

Original Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTI-DIARRHOEAL ACTIVITY OF ETHANOLIC EXTRACT OF LEAVES OF CLERODENDRUM INFORTUNATUM

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ABSTRACT

Objective: To determine preliminary phytochemical Screening and evaluation of Antidiarrheal activity of ethanolic extract of Leaves *Clerodendrum infortunatum*.

Methods: The ethanolic extract of leaves of *Clerodendrum infortunatum* was prepared and analyzed for phytochemical constituents using standard method and evaluate scientifically the anti-diarrheal effect using leaves of *Clerodendrum infortunatum* by castor oil-inducing method. Animals were divided into four groups. Group-I was treated with distilled water 2 ml/kg body weight. Group-II was treated with loperamide 1 mg/kg body weight, Group-III and Group-IV were treated with ethanolic extract of leaves *Clerodendrum infortunatum* 200 mg/kg and 500 mg/kg body weight respectively.

Results: Various phytochemical analysis revealed the presence of sterols, terpenoids, alkaloids, carbohydrates, tannins, glycoside, saponins, proteins and amino acids. The ethanolic extract of *Clerodendrum infortunatum* leaves given by oral route to mice at doses of 200 mg/kg and 500 mg/kg significantly showed antidiarrheal activity against castor oil induced diarrhea as compared with control group and standard.

Conclusion: The present study concluded that ethanolic extract of leaves of *Clerodendrum infortunatum* contain the high presence of phytochemical components and it showed that the leaves of *Clerodendrum infortunatum* having significant antidiarrheal activity. All doses of the plant extracts showed a significant delay in castor oil-induced diarrhea and this justifies the use of this plant as a herbal remedy against diarrhea.

Keywords: Anti-diarrheal, *Clerodendrum infortunatum*, Castor oil, Loperamide

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INTRODUCTION

Medicinal herbs as a potential source of therapeutics aids have attained a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as a potential material for maintaining proper health [1]. Diarrhoea is one of the leading causes of mortality in developing countries [2]. Each year there are approximately 4 billion cases of diarrhoea worldwide. According to the World Health Organization (WHO), diarrhoea still accounts for 1.6-2.5 million deaths annually and each child in the developing world experiences an average of three episodes of diarrhoea per year. In 1998, diarrhoea was estimated to have killed 2.2 million people, most of whom were under 5 y of age [3]. It is usually a symptom of gastrointestinal infection, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking-water, or from person to person as a result of poor hygiene [4]. Signs of dehydration often begin with loss of the normal stretchiness of the skin and irritable behaviour. This can progress to decreased urination, loss of skin color, a fast heart rate and a decrease in responsiveness as it becomes more severe. Loose but non-watery stools in babies what are breastfed, however, may be normal [5]. Although a diarrheal disease control programme (DDC) has been launched by the World Health Organization (WHO), diarrhoea is still a big public health challenge in developing countries. In Cameroon, for example, diarrhoea remains the number one killer disease among children under five years, while babies between the ages of 7-12 mo remain susceptible [6]. A drug or dietary fibre forming agents that relieve the symptoms of diarrhea [7]. The most effective antidiarrheal drugs are opioid derivatives, which slow intestinal motility to permit greater time for the absorption of water and electrolytes. Dietary fibre forming agents improve stool consistency, but may not decrease fluid and electrolyte loss. Antidiarrheals are used to relapse loss fluids and salts in acute cases [8].

MATERIALS AND METHODS

- **Collection of plant material:** The whole plant with leaves, stems and roots was collected from Mangaldai, Darrang district of Assam in January 2017 and the plant was thoroughly washed with water, roots and stems were discarded and the leaves were dried in shade for 3-4 w and then dried it at 40 °C at tray dryer.
- **Authentication of plant material:** The plant was authenticated by Prof. Dr. Nilakshi Sharma, Department of Botany, Gauhati University. A voucher specimen (Acc-18222, Dated: 22/03/2017) was kept in Department of Botany, Gauhati University for future reference.
- **Grinding of plant material:** The plant's materials were crushed by rubbing in between the hands then passed through the sieve no. 10. Then sieve materials transferred to the grinder and collect coarse powder. Stored in an airtight bottle, kept away from light, heat and moisture until use.
- **Chemicals:** Pet. Either purchased from North East Chemicals; Panbazar, Guwahati. All other chemicals and reagents used for and phytochemical screening were of analytical grade.
- **Extraction technique (Maceration):** Approximately 100 gm powder was weighed. The powdered drug was put into the round bottom flask (RBF) and about 600 ml of Pet. ether was poured into the RBF and kept for 7 d with occasional stirring. After that the liquid strained and the marc is pressed and the solvent is distilled off and then the Pet. ether extract was kept in a Petri dish and complete evaporation of the solvent is done in a water bath. The marc obtained is again put inside the RBF and treated with 600 ml of ethanol and kept for 7 d. The liquid extract distilled off and the remaining solvent was allowed to evaporate in a water bath.

Phytochemical screening

The Ethanolic extract of ethanolic extract of leaves of *Clerodendrum infortunatu* was subjected to preliminary phytochemical screening for their presence or absence of active constituents utilising the standard method of analysis [9].

I) Test for alkaloids

a) Mayer's reagent: It is used for the detection of alkaloids. 2-3 ml of plant extract, few drops of Mayer's reagent. It will produce white precipitation. This confirms presence of alkaloids

b) Wagner's reagent: In 2 ml of filtrate, Wagner's reagent was added. Reddish brown precipitation appeared, indicates presence of alkaloids

II) Test for sterols

Two tests Salkowski test and Liebermann-Burchard test were performed.

a) Salkowski test: In 2 ml of plant extract, 2 ml of chloroform and 2 ml of concentrated H_2SO_4 was added and shaken well. Chloroform layer appeared red and acid layer greenish yellow fluorescent. This confirms the presence of sterols.

b) Liebermann-Burchard Test: 2 ml of ethanolic plant extract was mixed with chloroform. 1-2 ml acetic anhydride and 2 drops of concentrated H_2SO_4 from the side of the test tube was added in the mixture. First red, then blue and finally green colour indicates the presence of sterols.

III) Test for terpenoids

a) Salkowski test: The extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 ml) is carefully added to form a layer. A reddish brown colouration of the interface is formed to show a positive result of the presence of terpenoids.

IV) Test for carbohydrates

a) Molisch test: Treat extract with few drops of alcoholic alpha-naphthol. Add 0.2 ml of conc sulphuric acid slowly along the sides of the test tube, purple to violet colour ring appears at the junction. It confirms the presence of carbohydrates.

b) Fehling's Test: Fehling A and Fehling B reagents were mixed and few drops of the extract is added and boiled. A brick red coloured precipitate of cuprous oxide forms, hence confirming the presence of carbohydrates.

V) Test for flavonoids

A small quantity of the extract is heated with 10 ml of ethyl acetate in boiling water for 3 min. The mixture is filtered and the filtrates are used for the following test.

a) Ammonium Test: The filtrate was shaken with 1 ml of dilute ammonia solution (1%). The layers were allowed to separate. A yellow colouration was not observed at ammonia layer which indicates the absence of the flavonoid from the plant extract.

b) Aluminum Chloride Test: The filtrates were shaken with 1 ml of 1% aluminium chloride solution and observed for light yellow color, which did not appear indicating the absence of flavonoids. The light yellow colour indicates the presence of flavonoid and when dilute NaOH and HCl have added the yellow solution turns colourless.

VI) Test for tannins

A small quantity of the extract is boiled with 5 ml of 45 % solution of ethanol for 5 min. Each of the mixtures is cooled and filtered. The different filtrates were used for the following test:

a) Ferric Chloride Test: 1 ml each of filtrate is diluted with distilled water and two drops of ferric chloride is added. A transient greenish to the black color indicated the presence of Tannins.

b) Lead Sub Acetate Test: 1 ml of the different filtrate was added with three drops of lead subacetate solution. A creamy gelatinous precipitation indicates a positive test for Tannins.

VII) Test for phenols

a) Ellagic Acid Test: The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution did not turned muddy nor did Nigler brown precipitate occur. Hence, the presence of phenols was not confirmed.

VIII) Test for glycosides

a) Keller-Kiliani Test: In 2 ml plant extract, glacial acetic acid, one drop of 5% $FeCl_3$ and conc. H_2SO_4 were added. Reddish brown color appears at the junction of the two liquid layers and upper layer appears bluish green, confirming the presence of glycosides.

b) Concentrate H_2SO_4 Test: In 5 ml plant extract, 2 ml glacial acetic acid, one drop of 5% $FeCl_3$ and conc. H_2SO_4 were added. Brown ring appears, indicating the presence of glycosides.

c) Legal's test: In 2 ml of plant extract, 1 ml of pyridine and 1 ml of sodium nitro-prussior was added. Pink to reddish colour appeared. Hence, the presence of glycoside was not confirmed.

IX) Test for saponin

a) Foam Test: The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 min. A layer of foam was formed which indicated the presence of Saponin.

X) Test for proteins and amino acids

a) Millon's test: In 2 ml of plant extract, few drops of Millon's reagent was added. Then warm it. White precipitation appeared the presence of proteins as well as amino acids.

b) Biuret test: 2 ml of plant extract, add 4% NaOH after that 1% $CuSO_4$ few drops added. Violet or pink colour appears i. e. presence of proteins.

Pharmacological screening

- **Drugs and Chemicals:** The drug loperamide is used during the experimental study.

- **Approval of the study:** All the animal experiments were carried out in accordance with the guidelines of CPCSEA and were approved by the Institutional Animal Ethical Committee of Girijananda Chowdhury Institute of Pharmaceutical Science, Azara, Guwahati, Assam, India. [GIPS/IAEC No.: GIPS/IAEC/B. Ph/2017/18]

- **Experimental Animals:** Swiss albino mice aged 8-10 w (20-30 gm) were used for the experimental study, these are kept in the animal house of the institution (Girijananda Chowdhury Institute of Pharmaceutical Science). The animals were acclimatised to the laboratory conditions (room temperature 24 ± 2 °C, relative humidity 55-60%, and 12 h light and dark cycles) and kept in plastic cages. The food was withdrawn 18 h before and during the experimental hours.

- **Acute Toxicity Study:** In the present study, the safety profile of *Clerodendrum infortunatum* leaf was evaluated by acute and subchronic toxicity study of the methanol extract of *C. Infortunatum* leaf (MECI) in swiss albino mice. In acute toxicity study, MECI up to 2000 mg/kg body weight. Did not produce any toxic effect or death. In sub-acute chronic toxicity study, MECI was administered at the single dose of 500 mg/kg body weight, i. p for 28 consecutive days and the 29th day, the haematological, histological, serum and hepatic biochemical parameters were evaluated by sacrificing the animals. No mortality was observed during the course of the whole study period. No detectable alterations were found in haematological biochemical and histological parameters in MECI treated group when compared to vehicle control group after 28 days. The result of the present study, therefore, indicated that *C. Infortunatum* leaf is safe in adult male albino mice demonstrating no noticeable toxicity [10].

- **Antidiarrheal activity:**

The antidiarrheal activity of ethanolic extract of *Clerodendrum infortunatum* was investigated by Castor oil inducing method [11]. Animals were divided into four groups.

Group I (Control)	-	Distilled water (2 ml/kg)	--	2 animals
Group II (Standard)	-	Loperamide (1 mg/kg)	--	2 animals
Group III (Test)	-	Ethanollic extract of leaves <i>Clerodendrum infortunatum</i> (200 mg/kg)	--	4 animals
Group IV (Test)	-	Ethanollic extract of leaves <i>Clerodendrum infortunatum</i> (500 mg/kg)	--	4 animals

Swiss albino mice aged 8-10 w (20-30 gm) were used for the experimental study. The day before the experiment, animals were fasted for 18 h, but with free access to water. Group-I was treated with distilled water 2 ml/kg body weight. Group-II was treated with loperamide (Standard drug) 1 mg/kg body weight, Group-III and Group-IV were treated with ethanolic extract of *Clerodendrum*

infortunatum 200 mg/kg and 500 mg/kg body weight respectively. After 30 min each of these animals were given 0.1 ml of Castor oil by the oral route. Each animal was placed in an individual metabolic cage, the floor was lined by white absorbent papers. It was then observed the consistency of fecal matter and frequency of defecation for 4 h [11].

Table 1: Phytochemical screening of ethanolic extract of leaves *Clerodendrum infortunatum*

S. No.	Phytochemical components	Result
1	alkaloids	+
2	sterols	+
3	terpenoid	++
4	carbohydrate	+
5	flavanoid	-
6	tannin	++
7	phenol	-
8	glycoside	++
9	saponin	+
10	Proteins and amino acids	++

(++)Strongly Present, (+) Weakly Present, (-) Absent.

Table 2: Effect of ethanolic extract of *Clerodendrum infortunatum* on Castor oil induced diarrhoea in mice

Group mg/kg	No. of defecation time interval							
	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Control (Distilled water) 2 ml//kg	2	2	2	2	3	2	1	2
Standard (Loperamide)	-	-	-	-	-	-	-	-
C. infortunatum (200 mg/kg)	1	1	1	1	2	1	-	1
C. infortunatum (500 mg/kg)	-	-	1	-	1	-	2	-

Table 3: Percentage of inhibition of ethanolic extract of leaves *Clerodendrum infortunatum* as compare to control

Group	Treatment/Dose (mg/kg)	Mean defecation in 4 hour	% of inhibition of defecation
1	Control (Distilled water) 2 ml//kg	4	-
2	Standard (Loperamide)	0	100%
3	C. infortunatum (200 mg/kg)	2	50%
4	C. infortunatum (500 mg/kg)	1	75%

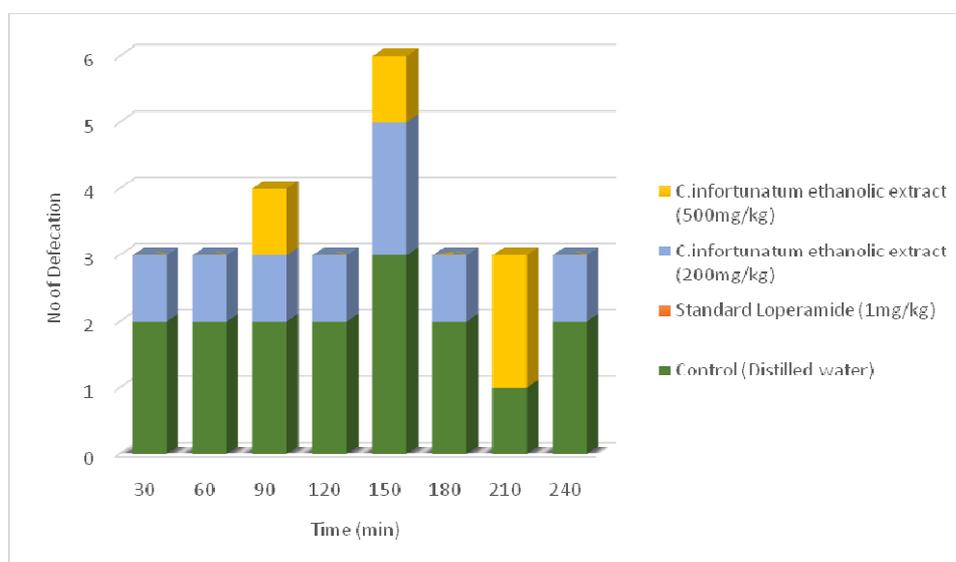


Fig. 1: Graph showing anti-diarrheal activity by castor oil inducing method

RESULTS

Phytochemical screening

The observations made for the phytochemical screening of the crude ethanolic extracts of *Clerodendrum infortunatum* [EECI] leaves are summarised in table 1.

In the study, it was found that the Ethanolic Extract of *Clerodendron infortunatum* possess sterols, terpenoids, alkaloids, carbohydrates, tannins, glycoside, saponins, proteins and amino acids whereas, flavonoids, phenols and was absent.

Antidiarrheal activity

The antidiarrheal activity of ethanolic extract of *Clerodendrum infortunatum* [EECI] leaves was performed by using Castor oil inducing method presented in table 2. There was a significant reduction in fecal output and frequency of defecation when the plant extracts of 200 mg/kg of the leaf and 500 mg/kg of the doses were administered orally compared with control and standard drug in mice.

DISCUSSION

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, followed by excess loss of fluid in the faeces. The ethanolic extract of *Clerodendrum infortunatum* leaves given by oral route to mice at doses of 200 mg/kg and 500 mg/kg significantly showed antidiarrheal activity against castor oil induced diarrhea as compared with control and standard (table 3) and (fig. 1).

Phytochemical screening of the plant extract in the present study revealed the presence of alkaloids, sterols, terpenoid, carbohydrate, tannin, glycoside, saponin, protein, amino acid (table 1). Tannin, glycosides are the compounds occupied in a various plant with such activities continue to be widely used in the treatment of diarrhoea and may act by several mechanisms explain its antidiarrheal action. It has been previously demonstrated that protein tannates make the intestinal mucosa more resistant and hence, reduce secretion and peristaltic movement.

The significant inhibition of the castor oil-induced enteropooling in mice suggests that ethanolic extract of *Clerodendrum infortunatum* leaf relief in diarrhoea and also anti-enteropooling effects.

CONCLUSION

In the present study, various phytochemical tests, as well as antidiarrheal activity, was performed. It showed that the leaves of *Clerodendrum infortunatum* having significant antidiarrheal activity. All doses of the plant extracts showed a significant delay in castor oil-induced diarrhea and this justifies the use of this plant as a herbal remedy against diarrhoea.

Therefore, further studies are needed for the isolation and identification of the active principle responsible for these properties, which can give rise to new drug molecule and can be used for human welfare. Also, the results found can be used as a guideline for further investigation.

CONFLICT OF INTERESTS

Declare none

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