

# Study on anti-diarrheal activities of *Abelmoscous esculentus* seeds



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## ABSTRACT

The main objective of this study is to analyze anti-diarrheal activities of *Abelmoscous esculentus*. Phytochemical screening showed that *Abelmoscous esculentus* contain alkaloid, carbohydrate, saponines, diterpene, phytosterol, proteins and gums. Crude ethanolic extract

of *Abelmoscous esculentus* seeds showed maximum (90.91%) anti-diarrheal activities whereas n-hexane soluble fraction and chloroform soluble fraction showed same (81.82%) anti-diarrheal activities which are much higher than the standard Loperamide (63.64%).

## INTRODUCTION

Phytochemical based researches have been holding the attraction of researchers since the ancient times. Phytochemicals are present in a plant based diet as non-nutritive components that exert protective or disease-preventing effects.<sup>1</sup> A number of plants can synthesize a huge number of biologically active phytochemicals with beneficial effects on long term human diseases on consumption and these phytochemicals have effectiveness to treat those diseases.<sup>2</sup> Based on phytochemical researches it is possible to obtain a number of promising and expensive drugs and active ingredients from plant sources.<sup>3</sup> Being encouraged from traditional medicines, researchers are seeking the remedy of diseases from the nature. Recent times study for anti-diarrheal agents from plant source is of a topic of great interest for the researchers. Being a major threat to human health, diarrheal diseases still represent a main cause for mortality and morbidity. Developed countries face lower problems with this disease than low and middle income countries like Bangladesh.<sup>4</sup> It's an alarming disease for perspective of Bangladesh which is one of the main causes of death in children in this country.<sup>5</sup> One third of the total child death occurs from diarrhea and every year nearly 2,30,000 children meet death suffering from diarrhea.<sup>6</sup> High prevalence of misuse of drugs in the treatment of diarrheal disease leads to improve prescribing pattern according to WHO recommendation.<sup>7</sup> Subsequent use of antibiotics for treatment of this disease may lead to antibiotic resistance which is alarming for our existence. Researchers are trying to find out the solution of the rising problem from nature and some successful attempt was found from the previous reports. The objective of this study was to find out a way to beat diarrhea with the help of natural ingredient which is cost effective without any visible side effects. It was first attempt to work with seeds of *Abelmoscous esculentus* and its connection with diarrhea treatment

in the perspective of Bangladesh. Phytochemical screening of *Abelmoscous esculentus* seeds showed the presence of alkaloids, saponins and steroids which have been reported to show anti-diarrheal activities.<sup>8,9</sup> These results pushed the research to be conducted and concluded with a fruitful result beneficial to further investigation process for the betterment of mankind.

## MATERIALS AND METHODS

### Sample collection

The seeds of *Abelmoschus esculentus* (DACB Accession Number: 46496) collected from Noakhali, Bangladesh on 21<sup>st</sup> January, 2016 identified by an expert from National Herbarium, Mirpur, Dhaka, Bangladesh. The seed sample was ground to coarse powder after sun drying for one week and kept in an airtight container for the further analysis.

### Sample preparation

700 g powdered sample were soaked in 1800 ml of 99% ethanol for about 15 days at room temperature with occasional stirring. After 15 days the solution was filtered using filter cloth followed by Whatman's filter paper and left for the evaporation of solvent. Resultant reddish brown granular extract was labeled as crude extract of ethanol which was partitioned with petroleum ether, n-Hexane, chloroform, ethyl acetate by using Modified Kupchan Partition method.<sup>10</sup>

### Phytochemical group screening

Phytochemical group screening was done to figure out different constituents present in the extract.<sup>11</sup> In addition, Xanthoproteic Test and Ninhydrin Test was carried out to find out the presence of Proteins and Amino acids respectively.<sup>12,13</sup>

### Anti-diarrheal activities

Castor oil induced diarrheal model is a popular and widely used method for the evaluation of drugs

and medicinal compounds.<sup>14</sup> The experiment was performed by using Swiss albino mice (20-25 g), collected from the Animal House, Department of Pharmacy, Jahangirnagar University and kept under standard environmental conditions (relative humidity 55-65%, room temperature  $23.0 \pm 2.0^\circ\text{C}$  and 12h light-dark cycle) and had free access to standard rodent diet and water *ad libitum*. Mice were randomly divided into eight groups having two mice in each group. Among these one group was marked as control, one as standard and rests of the six groups were marked as test groups. Each group received a particular treatment. Control group was treated with plain distilled water at the dose of 10ml/kg orally while the standard group received loperamide at the dose of 50 mg/kg orally. Different fractions (6 fractions) of ethanolic extract of *Abelmoschus esculentus* seed were given to the test group at the dose 200 mg/kg body weight. Weights of feces of the mice were measured every hour. Mice of all groups were orally treated with 0.5 ml of castor oil after an interval of 60 minutes. A thorough observation for 4 hours on consistency of fecal matter and frequency of defecation was done. Wet feces were counted at the end of the experiment and percentage of defecation as well as percentage of inhibition of defecation was measured.

## RESULT AND DISCUSSION

The screening test of experimental plant extract revealed the presence of alkaloid, carbohydrate, saponines, diterpene, phytosterol, proteins and gums. Alkaloids are very important and versatile phytochemical as it is used as local anesthetics, stimulants, analgesics, antibacterials, anticancer drugs, antihypertensive agents, cholinomimetics, spasmolysis agents, vasodilators, antiarrhythmia, antiasthma therapeutics, antimalarials and so on.<sup>15</sup> Moreover, presence of alkaloids, carbohydrate, saponins and steroids have been reported to show anti-diarrheal activity.<sup>9,16</sup>

In addition, gums possesses alleviate action against diarrhea, dysentery, sore-throat, kidney, wound and gum infections.<sup>17</sup>

### Anti-diarrheal activity

The total number of defecation for each mouse was taken up to four hours and then the data evaluated statistically to find its significance.

Crude ethanolic extract of *Abelmoschus esculentus* seed showed maximum inhibition to diarrhea (90.91%) whereas chloroform soluble fraction and n-hexane soluble fraction have given identical anti-diarrheal activities and inhibition of diarrhea was estimated to exactly 81.82%.

**Table 1** Phytochemicals present in *Abelmoschus esculentus* seed

Phytochemicals	Test	Observation
Alkaloid	a) Wagner's test	++
	b) Hager's test	++
	c) Meyers test	++
Cardiac Glycoside	a) Legal's test	-
	b) Balget test	-
Carbohydrate	a) Molisch's Test	++
	b) Benedict's Test	++
Saponines		++
Diterpene	Salkowski's Test	++
Phytosterol	Liebermann Burchard's test	++
Flavonoid	a) General Test	-
	b) Specific test	-
Protein	a) Xanthoproteic Test	++
	b) Ninhydrin Test	++
Tannin	Ferric Chloride Test	-
Phenol	Ferric Chloride Test	-
Gum	Molisch's Test	++

Key: (+) = Presence of Phytochemicals and (-) = Absence of Phytochemicals

**Table 2** Result of anti-diarrheal activities of different fractions of extract of *Abelmoschus esculentus* seed

Group	Total number of faeces	Number of diarrhoea faeces	Inhibition of diarrhoea (%)
Control (water)	8.50 ± 3.54	5.50 ± 0.7071068	-
Standard (Loperamide 5 mg/kg)	6.0 ± 2.83	2.00 ± 1.4142136	63.64
PESF	6.5 ± .707	5 ± 2.8284271	9.09
n-HSF	3.5 ± 3.54	1*	81.82
CSF	6.5 ± 6.36	1 ± 1.4142136	81.82
EASF	5.5 ± 3.54	5 ± 2.8284271	9.09
AQSF	4 ± 2.83	5 ± 7.07	9.09
Crude extract	1	1*	90.91

\* $P < 0.05$ , Probability values calculated as compared to control using excel followed by T Test. Comparing of defecation given by mice affected by the plant extracts against control at the level of  $p < 0.05$  level.

Different solvents are used for different purposes. Non-polar solvents are used for non-polar constituents like, oils, terpenoids and steroids, for flavanoids and glycosides. Mostly polar solvents and medium polar solvents are preferred and for alkaloids and related constituents.

Ethanol can dissolve polar compounds<sup>18</sup> and medium polar compounds<sup>19,20</sup> as a consequences alkaloid, carbohydrate, saponins, diterpene, phytosterol, proteins and gums are obtained in the current study. It is already stated that in this study alkaloids, carbohydrate, saponins and steroids are responsible for anti-diarrheal activity.<sup>9,16</sup> As ethanol soluble fraction that is crude extract contains all these ingredients, so, it shows maximum anti-diarrheal response.

Here there are a little bit similarities in the extraction by hexane and chloroform. Hexane can extract alkaloid and phytosterol from the crude extract.<sup>18,20</sup> In addition, chloroform is widely used in alkaloid research,<sup>21</sup> moreover relative polarity of chloroform is 0.259,<sup>22</sup> so, if one is looking for oils, fats, terpenoids, or steroids, then, chloroform can be used. These similarities of extraction help to give the same response against diarrhea, that is much more better than action of standard loperamide against diarrhea (63.64%).

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## REFERENCES

1. Arendt E, Zannini E. Cereal Grains for the Food and Beverage Industries. 2013; 1<sup>st</sup> edition:174.
2. Patel D, Kantharia ND, Patel D. Plants derived phytochemicals and their importance in current medical practice. *J Pharma Care Health Sys*. 2015; 2:5.
3. Katiyar C, Gupta A, Kanjilal S et. al. Drug discovery from plant sources: An integrated approach. *Ayu*. 2012; 33(1): 10-19.
4. Sarker AR, Sultana M, Mahumud RA et al. Economic costs of hospitalized diarrheal disease in Bangladesh: a societal perspective. *Glob Health Res Policy*. 2018; 3: 1.
5. Islam MR, Hossain MK, Khan MN et. al. An Evidence of Socio-Demographic Effects on Child's Diarrhoea in Bangladesh *J Health Science*. 2015; 5(1): 1-5.
6. Farthing M, Salam MA, Lindberg G et al. Acute Diarrhea in Adults and Children A Global Perspective. *J Clin Gastroenterol*. 2013; 47(1):12-20.
7. Alam MB, Ahmed FU, Rahman ME. *Bangladesh Med Res Counc Bull*. 1998; 24(2):27-31.
8. Shemsu U, Alemu T, Nigatu K. Antidiarrhoeal and antimicrobial activity of *Calpurnia aurea* leaf extract. *BMC Complement Altern Med*. 2013; 13:21.
9. Balaji G, Chalamaiah M, Ramesh B et. al. Antidiarrhoeal activity of ethanol and aqueous extracts of *Carum copiticum* seeds in experimental rats. *Asian Pac J Trop Biomed*. 2012; 2(2):1151-1155.
10. Kupchan SM. Recent Advances in the Chemistry of Terpenoid Tumor Inhibitors. *Pure Appl Chem*. 1970; 21(2):227-46.
11. Yadav RNS, Agarwala M. Phytochemical Analysis of Some Medicinal Plants. *J Phytology*, 2011; 3(12).
12. Waksman A, Stevensk R. A critical study of the methods for determining the nature and abundance of soil organic matter. *Soil Sci*. 1930; 30: 97.
13. Odén S, Hofsten BV. Detection of fingerprints by the ninhydrin reaction. *Nature*. 1954; 173(4401): 449-450.
14. Shoba FG, Thomas M. Study of antidiarrhoeal activity of four medicinal plants in case of induced diarrhea. *J Ethnopharmacology*. 2001; 76(1):73-6.
15. Kuete V. Health Effects of Alkaloids from African Medicinal Plants. Toxicological Survey of African Medicinal Plants. 2014; 611-633.

16. Shemsu U, Alemu T, Nigatu K. Antidiarrhoeal and antimicrobial activity of *Calpurnia aurea* leaf extract. *BMC Complement Altern Med.* 2013; 13:21.
17. Singh BR, Dubey S, Siddiqui MZ. Antimicrobial Activity of Natural Edible Gums, *World J Pharmaceutical Sciences.* 2015; 3(11):2217-2221.
18. Houghton PJ, Raman A. Laboratory Handbook for the Fractionation of Natural Extracts. New York: Chapman and Hall. 1998.
19. Lin HY, Kuo YH, Lin YL, Chiang W. Antioxidative effect and active component from leaves of lotus (*Nelumbo nucifera*). *J Agricultural and Food Chemistry.* 2009; 57: 6623-6629.
20. Cowan MM. Plant product as antimicrobial agents. *J Microbiology Reviews.* 1999; 12(4): 564 - 582.
21. Snyder LR. Classification of the solvent properties of common liquids, *J Chromatography*, 1974; 92: 223 – 230.
22. Reichardt C. Solvents and Solvent Effects in Organic Chemistry, Wiley-VCH Publishers. 2003, 3<sup>rd</sup> edition.



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