CONCLUSION

In conclusion it can be revealed that the crude ethanolic extract of *Diospyros blancoi* leaf and *Acacia nilotica* bark extract possess significant antidiarrhoeal activities. The potential of the extracts as antidiarrhoeal activities may be due to the presence of phytoconstituents like tannins, phenolics etc and might be responsible for its activity and justify its use as a traditional folk remedy. However, more detailed phytochemical analysis will be necessary to isolate and characterize the active compounds which are responsible for these activities and exact mechanisms of action of these activities.

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Table 1: Comparisons of phytochemical screening among the four extracts of three plants.

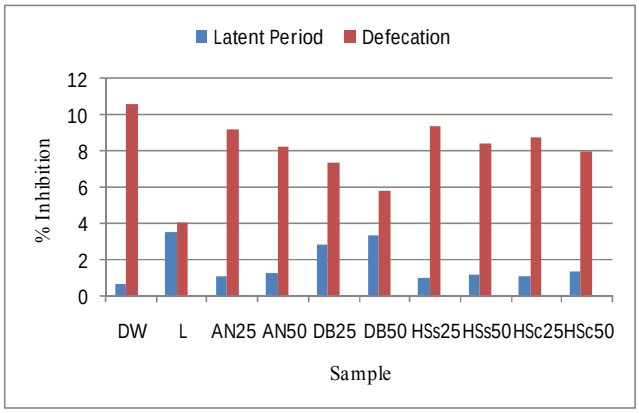
Phytoconstituents	*D. blancoi* (Leaf)	*A. Nilotia* (Bark)	*H. sabdariffa* (Seed)	*H. sabdariffa* (Calyxes)
Reducing Sugar	+	+	+	+
Steroid	-	+	-	-
Gum	+	+	+	+
Tannin	+	+	-	+
Alkaloid	+	-	+	+
Saponin	-	+	-	+
Flavonoid	-	-	-	-

+: Positive result; - : Negative result

Table 2: Antidiarrhoeal activity of three medicinal plants in castor oil induced diarrheal test method on mice (n=5).

Sample	Part Used	Dose	Mean± SE		% inhibition
			Latent period	Defication	
Distilled water	--	2ml/mice	0.65±0.06	10.0±0.25	--
Loperamide	--	3mg/kg	3.51±0.16**	4.0±0.32**	62.26
D. blancoi	Leaf	250 mg/kg	2.81±0.32**	7.4±0.75**	30.19
		500 mg/kg	3.29±0.11**	5.8±0.37**	45.28
A. nilotica	Bark	250 mg/kg	1.01±0.92*	9.2±0.37*	13.21
		500 mg/kg	1.24±0.18*	6.8±0.49**	32.64
H. sabdariffa	Seed	250 mg/kg	0.92±0.07*	9.4±0.25*	11.32
		500 mg/kg	1.34±0.13*	8.4±0.51**	20.75
H. sabdariffa	Calyxes	250 mg/kg	1.02±0.06**	8.8±0.58*	16.98
		500 mg/kg	1.30±0.07**	8.0±0.45**	24.53

*Values are expressed as mean±SEM (Standard Error Mean); * indicates P < 0.01, **indicates P < 0.001 one-way ANOVA followed by Dunnet's test as compared to control; p.o: per oral; .n = Number of mice.*



DW= Distilled water, L= Loperamide, AN25= *A. nilotica* 250 mg/kg, AN50= *A. nilotica* 500 mg/kg, DB25= *D. blancoi* 250 mg/kg, DB50= *D. blancoi* 500 mg/kg, HSs25= *H. sabdariffa* seed 250 mg/kg, HSs50= *H. sabdariffa* seed 500 mg/kg, HSc25= *H. sabdariffa* calyxes 250 mg/kg, HSs50= *H. sabdariffa* calyxes 500 mg/kg,

Figure 1: Antidiarrhoeal activity of three medicinal plants in castor oil induced diarrheal test method on mice.

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