

COMPARATIVE STUDY OF ANTIDIARRHEAL EFFECT OF THREE BANGLADESHI PLANT EXTRACTS

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ABSTRACT

Objective: The aim of the study to evaluate the medicinal properties of *Bombax ceiba*, *S. colocasiifolia* and *P. sikkimensis* as antidiarrheal.

Methods: Leaves of *Bombax ceiba* and *S. colocasiifolia* was extracted with pure ethanol (EEBC and EESC) and *P. sikkimensis* was extracted with pure methanol (MEPS). Antidiarrheal effect was carried out by using a standard method of bioassay: Castor oil-induced diarrhea test.

Result: In the castor oil-induced diarrhea conduct experiment, the leaves extract of *Bombax ceiba* success to produce a noticeable antidiarrheal result in the mice. At doses of 250 and 500 mg/kg, the extract produced significant inhibition of faces. The total number of wet feces produced upon administration of castor oil decreased (4.10 ± 0.38 , at 250 mg/kg and 2.90 ± 0.45 , at 500 mg/kg) compared to

the control group (5.8 ± 0.2) while Loperamide decreased to 2.2 ± 0.374 at the dose of 5 mg/kg. But two other plant extract (*S. colocasiifolia* and *P. sikkimensis*) failed to exhibit the antidiarrheal activity, because they increased the amount of faces in mice. **Conclusion:** So, we can conclude that *Bombax ceiba* may be good choice for searching antidiarrheal agent. Though, no other methods used to evaluate the antidiarrheal effect of *B. ceiba*, further studies

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need to evaluate the present finding and if the further studies also support that and also to establish the mechanism of action.

KEYWORDS: *Bombax ceiba*, ethanol extract, antidiarrheal, castor oil.

INTRODUCTION

Diarrhea is a major health challenge in developing countries, representing the second leading cause of mortality globally in children under age 5.^[1] Repeated episodes of hypovolemia from diarrhea can produce malnutrition and impaired development.^[2] The mainstay of diarrhea therapy is oral rehydration solution (ORS), which consists of an aqueous mixture of salts and carbohydrates.^[3] Though ORS has reduced mortality from diarrhea four-fold in the last 3 decades, its efficacy is limited, particularly in the young and elderly, and because of practicalities in its availability and compliance.^[4] Antisecretory drug therapy for diarrhea may be efficacious when ORS is not available, as during natural disasters, and it may potentiate the efficacy of ORS.

Medicinal plants are commonly used in treating and preventing specific ailments and diseases, and are generally considered to play a beneficial role in healthcare. They are already important to the global economy. Demand is steadily increasing not only in developing countries but also in the industrialized nations.^[5] World Health Organisation (WHO) estimates that approximately 80% of the developing world's population meets their Primary Healthcare needs through traditional medicine.^[6] About 25% of prescription drugs dispensed in the United States contain at least one active ingredient derived from plant material. Some are made from plant extracts while others are synthesized to mimic a natural plant compound.^[7]

Bombax ceiba is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. It is one of the important medicinal plants in tropical and subtropical India and also occurs in Sri Lanka, Pakistan, Bangladesh, Myanmar, Malaysia, Java, Sumatra and Northern Australia. It has number of traditional uses and its medicinal usage has been reported in the Indian traditional systems of medicine such as Ayurveda, Siddha and Unani.^[8] The various parts of *B. ceiba* have been reported for hypotensive and hypoglycaemic^[9] antiangiogenic^[10], hepatoprotective^[8] and antimicrobial^[11] activities.

Stuednera colocasiifolia K. Koch (family: *Araceae*) is an evergreen herb, which is short Stem, creeping and ascending; persistent cataphylls brown, not netted. Locally it is used to treat injuries, cuts, snake and insect bites and skin ulcers. Whole plant extract of *S. colocasiifolia* has anti-arthritic and anti-inflammatory activities^[12], hypoglycemic and antibacterial activity.^[13]

Phyllanthus sikkimensis Muell. Arg. is native to Bangladesh. It grows 50 to 70 centimeters tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds. Leaves extract of *P. sikkimensis* has anthelmintic activity.^[14]

So far no attempts have been made to evaluate the medicinal properties of *Bombax ceiba*, *S. colocasiifolia* and *P. sikkimensis* as antidiarrheal. Hence, the present study was planned to assess antidiarrheal potential of *Bombax ceiba*, *S. colocasiifolia* and *P. sikkimensis*.

METHODS AND MATERIALS

Plant collection and identification

Fresh leaves of *Bombax ceiba*, *S. colocasiifolia* and *P. sikkimensis* were collected from different parts Chittagong, Bangladesh in the month of September 2014. It was authenticated by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Extract preparation

Each of the plant materials was dried and ground into powder (40-80mesh, 600 g) and soaked for 7 days with 2-3 days interval in 3.0 L of ethanol (*Bombax ceiba* and *S. colocasiifolia*) and methanol (*P. sikkimensis*) at room temperature ($23 \pm 0.5^\circ\text{C}$). Filtrate obtained through cheesecloth and Whatman filter paper No. 1 was concentrated under reduced pressure at the temperature below 50°C using rotary evaporator (RE 200, Sterling, UK). The extracts (yield 3.8-7.0% W/W) were all placed in air tight glass tube.

Chemicals and Reagents

All other chemicals and reagents were of analytical grade. Ethanol and methanol was purchased from Merck (Germany). Loperamide (Square Pharmaceuticals Ltd., Bangladesh), castor oil (WELL's Heath Care, Spain), normal saline solution (0.9% NaCl) were used.

***In vivo* Antidiarrheal activity**

Castor oil-induced diarrhea

The experiment employed the method described by Awouters *et al.*^[15] Mice were fasted for 18 h before the test with free access to water and divided into five groups of five animals each. 1st group treated as control (saline 2 ml/kg body weight intraperitoneally), 2nd group received standard drug (loperamide 5 mg/kg b. wt. oral) and 3rd and 8th group received extract of *Bombax ceiba*, *S. colocasiifolia* and *P. sikkimensis* leaves (250 and 500 mg/kg b. wt. oral). Then 1 h later, castor oil was administered orally to these animals to induce diarrhea. The mice were then housed singly in cages lined with white blotting paper. The papers were changed every hour. The total number of both dry and wet feces excreted were counted every hour for a period of 4 h and compared with the control group. The total number of diarrheal feces of the control group was considered 100%.

Statistical analysis

All results are expressed as mean \pm standard error of the mean (SEM). The results were statistically analyzed using repeated measures analysis of variance with Dunnett's multiple comparison when compared against negative control in antidiarrheal test. $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered as statistically significant. Statistical programs used were SPSS (Statistical Package for Social Science, version 16.0, IBM Corporation, NY).

RESULTS

***In vivo* Antidiarrheal activity**

Castor oil-induced diarrhea

In the castor oil-induced diarrhea conduct experiment, the leaves extract of *Bombax ceiba* success to produce a noticeable antidiarrheal result in the mice, as shown in Table 1 and Figure 1. At doses of 250 and 500 mg/kg, the extract produced significant inhibition of faces. The total number of wet feces produced upon administration of castor oil decreased (4.10 ± 0.38 , at 250 mg/kg and 2.90 ± 0.45 , at 500 mg/kg) compared to the control group (5.8 ± 0.2) while Loperamide decreased to 2.2 ± 0.374 at the dose of 5 mg/kg. But two other plant extract (*S. colocasiifolia* and *P. sikkimensis*) failed to exhibit the antidiarrheal activity, because they increased the amount of faces in mice.

Table 1: Effect of extract of leaves of *Bombax ceiba*, *S. colocasiifolia* and *P. sikkimensis* on castor oil (0.5ml) induced diarrhea in Swice albino mice.

Treatment	Total number of feces	% Inhibition of defecation	Total number of diarrheal feces	% Inhibition of diarrhea
Saline (2 ml/kg)	13.40±0.245	-	5.80±0.2	-
Loperamide (5 mg/kg)	5.40±0.4 ^c	59.70	2.20±0.374 ^b	62.07
EEBC (250 mg/ kg)	9.20±0.45	31.34	4.10±0.38	29.31
EEBC (500 mg/ kg)	6.80±0.245	49.25	2.90±0.45	50.00
EESC (250 mg/ kg)	14.52±0.33	-	6.78±0.3	-
EESC (500 mg/ kg)	15.91±0.45	-	7.56±0.38	-
MEPS (250 mg/ kg)	14.96±0.82	-	6.92±0.68	-
MEPS (500 mg/ kg)	16.60±0.95	-	7.86±0.84	-

Values are mean ± SEM ($n = 5$); ^a $P < 0.05$, ^b $P < 0.01$ and ^c $P < 0.001$, Dunnett test as compared to negative control (saline). Statistical representation of the total number of feces and total number of diarrheal feces by *Bombax ceiba* (EEBC), *S. colocasiifolia* (EESC) and *P. sikkimensis* (MEPS) ethanol extract, positive antidiarrheal control (Loperamide, 5 mg/kg p.o.) processed by paired t-test analysis (Dennett's test). Data were processed by paired t-test analysis by using SPSS for windows, version 16.0.

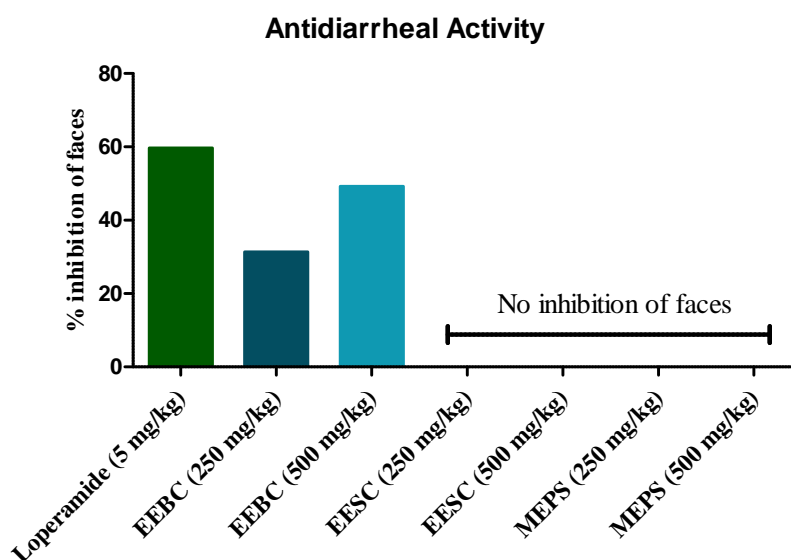


Figure 1: Effect of extract of leaves of *Bombax ceiba*, *S. colocasiifolia* and *P. sikkimensis* on castor oil (0.5ml) induced diarrhea in Swice albino mice.

DISCUSSIONS

Many plants with antidiarrhoeal activities are widely distributed in Bangladesh, but very few have been subjected to pharmacological screening and elemental analysis. Human health

could benefit maximally from nature if the correct amounts of the elements are taken in its ionic varieties in the right form and at the right time. The medicinal values of some plant species used in homeopathic system has been traced to the presence of Ca, Cr, Cu, Fe, Mg, Ca, K and Zn in plants.^[16] Elements have been reported to play major role as co-factors of various enzymes and in various metabolic processes.^[17]

There is an unmet need for effective drug therapy for secretory diarrheas, especially in developing countries where cholera and other enterotoxin-mediated secretory diarrheas remain a major cause of morbidity and mortality. Potential targets for antisecretory therapy include the causative bacterial or viral agent (vaccines and antibiotics), elaborated endotoxins and endotoxin-enterocyte interactions, as well as enterocyte signaling effectors (cAMP, cGMP, Ca²⁺) and membrane transporters involved in fluid secretion (Cl⁻ and K⁺ channels, NKCC1) and absorption (NHE3, SGLT1).^[18] Cl⁻ channels are attractive targets for antisecretory therapy because they are the final, rate-limiting step in Cl⁻ (and hence Na⁺ and water) secretion. Unlike vaccines and antimicrobials that target the causative microbial agent, therapies targeting host secretory mechanisms, such as enterocyte Cl⁻ channels, are not subject to the emergence of resistance. Here, we identified a widely used Thai herbal remedy, Krisanaklan, as having broad antidiarrheal efficacy in bacterial and viral models of secretory diarrhea, which, at the cellular level, inhibits the two major enterocyte Cl⁻ channels, CFTR and CaCC.

From the castor oil induced diarrheal test it was found that *S. colocasiifolia* and *P. sikkimensis* might not be good choice for searching antidiarrheal agent. Because, from our experiment data, it is clear that these plants have no antidiarrheal effect. Though, these extracts did not reduce the faces after castor oil induction. But *B. ceiba* reduces the faces after castor oil induction and it could be a great source for antidiarrheal drug.

CONCLUSION

From the conducted study, we can conclude that *Bombax ceiba* may be good choice for searching antidiarrheal agent. Though, no other methods used to evaluate the antidiarrheal effect of *B. ceiba*, further studies need to evaluate the present finding and if the further studies also support that. But in case of *S. colocasiifolia* and *P. sikkimensis*, these plants have no antidiarrheal effect, then we have quit to finding potential antidiarrheal effect in these plants. Though, they increased the number of faces, may be it has laxative effect, which can be identify by further established methods.

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