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## Preliminary Evaluation of Some Traditionally used Medicinal plants as antidiarrhoeal in Assam

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### ABSTRACT

The selected plant *Alternanthera sessilis* (L.), *Houttuynia cordata* (Thunb) *Murraya koenigii* (L.) and *Ziziphus oenoplia* (L) Miller has been used traditionally in Assam, India for the treatment of diarrhoea and dysentery. However, the claims need to be validated by a suitable experimental model. Therefore, the present study was under taken to evaluate the effect of 50% methanol extracts of *Alternanthera sessilis* (L.) whole plant (ASM), *Houttuynia cordata* (Thunb) Aerial part (HCM), *Murraya koenigii* (L.) Leaves (MKM) and *Ziziphus oenoplia* (L) Miller Root Bark (ZOM) for its antidiarrhoeal potential against experimental models of diarrhoea in Albino Wister rats. The plant extracts showed significant ( $P < 0.05$ ) inhibitor activity against castor oil induced & magnesium sulphate induced diarrhoea in rats. The results point out the possible antidiarrhoeal effect of the plants extract traditionally used in Assam, India.

**Keywords:** Antidiarrhoeal activity, Castor oil induced, ASM, 50% methanol.

### 1. Introduction

Plants are important sources of therapeutic drugs and play a significant role in the survival of the tribal and ethnic communities. Traditional system practiced in India as Ayurveda medicine and in other parts of the world as a form of alternative medicine. India is rich in cultural and a store house of ethno-botanical knowledge. Large sections of Indian population still rely on plant-based medicines as they are abundantly available, economical, and have little or no side-effects<sup>[1, 2]</sup>. Certain plant drugs used in modern medicine have ethno-botanical background<sup>[3]</sup>. Hence, medico-botanical knowledge of different ethnic & folklore communities and tribal's need urgent documentation, before it is completely lost.

In India, about 3000 plants are used in the form of ethno or folk-medicines based on oral information from generation to generation. About 1500 plants are systematically used in indigenous system of medicine, like Aayurveda, Unani and Siddha. It is obvious that folk medicines are the original source to point out, the plants as the source of medicine in the primary level<sup>[4, 5]</sup>.

Diarrhoea can cause dehydration, which means the body loses too much fluid & too many electrolytes, and is not able to function properly. Due to rapid depletion of water & sodium, the body starts to "dry up" or get dehydrated. If more than 10% of the body's fluid is lost, death occurs. Children are more likely than adults to die from diarrhoea because they become dehydrated more quickly. Severe dehydration is the reasons for deaths<sup>[3]</sup>. W.H.O. estimates that about 7.1 million deaths were caused by diarrhea<sup>[6]</sup>.

It is a leading cause of malnutrition and death among children in the developing countries of the world today. More than 5.8 Million infants & children below 5 years old, die every year of diarrhoea especially in developing countries. About 80% of deaths due to diarrhoea occur in the first two years of life. A nationwide study conducted has estimated that diarrhoea kills more than 1 million children per year in India alone<sup>[7]</sup>. Although diarrhoeal disease is usually less harmful to adults than to children, it can also affect a country's economy by reducing the health of its work force.

Many synthetic chemicals like Diphenoxylate, Loperamide, Codeine and Antibiotics are available for the treatment of diarrhoea but they have some side effects. Since ancient times, diarrhoea has been treated orally with several medicinal plants or their extracts based on folklore medicine [8]. A range of medicinal plants with anti-diarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these anti-diarrhoeal traditional medicines has not been scientifically evaluated. So the present study was focused on the following traditionally used plants in Assam, for their anti-diarrhoeal and phytochemical evaluation i.e.

Plant	Family	Part used as anti-diarrhoeal
<i>Alternanthera sessilis</i> (L.)	Amaranthaceae	Whole plant
<i>Houttuynia cordata</i> (Thunb)	Saururaceae	Aerial part
<i>Murraya koenigii</i> (L.)	Rutaceae	Leaves
<i>Ziziphus oenoplia</i> (L) Miller	Rhamnaceae	Root Bark

## 2. Materials and Methods

### 2.1. Collection and authentication of plant material

The all selected plants were collected from District Dibrugarh Assam (India) in the month of Feb to April 2010 and were authenticated by Dr Tariq Hussain Scientist, Taxonomy division at National Botanical Research Institute (NBRI), Lucknow, Uttar Pradesh (India). A voucher specimen (NBRI/CIF/145/2010) has been deposited in the institute for further reference.

### 2.2 Preparation of the extract

The all selected plants were washed with distilled water to remove dirt and soil and shade dried in a ventilated place at room temperature. The dried plant materials were reduced to coarse powder by mechanical grinder, extracted with 50% methanol as solvent in soxhlet extractor for 18 h. The extract was filtered and concentrated under reduce pressure using rotavapor. The extract was freeze-dried and stored in deep freezer for further use. Solutions of the extracts were prepared freshly for each study.

### 2.3. Preliminary phytochemical screening

Phytochemical screening of the crude extract of selected plant materials were performed qualitatively for the presence of

alkaloids, flavonoids, tannins, coumarins, sterols, terpenes and saponins according to the standard methods [9, 10]. Briefly, alkaloids were detected by treating the selected plant materials extract with Dragendorff's reagent, resulting in the appearance of a precipitate at the bottom of the test tube. The plant material was deemed positive for flavonoids when it gave a yellow color with  $AlCl_3$  reagent and for tannins when a green or black color was produced with aqueous  $FeCl_3$ . The observation of yellow fluorescence under ultra violet light on examination of the filter paper previously exposed to vapors from the boiling plant material indicated the presence of coumarins. For the detection of sterols and terpenes, plant material was treated with petroleum ether and subsequently extracted with  $CHCl_3$ . The gradual appearance of green to pink (for sterols) and pink to purple colors (for terpenes) was noted after the treatment of  $CHCl_3$  layer with acetic anhydride and concentrated HCl in succession. Lastly, the presence of saponins was based on the appearance of froth upon vigorous shaking of diluted samples. Using procedure to identify their major groups of chemical constituents [9].

### 2.4. Animals

Wister albino rats of either sex weighing 150-200 g were kept at departmental animal house at a temperature ( $25 \pm 2$ ) °C and 12 h light/dark cycle respectively for one week before and during the experiments and fed with standard diet and water ad libitum. Animal studies were conducted according to the Institute Animal Ethics Committee. All the experiments were performed in the morning according to the current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

### 2.5. Drugs and chemicals

Atropine sulphate and loperamide (Ranbaxy (I) Ltd, castor oil (Galaxo) all other chemicals were of analytical grade.

### 2.6. Statistical analysis

The experimental results were expressed as the mean  $\pm$  standard error of the mean (S.E.M.). Data were evaluated by one-way analysis of variance (ANOVA) and means were compared using Waller–Duncan test at  $p \leq 0.05$ .

## 2.7. Experiment Design

Group	Particulars
Control (10ml/kg b.w.)	Saline in vehicle
Standard (3mg/kg b.w.)	Loperamide HCl
Test-1 (ASM) (200mg/kg b.w.)	<i>Alternanthera sessilis</i> 50% methanol extract of whole plant
Test-2 (HCM) (200mg/kg b.w.)	<i>Houttuynia cordata</i> 50% methanol extract of areal parts
Test-3 (MKM) (200mg/kg b.w.)	<i>Murraya koenigii</i> 50% methanol extract of leaves
Test-4 (ZOM) (200mg/kg b.w.)	<i>Ziziphus oenoplia</i> 50% methanol extract of root bark

### 2.7.1. Antidiarrhoeal activity study by castor oil-induced diarrhoea

To assess the antidiarrhoeal activity, a previously described method [11] was modified and used to assess the antidiarrhoeal activity of the plant extract. Wistar albino rats of either sex (150-200 g) were fasted for 24 h before starting the experiment. The animals were housed in individual cages and divided into six groups (n = 4 each). The first group received saline in vehicle (10 ml/kg, p.o.) and served as the control. The next four groups treated with selected plants extract (200mg/kg). Last groups received loperamide (3mg/kg) acting as the standard. One hour after the treatment, each animal received castor oil (10ml/kg, p.o.) through a feeding needle. At 4th hour after dosing the castor oil, the individual mouse cages were inspected for the presence of the total number of faecal output and the numbers of diarrhoeic faeces excreted by the animals were recorded.

### 2.7.2. Antidiarrhoeal activity study by magnesium sulphate-induced diarrhoea

To assess the antidiarrhoeal activity, a previously described method [12] was modified and used to assess the antidiarrhoeal activity of the plant extract. A similar protocol as for castor oil-induced diarrhoea was followed. Diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals, one hour after pretreatment with saline in vehicle (10ml/kg, p.o.) to the control group, loperamide (3mg/kg) to the positive control group, and the selected plants extract at the doses of 200mg/kg to the test groups. All the administrations were carried out through oral route.

## 3. Results

### 3.1. Qualitative analysis of plant extracts

The phytochemical analyses of selected plants materials (50% methanols extract) were revealed the presence of various chemical constituents such as saponins, flavonoids, glycosides, alkaloids, tannins and reducing sugar. (Table 1)

**Table 1:** Qualitative analysis of selected plants material extract

Test	ASM	HCM	MKM	ZOM
Alkaloids	-	+	+	+
Steroids/Terpines	+	+	+	+
Flavonoids	+	+	+	-
Saponins	+	+	-	+
Tannins	-	+	+	+
Reducing sugars	+	+	+	+

### 3.2. Effect on castor oil-induced diarrhoea

In the castor oil-induced diarrhoeal rats, the 50%MeOH extract of the selected plant materials at the doses of 200mg/kg, reduced the total number of faeces as well as of diarrhoeic faeces and the results were statistically significant (Table 2). Among the selected

plants material, the HCM and ZOM, exhibited prominent antidiarrhoeal activity. Both the extracts reduced the total number of faeces and total number of wet faeces in test animals.

**Table 2:** Effect of methanol extract of selected plants material on castor oil-induced diarrhoea

Treatment	Dose (mg/kg p.o).	Total no of faeces in 4 h	Total no of wet faeces 4 h
Control	-	11.64 ± 1.60	9.83 ± 2.23
ASM	200	9.24 ± 2.61 <sup>a</sup>	5.74 ± 1.47 <sup>a</sup>
HCM	200	6.42 ± 1.37 <sup>b</sup>	4.84 ± 1.22 <sup>a</sup>
MKM	200	9.34 ± 1.37 <sup>a</sup>	6.56 ± 1.10 <sup>b</sup>
ZOM	200	5.87 ± 1.37 <sup>a</sup>	4.12 ± 0.81 <sup>a</sup>
Standard	03	5.17 ± 0.98 <sup>b</sup>	1.33 ± 0.52 <sup>a</sup>

Values are mean ±SEM (n=4)

<sup>a</sup> P < 0.005 vs control, ANOVA test

<sup>b</sup> P < 0.001 vs control, ANOVA test

### 3.3. Effect on magnesium sulphate-induced diarrhoea

In the magnesium sulphate induced diarrhoeal model, the 50%MeOH extract of the selected plant materials at the doses of 200mg/kg, reduced the severity of diarrhoea in test animals and the results were statistically significant (Table 3). Among the selected

plant materials, the HCM and ZOM, exhibited significant activity. Both the extracts were able to reduce the number of total number of faeces and wet faeces as compared to the control.

**Table 3:** Effect of methanol extract of selected plants material on magnesium sulphate-induced diarrhoea

Treatment	Dose (mg/kg p.o.)	Total no of faeces in 4 h	Total no of wet faeces 4 h
Control	-	12.42 ± 0.62	8.57±1.54
ASM	200	8.67±1.22 <sup>a</sup>	6.23±0.94 <sup>a</sup>
HCM	200	6.44±0.75 <sup>a</sup>	4.77±0.52 <sup>b</sup>
MKM	200	9.11±0.83 <sup>a</sup>	7.35±0.62 <sup>a</sup>
ZOM	200	6.23±0.44 <sup>b</sup>	4.54±0.72 <sup>a</sup>
Standard	03	2.74±0.37 <sup>b</sup>	0.92±0.22 <sup>b</sup>

Values are mean ±SEM (n=4)

<sup>a</sup> P < 0.005 vs control, ANOVA test

<sup>b</sup> P < 0.001 vs control, ANOVA test

#### 4. Discussion

It is well known that the traditional uses of plants and their effects are due to the presence of secondary metabolites. These metabolites may be alkaloids, glycosides, flavonoids, tannins, triterpenes etc. The medicinal value of plants depends on the presence of these metabolites qualitatively & quantitatively. So the main aim of the present study was to provide scientific proof to the traditional claim. The study has proven that the plants HCM and ZOM have significant antidiarrhoeal value. Number of factors, such as infective, immunological and nutritional, have been involved in the perpetuation of the diarrhoeal syndrome [13]. Many plants conveniently available in India are used in traditional folk medicine for the treatment of diarrhoea and dysentery. Of the indigenous plants used, *Andrographis paniculata*, *Asparagus racemosus*, *Butea monosperma*, *Cassia auriculata*, and others are mentioned [14]. Several studies have shown that prior administration with some plant extracts had a protective effect on the intestinal tract [15-17]. In the present study, the newer plant used by tribals and rural areas has not been studied so far, was evaluated for its anti-diarrhoeal potential against castor oil induced diarrhoea, in Wistar albino rats.

It is widely known that castor oil or its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea. Ricinoleic acid markedly increased the PGE2 content in the gut lumen and also caused an increase in the net secretion of water and electrolytes into the small intestine. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion [18].

On the other hand, magnesium sulphate has been reported to induce diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been demonstrated that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water [19, 20]. The methanol extract was found to alleviate the diarrhoeic condition in this model. The extract may have increased the absorption of water and electrolyte from the gastrointestinal tract, since it delayed the gastrointestinal transit in mice as compared to the control. The delay in the gastrointestinal transit prompted by the extract might have contributed, at least to some extent, to their antidiarrhoeal activity by allowing a greater time for absorption.

Traditionally to treat diarrhoea the ZOM bark is used as water decoction for overnight, HCM leaves and areal part used as 'salad' ASM used as vegetable and MKM leaves as decoction. 50% Methanol is a strong polar solvent considered to extract most plant secondary constituents. Though several constituents were present in the extract, the compound responsible for the observed actions is unknown. Flavonoids possess a wide range of activities in vitro [21] including antidiarrhoeal activity [19, 22-24] may have contributed to this activity, but further studies are required.

#### 5. Conclusion

The 50% methanol extract of selected plant materials showed antidiarrhoeal activity in primary evaluation of diarrhoeic conditions in test animals. The obtained results thus give the experimental basis to understand the use of selected traditional medicine, as an antidiarrhoeal agent. However, further bioassay guided phytochemical and pharmacological studies are required to identify the active principle(s) and exact mechanism(s) of action.

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