

Short Communication

**Comparative Evaluation of Anti-diarrhoeal Activity of Methanolic Extract of
Averrhoa carambola and *Averrhoa bilimbi* in Castor Oil Induced Diarrhea in Mice**

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Abstract: Diarrhea is a worldwide health concern. This study was conducted to evaluate the anti-diarrheal activity of two commonly used medicinal plants of Bangladesh *Averrhoa carambola* and *Averrhoa bilimbi*. The Percentage inhibition defecation of *A. carambola* is 10.3% and 69.2% ($p = 0.0001$) in the dosages of 25 mg/kg and 50 mg/kg respectively. *A. bilimbi* inhibited diarrhea 2.6% and 8% at low and high dosage respectively. The percentage inhibition of loperamide (3mg/kg) is 74.4% ($p = 0.001$). Results shows that the effect of *Averrhoa carambola* shows a dose-dependent relationship, wherein higher doses exert much stronger effects compared to lower doses. On the other hand, the methanolic extract of *Averrhoa bilimbi* does not show any anti-diarrheal activity at the dose of 25 mg/kg and 50 mg/kg. Further research should be done to determine its therapeutic dose.

Keywords: Anti-diarrheal activity, castor oil induced diarrhea, traditional medicine.

1. Introduction

Diarrheal diseases are a major health concern in third world nations which is responsible for the death of millions of people every year [1]. The problem is more severe among the children. According to the World Health Organization diarrheal infections are the second largest cause of death in children under five years old and 370,000 children died due to diarrhea 2019 [2]. There are several treatment options for diarrhea including synthetic anti-diarrheal agents and traditional medicines [3]. Although the anti-diarrheal drugs can mitigate the signs and symptoms of diarrhea, there is a scarcity of these drugs in the developing countries and they may cause serious side effects and sometimes contraindicated in many cases [4, 5]. The Infectious Disease of the Society of America states that loperamide which is widely used anti-diarrheal agent is contraindicated in the treatment of diarrhea in pediatric patients [6, 7].

Use of traditional medicines to treat various diseases including diarrhea is still very common. About 80% people rely on plant based medicines for the management of a lot of illness [8]. *Averrhoa bilimbi* and *Averrhoa carambola* L. are two widely used traditional medicine in Asian subcontinent. They come under the same genus *Averrhoa* of family *Oxidaceae* and both species have significant medical applications [9]. Studies shows that the total phenolic content is higher in *Averrhoa carambola* than *Averrhoa bilimbi*. On the contrary *Averrhoa bilimbi* is rich in flavonoids and vitamins A, C and E. *Averrhoa carambola* possesses more antioxidant properties than bilimbi [10, 11].

The present study aims to conduct a comparative evaluation of anti-diarrheal activity of leaves extract of *Averrhoa carambola* and *Averrhoa bilimbi*.

2. Materials and Methods

2.1 Plant material collection and extraction

Averrhoa carambola and *Averrhoa bilimbi* leaves were collected from Banani, Dhaka and identified by the experts of Bangladesh National Herbarium (accession number DACB 94779 and DACB 94780 respectively). The leaves were dried under shades for twenty days and after that converted to coarse powder using a suitable mixer grinder. The powder was soaked in methanol for 15 days with occasional shaking and stirring. The mixtures were filtered using a clean white cloth. Then the extracts were filtered through Whitman filter paper and was concentrated and evaporated to obtain methanolic crude extracts using water bath. The extracts were stored at 4°C until used.

2.2 Chemicals and reagents

Methanol was purchased from Merck, Germany. Loperamide was obtained from Square Pharmaceuticals Bangladesh. Castor oil was acquired from WELL's Health Care, Spain.

2.3 Animals used in the experiments

Swiss albino mice weighing (25-30g) collected from Jahangirnagar University, Savar, Dhaka, Bangladesh. The animals were kept in standard environmental conditions (temperature $25 \pm 2^\circ\text{C}$, relative humidity 55-65%) with a 12-hour light / dark cycle for seven days to acclimatize. They were fed with normal laboratory chow pellet diet and drinking water was given *ad libitum*. All protocols adopted in these experiments were approved by the institutional animal ethical committee of Primeasia University Dhaka, Bangladesh (Reference number PAU/IEAC/24/108 and PAU/IEAC/24/114).

2.4 Anti-diarrheal activity study by castor oil induced diarrhea

Animals were kept for overnight fasting with water *ad libitum*. The mice were divided into control, standard and test groups each group consisting 5 mice. After 30 minutes of administration of saline water, loperamide and extract treatment with *Averrhoa carambola* and *Averrhoa bilimbi* at dosages of 25mg/kg and 50 mg/kg, each mouse received 0.5 ml of castor oil orally. The animals were placed in separate cages. The floors of the cages were covered with blotting paper which were changed in every 60 minutes. The total number of faecal output and the number of diarrheic faeces excreted by the animals in 4 hours were observed. The means of total number of stool in various extract group were compared with standard and control [12-14].

3. Results

3.1 Preliminary Phytochemical screening

The result of the phytochemical screening shows that the methanolic extract of *Averrhoa carambola* is rich in alkaloid, flavonoids, reducing sugar, carbohydrate, glycoside and Tannin and the methanolic extract of *Averrhoa bilimbi* contains flavonoids, steroids, saponin, carbohydrate and tannin. Results are shown in table 1.

Table 1: Test results of phytochemical screening

SI	Name of the test	Methanolic extract of <i>Averrhoa carambola</i>	Methanolic extract of <i>Averrhoa bilimbi</i>
1	Alkaloid	++	---
2	Flavonoid	++	++
3	Reducing sugar	++	--
4	Steroid	-	+
5	Saponin	-	+
6	Carbohydrate	+	+
7	Glycoside	+	-
8	Tannin	+	+

(+) indicate the presence of the compound in a single test method, (++) indicates the presence of the compound in two test methods and (-) indicate absence.

3.2 Anti-diarrheal activity

Phytochemical screening was carried out following the method described in the literature [16]. The result shows that the methanolic extract of *Averrhoa carambola* shows anti diarrheal effect in a dose dependent way while *Averrhoa bilimbi* has no effect in the dosages of 25 mg/kg and 50 mg/kg. The Percentage inhibition of *A carambola* is 10.3% and 69.2% ($p = 0.0001$) in the dosages of 25 mg/kg and 50 mg/kg respectively. *A bilimbi* inhibited diarrhea 2.6% and 8% at low and high dosage respectively. The percentage inhibition of Loperamide (3mg/kg) is 74.4% ($p = 0.001$). The equation for this calculation is below-

$$\% \text{ inhibition of defecation} = [(\text{Control-test})/\text{Control}] \times 100$$

The result is shown in table 2:

Table 2: Anti-diarrhoeal activity of *Averrhoa Carambola* and *Averrhoa bilimbi*

Treatment	Dose (Oral)	Number of fecal dropping in 4 hours	% inhibition of defecation
Castor oil (Control)	0.5 ml/mouse	7.8 ± 0.200	-----
Loperamide	3mg/kg	$2 \pm 0.316^{***a}$	74.4
<i>Averrhoa carambola</i>	25 mg/kg	7 ± 0.532^{ns}	10.3
<i>Averrhoa carambola</i>	50 mg/kg	$2.4 \pm 0.245^{***a}$	69.2
<i>Averrhoa bilimbi</i>	25 mg/kg	7.6 ± 0.245^{ns}	2.6
<i>Averrhoa bilimbi</i>	50 mg/kg	7.2 ± 0.374^{ns}	8

* Each value is represented by mean \pm S.E.M (n=5). (*) indicates the statistically significant difference from the respective group using ANOVA, followed by Turkey; compare all pairs of column (* $p < 0.05$ a ** $p < 0.01$ and *** $p < 0.001$). ^{ns}indicates that statistically no significant difference from the respective groups ($p > 0.5$), ^aindicates when compared with control.

4. Discussion

Castor oil induces diarrhea by increasing intestinal motility and secretion by releasing ricinoleic acid, which results in fluid accumulation and inflammation in the intestines. [15] The anti-diarrheal activity of methanolic extract of *Averrhoa carambola* is comparable to standard at higher dose (50 mg/kg). The methanolic extract of *Averrhoa carambola* can exert the anti-diarrheal effect by the inhibition of ricinoleic acid which results in reduced intestinal inflammation and edema. Additionally, it regulates intestinal motility by limiting excessive peristalsis and fluid secretion. Moreover, its antibacterial activity might help in treating diarrhea-related secondary infections.

Averrhoa carambola is rich in bioactive compounds such as flavonoids, tannins, and alkaloids, which possess various pharmacological activities, including anti-diarrheal effects. Flavonoids in the extract regulate intestinal motility, lowering hypermotility associated with diarrhea and enhancing fluid and electrolyte absorption in the intestines. Furthermore, tannins have an astringent characteristic, which aid in the anti-inflammatory capabilities to reduce intestinal inflammation associated with diarrhea. [17]

From the data it can be safely concluded that the effect of *Averrhoa carambola* extract in diarrhea management appears to exhibit a dose-dependent relationship, wherein higher doses exert stronger effects compared to lower doses. This can be caused by a number of reasons, including the extract's bioactive ingredient content, pharmacokinetics, and pharmacodynamics. At high doses, *Averrhoa carambola* extract may achieve therapeutic concentrations capable of altering intestinal motility, fluid secretion, and inflammation, thereby alleviating diarrhea symptoms. In contrast, low dose may not generate adequate plasma levels to induce a pharmacological response, resulting in non-significant effects observed in experimental models. Same dose was used for the study to facilitate the comparison of the effect on castor-oil induced diarrhea of the above stated plant extracts of same family i.e. oxalidaceae.

Understanding an appropriate dose range is critical to enhancing therapeutic efficacy while reducing potential side effects. More study is needed in the areas of dose selection, extraction technique standardization, and bioactive component concentration variability. Finally, clinical trials can be conducted to assess the dose-response relationship reported in preclinical models and the safety and efficacy of *Averrhoa carambola* extract in human diarrhea patients.

In this investigation, the methanolic extract of *Averrhoa bilimbi* does not show any anti-diarrheal activity at the dose of 25 mg/kg and 50 mg/kg. The reason behind this factor may be the concentration of bioactive compounds present in the extract, such as flavonoids, tannins, and phenolic acids may not reach therapeutic concentration to regulate intestinal motility, fluid secretion, and inflammation. Furthermore, its antioxidant qualities may help to protect the intestinal mucosa from the oxidative damage associated with diarrhea.

Future research should be done regarding the dose, extraction mechanism and solvent variability to reveal the underlying pharmacological mechanism. Moreover, clinical trials are needed to evaluate its safety and efficacy in human patients with diarrhea. Exploring alternative extraction methods and formulations may increase the bioactive potential and therapeutic activity of *Averrhoa bilimbi* extract, which is necessary for the development of an effective treatment of diarrhea.

5. Conclusions

The promising anti-diarrheal activity of the methanolic extract of *Averrhoa carambola* holds significant clinical outcome, particularly in resource-constrained countries where access to conventional medications may be limited. Combining this natural remedy with the primary health care support could offer a cost-effective and accessible solution. Although this study does not show any therapeutic efficacy for *Averrhoa bilimbi*, further research should be done to determine its therapeutic dose for optimal concentration of its rich bioactive compounds, dose-response

relationship, efficacy, safety, mechanisms of action and translate preclinical findings into clinically relevant strategies creating the way for its integration into mainstream healthcare.

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