Research Article

Phytochemical Screening and Antidiarrhoeal Activity of Aqueous Extracts of *Croton sparsiflorus* Morong

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ABSTRACT

In this modern era, gastrointestinal disorders are the universal problem. Diarrhea & dysentery is one of the major diseases affecting the human population. In developing countries, a quarter of infant and childhood mortality is related to the diarrhea. Number of factors, such as infective, immunological and nutritional has been involved in the perpetuation of the diarrheal syndrome. Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhoea and dysentery. In the present study, the crude aqueous extract of the entire plant of *Croton sparsiflorus* Morong. (Family-Euphorbiaceae) was evaluated for its possible phytochemical nature (group determination of plant constituents) and antidiarrhoeal activity. Phytochemical analysis of the aqueous extract of *C. sparsiflorus* indicated the presence of tannin, steroid & alkaloid types of compounds. The aqueous extract of *C. sparsiflorus* was studied for its antidiarrhoeal properties in experimental diarrhoea, induced by castor oil and magnesium sulphate in mice. At the doses of 250 and 500 mg/kg per oral, the aqueous extract showed significant and dose-dependent antidiarrhoeal activity in both models. The results were statistically significant ($P < 0.001$) and was comparable to the standard drug atropine sulphate (0.1mg/kg; i.p.). The results showed that the extracts of *C. sparsiflorus* have a significant antidiarrhoeal activity and supports its traditional uses in herbal medicine.

Keyword: Antidiarrhoeal, croton sparsiflorus, euphorbiaceae, phytochemical.

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INTRODUCTION

Croton sparsiflorus Morong (Family-Euphorbiaceae) is a small annual herb, growing mainly road side up to 1-2 ft tall. Alternately arranged leaves, 3-5 cm long, are lance-shaped, with toothed margin. Small white flowers are borne in 3-8 cm long racemes at the end of branches. Flowers have 5 sepal and 5 petals and numerous long stemens producing out. Fruit is 5mm oblong capsule with warty surface. The plant is well known under vernacular as "Ban Tulasi" The powdered leaves are useful in controlling high blood pressure and used for treatment of skin disease, cuts & wounds as well as antiseptic and antidote [1-3]. It contains broad spectrum antibiotic compounds in leaves of this species [4]. This plant main chemical constituents i.e. glycoside, saponins tannins, flavonides, terpenoids and alkaloids [5-6].

Fig. 1: The plant of *Croton sparsiflorus.*

The most of phytoconstituents were extracted from leaves of *C. sparsiflorus* Morong, hence the leaves of this plant have

been used for all pharmacological activities. Hence, an endeavor has been made to establish the scientific validity to explore the possible antidirrhoeal activity in animal models.

In developing countries, diarrhoea is a major cause of infant mortality and morbidity [7]. Despite the availability of vast spectrum of approaches for diarrhoeal management, a vast majority of the people in these developing countries rely on herbal drugs for the management of diarrhoea. WHO has encouraged studies for treatment and prevention of diarrhoeal diseases using traditional medical practices [8]. As a part of our continuing evaluation of antidiarrhoeal activities of the medicinal plants from north east India flora [9-11]. The present study was aimed to evaluate the antidiarrhoeal activity of C. sparsiflorus Morong in mice due the plant contain flavonides, as a major constituents which is responsible for antidiarrhoeal activity [12].

MATERIALS AND METHODS

Collection and Identification of Plant Material:

The entire plant of Croton sparsiflorus Morong were collected from north east India Assam State in May, 2010. The botanical identity of the plant was by Dr Tariq Hussain of the National Botanical Research Institute (N.B.R.I.) Lucknow, U.P.

Extraction and Preparation of the Extract:

After collection, the plant materials were air dried for one week. This was further subjected to another one week of drying in an oven maintained at 40^0C. The leaves were pulverized into a smooth powder. The pulverized plant material (150g) was mixed with distilled water (3.0 liters) and left for 72 hours. The mixture was stirred at 6 hours intervals using a sterile glass rod. At the end, the extract was passed through filter paper. The filtrates were concentrated with the aid of a vacuum pump and rotavapour at 40^0 C. The concentrated extract was stored in cool places prior to use.

Phytochemical Screening:

The aqueous extract was subjected to phytochemical screening testing for the presence of alkaloids, Tannins, saponions, reducing sugars and carbohydrate using the method of Trease and Evans. The aqueous extract (4 g) was warmed with water on a steam bath for 30 min then filter. After filtration, the filtrate obtained was tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with Libermann-Burchard reagent. Reducing sugars with Benedict's reagent [13, 14].

Table 1: Results of different group tests of aqueous extract of *C. sparsiflorus* Morong.

Phytoconstituents	Results
Alkaloids	Present
Reducing sugars	Present
Flavonoids	Present
Saponins	Absent
Steroids	Present
Gums	Absent
Tannins	Present
Carbohydrates	Present

Antidiarrhoeal Activity:

Animals:

Albino Swiss mice weighing between 20-30g of either sex were purchased from the Chennai. They were kept in the departmental animal house in a well cross-ventilated room at 27 ± 2 °C, and relative humidity 44–56%, light and dark cycles of 10 and 14 h, respectively, for one week before and during the experiments. Animals were provided with the standard rodent pellet diet (Amrut, India) and the food was withdrawn 24 h before the experiment but water was allowed ad libitum. 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 [15].

Castor-oil Induced Diarrhoea:

Mice were divided into four groups of five animals each group. Diarrhoea was induced by administering 0.3ml of castor oil orally to mice. Group one served as control (distilled water 10ml/kg), groups 2 and 3 received the aqueous extract of 250 and 500 mg/kg body weight oral, respectively as test while group 4 received atropine (0.1mg/kg I.p) reference. This was done 30 minutes before castor oil administration.

Table 2: Antidiarrhoeal activity of aqueous extracts *C. sparsiflorus* Morong on castor oil induced diarrhoea in mice.

Treatment	Dose	Total number of faeces in 4 hr.	Total number of wet faeces in 4 hr.
Control	10 ml/kg p.o.	15.35 ± 0.46	11.0 ± 0.94
Test-1	250 mg/kg p.o.	9.20 ± 0.34[a]	6.00 ± 0.47[a]
Test-2	500 mg/kg p.o.	7.20 ± 1.06[a]	4.00 ± 0.44[a]
Atropine	0.1mg/kg I.p	3.20 ± 0.37[a]	1.75 ± 0.20[a]

Values are mean ± SEM (n=5)

[a] P < 0.001 vs control, student's t- test.

Magnesium sulphate-induced diarrhea:

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 30min after pre-treatment with vehicle (distilled water 10ml/kg) to the control group, atropine (0.1mg/kg I.p) to the reference group, and the aqueous extract at the doses of 250 and 500 mg/kg body weight oral to the test groups.

Table 3: Antidiarrhoeal activity of aqueous extracts *C. sparsiflorus* Morong on magnesium sulphat induced diarrhoea in mice.

Treatment	Dose	Total number of faeces in 4 hr.	Total number of wet faeces in 4 hr.
Control	10 ml/kg p.o.	12.45 ± 0.46	9.0 ± 1.34
Test-1	250 mg/kg p.o.	6.40 ± 1.35[b]	4.20 ± 0.77[b]
Test-2	500 mg/kg p.o.	4.22 ± 0.66[b]	2.95 ± 0.54[b]
Atropine	0.1mg/kg I.p	2.20 ± 0.37[a]	1.15 ± 0.20[a]

Values are mean ± SEM (n=5)

[a] P < 0.001 vs control, student's t- test.

[b] P < 0.01 vs control, student's t- test.

The following parameters were observed for a period of 4 hours, the time elapsed between the administration of the cathartic agent and the excretion of the first diarrhoeic faeces, the total number of both dry and wet diarrhoea droppings in 4 hours and the total weight of both the wet and dry diarrhoeal stool in that period of time [16-17].

Statistical Analysis:

All data were expressed as mean +SEM and where applicable, the data were analyzed statistically by Student's t-test using graph pad. The level of significance was from P < 0.05.

RESULTS AND DISCUSSION

Results of different chemical tests on the ethanolic extract of C. sparsiflorus roots showed the presence of alkaloid, tannin, steroid etc. (**Table 1**).

In the castor oil-induced diarrhoeal experiment in mice, the aqueous extract of C. sparsiflorus Morong, at the doses of 250 and 500 mg/kg, reduced the total number of faeces as well as the total number of diarrhoeic faeces in a dose dependent manner (**Table 2**). These results were shown to be statistically significant (P < 0.001).

In the magnesium sulphate-induced diarrhoeal model in mice, the aqueous extract at the above dose levels significantly ($P < 0.01$, $P < 0.001$) reduced the extent of diarrhoea in test animals (**Table 3**). Both the doses were shown to reduce the total number of faeces and wet faeces when compared to the control.

However several mechanisms have been previously proposed to induce the diarrhoeal effect of castor oil i.e. inhibition of intestinal Na+, K+, ATPase activity to reduce normal fluid absorption [18]. Activation of adenylate cyclase or mucosal cAMP mediated active secretion, stimulation of prostaglandin formation, platelet activating factor and most recently nitric oxidehas been claimed to contribute to the diarrhoeal effect of castor oil [19]. Despite the fact that these numerous mechanisms have been proposed, it has not been possible to define castor oil's correct mechanism of action [20]. However, it is well documented that castor oil produces diarrhoea due to its most active component recinoleic acid by a hypersecretory response [21-22]. Since the aqueous extract of *C. sparsiflorus* Morong successfully inhibited the castor oil-induced diarrhoea, the extract might have exerted its antidiarrhoeal action by antisecretory mechanism. This was also evident from the reduction of total number of wet faeces in the test groups in the experiment.

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 [23-24]. The aqueous 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. Though several constituents were present in the plant extract, the compound responsible for the observed actions is unknown. Flavonoids possess a wide range of activities in vitro including antidiarrhoeal activity may have contributed to this activity [12, 23, 25, 26]. The obtained results thus give the experimental basis to understand the use of *C. sparsiflorus* Morong, 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.

CONCLUSION

The results indicate that the plant contains numbers of secondary metabolites and significant against traditional uses. The aqueous extract of *C. sparsiflorus* possesses significant antidiarrhoeal activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion. The inhibitory effect of the extract justified the use of the plant as a non-specific antidiarrhoeal agent in folk medicine. Further detailed investigations are underway to determine the exact phytoconstituents which are responsible for the antidiarrhoeal activity.

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