Phytochemical Screening And Evaluation of Antidiarrheal Activity of *Eichhornia Crassipes* in Experimental Animals

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ABSTRACT

Background and Objectives: Eichhornia crassipes, belonging to the family Pontederiaceae, is used in Indian traditional medicine to treat diarrhea. This study aims to investigate the anti-diarrheal activity of the methanolic extract of leaves of Eichhornia crassipes (Water hyacinth) by castor oil-induced diarrhea and charcoal meal tests in wistar albino rats.

Methods: All animals were divided into five groups of six animals each. In castor oil-induced diarrhea, castor oil (0.4ml/animal) was used as an inducer, and loperamide (3mg/kg) was used as standard drug. In charcoal induced diarrhea, loperamide (3mg/kg) was used as standard drug. Methanolic extract of Eichhornia crassipes was administered to rats at 100, 200 and 400mg/kg respectively. These rats were monitored for 6 hour and the first defecation time was noted. Charcoal meal test was performed on the rats.

Result: The extract produced a significant decrease in the severity of diarrhea. The activity was found to be dose-dependant. Its effect when evaluated on intestinal transit produced a decrease in intestinal transit (59.97%).The results revealed that the methanolic extract significantly reduced diarrhea in rats with reduction in weight of stools.

Conclusion: Methanolic extract of Eichhornia crassipes alleviates diarrhea at higher dose 400mg/kg. Tannins, flavonoids and terpenoids may be responsible for antidiarrheal effect.

KeyWords: Eichhornia Crassipes, Castor Oil, Intestinal Transit, Loperamide

INTRODUCTION

Diarrheal disease remains a major killer of children under five, claiming approximately 800,000 lives annually, making it the third leading cause of mortality in this age group. Diarrhea can persist for several days, depleting the body's essential water and salts, threatening survival. Historically, severe dehydration and fluid loss were the primary causes of diarrhea-related deaths. Currently, septic bacterial infecions are emerging as a leading contributor to diarrhea-related mortality. Life-threatening diarrhea disproportionately affects vulnerable populations, including malnourished children, immunocompromised individuals, and people living with HIV[1]. Diarrhea is characterized by ≥ 3 loose or liquid stools daily, it is a symptom of intestinal infection caused by bacteria, viruses, or parasites, and is distinct from normal stool patterns, such as frequent formed stools or loose stools in breastfed infants. Poor sanitation, contaminated food or water, and person to person contact spread diarrheal infections; prevention strategies like safe water, improved sanitation, and handwashing with soap can mitigate this risk[2]. Diarrhea should be treated with Oral Rehydration Solution (ORS), a clean water with sugar and salt, and a 10-14 day course of dispersible zinc tablets, which shortens duration and improves outcomes. Yet, despite advances in modern medicine, many in developing countries still rely on traditional healing practices and medicinal plants for daily health needs[3].

Water hyacinth, also known as Eichhornia crassipes (Mart.), is a monocotyledonous plant belonging to the family Pontederiaceae, a large free-floating aquatic weed[4]. This weed is native to Brazil and the Amazon, but is also found in many tropical and subtropical regions, including India, Australia, Africa, Egypt, Sudan, Kenya, Ethiopia, Nigeria, Zimbabwe, Zambia, and South Africa. Plants are mainly characterized by rapid and rapid growth with wide distribution, strong tolerance to pH, nutrient fluctuations and temperature conditions[5]. As such, it has been recognized by the International Union for Conservation of Nature as one of the 100 most aggressive invasive species and identified as one of the 10 worst weedy plants in the world, however, there are many potential advantages. It is known for its phytoremediation properties useful in wastewater treatment as it absorbs heavy metals and grows in polluted water. It is also considered as a potential source of bioenergy and biofertilizer. Traditional uses of the plant include treating gastrointestinal disorders such as intestinal parasites, indigestion, diarrhea and gas. The plant is a rich in diverse bioactive plant constituents that exhibits a wide range of pharmacological and biological activities. Some of these are antioxidants, antimicrobials, antitumor agents, anticancer agents, anti-inflammatory, hepatoprotective, larvicidal, wound healing. To date, many patents have been filed, mainly in the field of medicinal uses of plants and their product formulations.[6]

2. MATERIAL AND METHODS

2.1 Plant Material

The fresh leaves of E. Crassipes (water hyacinth) collected at the flowering stage in the month of March 2025 from the local area of Bareilly district, Uttar Pradesh, North India were authenticated by MJPRU. Leaves were dried in shade for 20 days and then powdered to get a coarse powder. This powder was stored in an air-tight container and used for further successive extraction.

Eichhornia crassipes (water hyacinth)

2.2 Animals

Healthy Wistar albino rats of male sex weighing between 95-100g obtained from central Animal House, BIU College of Pharmacy, Bareilly International University, Bareilly . The animals were housed under standard conditions of light/dark at 12/12-hr cycle. They were fed with commercial pellet diet and water. All animal experiments were carried out in accordance with guidelines of CPCSEA and the study was approved by the institutional Animal Ethics Committee.

Wistar albino rats

2.3 Drugs and Chemicals

Loperamide, castor oil, normal saline solution (0.9% NaCl), charcoal meal (10% activated charcoal in 5% gum acacia) were used.[7]

2.4 Preparation of Crude Extract

A 10gm of dried and powdered plant material was soxhlet extracted with 70% methanol. The extraction was carried out for 48 h at room temperature with mild shaking. The extract was filtered and concentrated at 45°C, and the weight of the residue was recorded[8]. The percentage yield of methanolic extract was found to be 36.50%w/w and was used for further studies.

2.5 Phytochemical screening test

Preliminary phytochemical screening tests were carried out on the methanolic extract of E. Crassipes according to the standard methodology of Indian pharmacopeia (1996) for the detection of various plant constituents (alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, and proteins).[9]

2.6 Acute toxicity test

Acute toxicity study was conducted for the crude extract (single dose 2,000 mg/kg) using the OECD guideline (423) for the testing of chemicals. Three female Swiss albino rats weighing 150-200 g were used. Each animal received the extracts at a dose of 2,000 mg/kg body weight. These animals were observed first for 24h and then for the next 14 days for toxic symptoms such as behavioral changes, locomotion, convulsions, and mortality.[10]

2.7 Antidiarrheal activity testing

2.7.1 Castor-oil induced diarrhea

Thirty mice were allowed to fast for 18h and divided into five groups of six animals each. All groups received castor oil (0.4ml / animal) orally, the first group (control group), receive vehicle (2ml/kg normal saline), the second group received reference drug loperamide (3mg/kg body weight) and third, fourth and fifth group received 100, 200 and 400 mg/kg body weight respectively of the methanolic extract of E. crassipes. After the administration, the animals were placed separately.[11] The severity of diarrhea was assessed each hour for 6h. The total weight of feces was recorded within a period of 24h and compared with the control group. The total number of diarrhea feces of the control group was considered 100%. The result were expressed as a percentage of inhibition of diarrhea.[12]

2.7.2 Gastro intestinal motility test

Thirty mice were randomly selected and fasted for 24 hours prior to the experiment. Animals were randomly divided into five groups of six animal each. First group received vehicle (2ml/kg normal saline) orally, second group received reference drug loperamide (3mg/kg body weight) and third, fourth and fifth group received 100, 200 and 400 mg/kg of the crude extract respectively.[13] Five minutes after drug administration, 0.5ml of a 5% charcoal suspension in distilled water was given orally to each animal.[14] After 30 minutes animals were killed by cervical dislocation and intestine was removed and placed lengthwise on moist filter paper. The length of intestine and the distance travelled by charcoal as a percentage of that length were evaluated for each animal.[15]

Statistical Analysis

All the experimental results were expressed as mean±S.E.M. Data were analyzed by analysis of variance (ANOVA) followed by Dunnett's test.

3. RESULTS

3.1 Phytochemical screening test

Preliminary phytochemical screening tests on methanolic extract of leaves of *E. crassipes* showed the presence of phenols, flavonoids, terpenoids, and saponins (Table 1).

Table 1. Results of identification tests of secondary metabolites in leaves of *E.crassipes*

Secondary Metabolites	Methods Used for Identification	Result
Carbohydrates	Fehling's test	Positive
Proteins	Biuret's test	Positive
Amino Acids	Tyrosine test	Positive
Glycosides	Coumarin test	Positive
Alkaloids	Dragendorff's test	Positive
Saponins	Froth test	Positive
Flavonoids	NaOH test	Positive
Triterpenoids	Ferric chloride test	Positive
Tannins	Ferric chloride test	Positive
Phenols	Potassium permanganate test	Positive

3.2 Preliminary acute toxicity test

Oral administration of the methanolic leaf extract of *E.crassipes* at a dose of 2000mg/kg body weight did not produce any mortality and remarkable signs of toxicity.

3.3 Castor oil induced diarrhea

Compared to the negative control, the three serial doses (100, 200, and 400 mg/kg) of the extract reduced the total number of fecal output significantly (Table 2).

Table 2. Effect of methanolic extract of E.crassipes on castor oil induced diarrhea in rats

Groups	Dose	Onset of Diarrhea (min)	Total No. of Feces	Weightof Stool (g)	% Protection
Control	2 ml/kg	43.5 ± 2.11	15.6 ± 0.66	1.164 ± 0.055	-
Standard	3 mg/kg	67.5 ± 1.53	3.4 ± 0.77	0.244 ± 0.0233	79.03
E. crassipes	100 mg/kg	45.7 ± 2.35	14.2 ± 1.74	0.477 ± 0.0238	28.8
E. crassipes	200 mg/kg	56.7 ± 1.77	11.3 ± 0.47	0.377 ± 0.0245	41.3
E. crassipes	400 mg/kg	63.6 ± 1.88	9.6 ± 0.77	0.258 ± 0.0648	52.2

Values are expressed as mean ± SEM (n=6) and analyzed using one way ANOVA followed by Post Hoc Tukey test. Compared with control P<.05, P<.01, P<.001

3.4 Gastrointestinal transit

Compared to the value of negative control group, the three consecutive doses of the extract significantly reduced the percentage mean of the small intestine transit by charcoal meal in a dose-dependent manner (Table 3).

Table 3. Effect of methanolic extract of *E.crassipes* on gastrointestinal motility

Groups	Dose (mg/kg)	Mean Distance Travelled by Charcoal (% of total small intestine length)	% Reduction
Control	2 ml/kg	84.257 ± 1.999	-
Standard	3 mg/kg	15.244 ± 2.040	81.91
E. crassipes	100 mg/kg	39.667 ± 2.112	31.92
E. crassipes	200 mg/kg	30.333 ± 1.090	48.00
E. crassipes	400 mg/kg	20.244 ± 2.444	59.97

Values are expressed as Mean ± SEM (n=6) . P<0.001, P<0.05, *E.crassipes*, alcoholic extract of leaves of *E.crassipes*

4. DISCUSSION

Preliminary phytochemical screening of the methanolic extract of *E.crassipes* revealed the presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids, phenols and absence of fixed oils and steroids. The median lethal dose of the extract was found to be greater than 5000 mg/kg bodyweight. Castor oil causes diarrhea due to its active metabolite, ricinolic acid,which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin. In this study, the methanol extract of E.crassipes(36.5%w/w) exhibited a significant antidiarrheal activity. Its effect was dose-dependant. The results were similar to some extent to that of the standard drug loperamide (3 mg/kg) with regard to the severity of diarrhea. Phytochemical screening revealed the presence of tannins, sterol and/or triterpenes and reducing sugars, which may be responsible for the mechanism of action of *E.crassipes* antidiarrheal activity. The antidiarrheal activity of this extract may also be due to the presence of denatured proteins, which form protein tannates. Protein tannates make the intestinal mucosa more resistant and hence, reduce secretion. This can be due to the fact that the extract increased the reabsorption of water by decreasing intestinal motility as observed in the decrease of intestinal transit by charcoal meal. Loperamide, apart from regulating the gastrointestinal tract, also reported to slow down transit in the small intestine, reduce colon flow rate, and consequently any

effect on colonic motility. The methanol extract of *E.crassipes* administered at the dose of 100, 200 and 400 mg/kg showed 28.8%, 41.3% and 52% protection, respectively. This reduction in diarrheal episodes was significant and maximum effect was observed at the dose of 400 mg/kg. Mean distance travelled by charcoal, as % total length of small intestine (cm) was less at 400 mg/kg of the dose of *E.crassipes* and is comparable with standard drug atropine sulfate which is used as positive control. The study reveals that the methanol extract exhibited significant diarrheal activity. Thus, *E.crassipes* can increase the absorption of water and electrolytes from the gastrointestinal tract since the extract decreased the small intestinal transit that proves to its efficacy in an extensive range of diarrheal conditions. In conclusion, the results of this investigation revealed that *E.crassipes* contains pharmacologically active substance(s) with antidiarrheal properties. These properties confirm the use of *E.crassipes* as an antidiarrheal drug as proposed by traditional healers. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecules responsible for the antidiarrheal activity observed.

5. CONCLUSION

In the present study, the E.crassipes was found to be safe upto a dose of 2,000 mg/kg p.o. because the treated rats exhibited normal behaviour. During the observational period, the rats were found alert with normal touch and pain responses. There were no signs of toxicity. Their motor activity and salivary secretions were also normal. The rats showed no sign of depression. Moreover, alertness limb tone and grip strength were also found normal. It has been found that ricinoleic acid, a bioactive of castor oil, produces irritation and swelling in the intestinal mucosa. As a result, it stimulated motility and secretion of water and electrolytes into the intestine by releasing prostaglandins. In the present study, the drug decreased the cumulative faecal weight, stool frequency and delay in the onset of defecation. It also decreased the weight and volume of intestinal content. E. crassipes led to a delay in first defecation time, decreased the cumulative faecal weight and changed the nature of stools from watery to solid.

Source of Support: Nil

Conflict of Interest: Nil.

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