

Antidiarrhoeal and Antioxidant Properties of *Curcuma alismatifolia* Leaves

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Abstract: Hydromethanol extract of *Curcuma alismatifolia* leaves was scientifically evaluated for its antidiarrhoeal and antioxidant properties. Antidiarrhoeal property was studied using castor oil and MgSO₄-induced diarrhoeal models and charcoal induced gastrointestinal motility test in Swiss Albino mice. At the doses of 250 and 500 mg/kg body weight, the extract displayed remarkable antidiarrhoeal activity, evidenced by a reduction in the rate of defecation as well as by retardation of intestinal transit of charcoal meal in test animals throughout the study period. In all of these experimental models the extract, at higher dose (500 mg/kg body weight), exhibited significant ($p < 0.05$) antidiarrhoeal property compared to the control. Again, antioxidant potential of the extract was assessed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, nitric oxide (NO) radical scavenging, total phenol and total flavonoid content determination assays. The extract displayed a dose dependent scavenging of DPPH radical and NO. Moreover, the extract was found to possess high amount of phenols and flavonoids, expressed as gallic acid and quercetin equivalents respectively. The results of the present study might indicate the usefulness of *C. alismatifolia* leaves in diarrhoeal disease and other disorders linked to free radical-mediated oxidative stress.

Key words: Medicinal plant, *Curcuma alismatifolia*, antioxidant, antidiarrhoeal property.

INTRODUCTION

In developing countries, a majority of people living in rural areas almost exclusively use traditional medicine for treating diseases of different types including diarrhoea. Diarrhoea is a major health problem especially for children under the age of 5, and up to 17% of children admitted in the paediatrics ward die of diarrhoea. Worldwide distribution of diarrhoea accounts for more than 5-8 million deaths each year in infants and children below 5 years old especially in developing countries (Fauci *et al.*, 1998). According to W.H.O. estimates for 1998, about 7.1 million deaths were caused by diarrhoea (Park *et al.*, 2000). The incidence of diarrhoeal diseases still remains high despite the efforts of many governments and international organisations to curb it. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used anti-diarrhoeal drugs, which are not always readily accessible and free from adverse effects (Hardman *et al.*, 1992). A range of medicinal plants with anti-diarrhoeal properties is widely used by traditional healers. However, the effectiveness of many of these antidiarrhoeal traditional medicines has not been validated scientifically. On the other hand it has been proposed that oxidative stress damage to cellular and extracellular macromolecules, such as proteins, lipids, and nucleic acids (Halliwell *et al.*, 1999; Jones *et al.*, 2000; Schafer and Buettner, 2001) results from the tipping of balance toward prooxidant status. The production of reactive oxygen species (ROS) has been implicated in the pathogenesis of age-related diseases (Ames *et al.*, 1993; Weinbrenner *et al.*, 2003) such as cancer and coronary heart disease and neurodegenerative disorders such as

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Alzheimer's disease (Smith *et al.*, 1996). It is generally assumed that frequent consumption of plant-derived phytochemicals from vegetables, fruit, tea, and herbs may contribute to shift the balance toward an adequate antioxidant status (Halliwell *et al.*, 1993). Currently, the possible toxicity of synthetic antioxidants has been criticized. The interest in natural antioxidant, especially of plant origin, has greatly increased in recent years. *C. alismatifolia* is a member of the family Zingiberaceae (ginger). Curcuma is a perennial herb having a fleshy corm with fibrous and fleshy ovoid storage roots. The plant has 6-8 lanceolate leaves with a pseudostem formed from leaf sheaths. The inflorescence is a splendid compact spike with lotus flower-like bracts, and a long stalk of 60-70 cm. Previous phytochemical screenings on *Curcuma longa*, *Curcuma aromatica* etc. of the same family reveals the presence of curcumin, curcumol, resin, various essential oils including turmerone, alantone, zingiberone and other sesquiterpenes as the major constituents. *Curcuma aromatica* and *Curcuma longa* are applied to bruises and sprains, in snake bite, skin diseases and various inflammatory disorders. But to the best of our knowledge, no investigation on antidiarrhoeal and antioxidant activity has so far been carried out on *C. alismatifolia*. Considering the importance of having natural remedies for diarrhoea and oxidative stress-induced ailments and as a part of our ongoing investigation on local medicinal plants of Bangladesh (Hasan *et al.* 2009), we herein present a study of antidiarrhoeal and antioxidant activity of the hydromethanol extract of leaves of *C. alismatifolia*.

MATERIALS AND METHODS

Chemicals and Drugs:

DPPH (1, 1-diphenyl, 2-picryl hydrazyl), Gallic acid and Quercetin were obtained from Sigma chemical co. USA, Ascorbic acid from SD Fine chem. Ltd., Biosar, India, Naphthyl ethylene diamine dihydrochloride from Roch-light Ltd., Suffolk, England and Sodium nitro prusside was obtained from Ranbaxy Lab., Mohali, India. Loperamide and Atropine were purchased from local market.

Plant Material:

For this present investigation the leaves of *C. alismatifolia* was collected from the district Comilla, Bangladesh. The plant leaves and flowering top were collected for identification. Expert of Bangladesh National Herbarium Mirpur, Dhaka, identified the plant where a voucher specimen (Accession No. 32787) has been deposited. The flowering tops were discarded and the leaves were dried in hot air oven at 55°C for 3 days and at 40°C for the next 4 days.

Extraction:

The dried leaves were coarsely powdered and extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus for 72 hours. The solvent was completely removed and obtained dried crude extract which was used for investigation.

Animal:

For the present study, male Swiss albino mice, age of 3-4 weeks, weighing between 20-25 gm, were collected from the Animal Research Branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Animals were housed in stainless steel cages at room temperature, (24 ± 1)°C and 12/12 h light dark cycle. All animals were fed with standard pellet diet (ICDDR, B) and water *ad libitum*. Animals were acclimatized to laboratory condition for one week prior to performing the experiment (Chatterjee, 1993). Animals were treated in line with the guide and care for laboratory animals.

Tests for Antidiarrhoeal Activity:

Castor Oil-induced Diarrhoea:

20 mice were allowed to fast for 18 h and divided into 4 groups of 5 animals each. All groups received castor oil at a dose of 1 ml/animal orally (p.o.). 30 min after castor oil administration, group I (control group) received vehicle (1% CMC in distilled water), Group III and Group IV orally received the plant extract at 250 mg/kg and 500 mg/kg doses, respectively. Group II received the reference drug, loperamide (3 mg/kg p.o.). Then the animals were placed separately in cages with filter papers underneath, which was changed every hour. The severity of diarrhoea was assessed each hour for 4 h and the characteristic diarrhoeal droppings were recorded (Shoba and Thomas, 2001).

Magnesium Sulphate-induced Diarrhoea:

Diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals 30 min after pre-treatment with vehicle (1% Tween 80 in water, 10 ml/kg, p.o.) to the control group (Group I), loperamide (3 mg/kg) to the positive control group (Group II), and the methanol extract at the doses of 250 and 500 mg/kg to the test groups (Groups III and IV) (Doherty, 1981). Foecal droppings were observed hourly for 4 h and the characteristic diarrhoeal droppings were recorded

Effect on Gastrointestinal Motility:

Mice were fasted for 18h and divided into four groups of five mice each and each animal was given 1 ml of charcoal meal orally (5% activated charcoal suspended in 1% CMC) 60 min after an oral dose of the test drugs and vehicle. Group I was administered 1% CMC (10 ml/kg) and groups III and IV received extract at the dose of 250 mg/kg and 500 mg/kg body weight respectively. Group II received atropine sulfate (0.1 mg/kg,) as the standard drug. Mice were sacrificed after 30min and the intestine was removed without stretching and placed lengthwise on moist filter paper. The intestinal transit was calculated as a percentage of the distance travelled by the charcoal meal compared to the length of the small intestine (Lutterodt, 1989).

Tests for Antioxidant Activity:

DPPH Radical Scavenging Activity:

A method described by Braca *et al.* (2001) was followed to determine the free radical scavenging activity of the extract, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Plant extract (0.1 ml) was added to 3ml of a 0.004% methanol solution of DPPH. Absorbance at 517nm was determined after 30 min, and the percentage inhibition activity was calculated from the equation: $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. The inhibition curve was prepared and IC_{50} value was calculated.

Nitric Oxide Scavenging Assay:

To evaluate nitric oxide radical scavenging potential of the plant extract, Griess Illosvoy reaction method was used followed by Govindarajan *et al.* (2003). In this study, Griess-Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and *C. alismatifolia* extract (5 to 250µg/ml) or standard solution (ascorbic acid, 0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions.

Total Phenol and Total Flavonoid Content Determination:

To measure total phenol content of plant extract, extract (100 µL) was mixed with 500 µL of the Folin-Ciocalteu reagent and 1.5 mL of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 h. Then the absorbance at 765 nm was determined. These data were used to estimate the phenolic contents using a standard curve obtained from various concentration of gallic acid (Yu *et al.*, 2002).

The total flavonoid content was estimated using a method previously described by Kumaran and Karunakaran (2007) using quercetin as a reference compound. 1mL of plant extract in methanol (50-250 µg/mL) was mixed with 1mL aluminium trichloride in ethanol (20 mg/mL and a drop of acetic acid, and then diluted with ethanol to 25 mL. The absorption at 415nm was read after 40 min. Blank samples were prepared from 1ml of plant extract and a drop of acetic acid, and then diluted to 25 mL with ethanol. The absorption of standard quercetin solution (0.5 mg/mL) in ethanol was measured under the same conditions. All determinations were carried out in duplicates. These data were used to determine the flavonoid content using a standard curve obtained from various concentration of quercetin.

Statistical Analysis:

The values were expressed as mean ± SD/SEM. The results obtained were analyzed statistically using one-way ANOVA followed by Dunnet's multiple comparisons. *p* values < 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

The extract at the doses of 250 and 500 mg/kg, produced a dose dependent decrease in the number of faecal matters passed by the animals in castor oil-induced diarrhoeal model (Table 1). At higher dose (500 mg/kg) of the extract, a significant ($p < 0.05$) inhibition (58.10%) of characteristic diarrhoeal feces was observed. Similarly, the extract at 500 mg/kg dose level significantly ($p < 0.05$) reduced the extent of diarrhoea (54.64% inhibition) in test animals in magnesium sulphate-induced diarrhoea (Table 2). However, both the doses were shown to reduce the total number of faeces when compared to control. In the gastrointestinal motility test, the extract at the doses of 250 and 500 mg/kg retarded the intestinal transit of charcoal meal in mice where a significant ($p < 0.05$) retardation of intestinal transit was observed at 500 mg/kg dose when compared to the control (Table 3).

In DPPH radical scavenging assay, the extract showed a dose dependent scavenging of DPPH radical as was with the reference ascorbic acid (Figure 1); the IC_{50} value of the extract was 24.06 μ g/ml while the IC_{50} value for the reference ascorbic acid was 3.76 μ g/ml. moreover, scavenging of nitric oxide was also found to rise with increasing concentration of the extract and the result was comparable to ascorbic acid which was used as the reference (Figure 2). The IC_{50} values of the extract and ascorbic acid were 105.86 μ g/ml and 67.57 μ g/ml respectively. However, the extract was found to contain high amount of phenols (374.09 mg/g, expressed as gallic acid equivalents) and flavonoids (888.72 mg/g, expressed as quercetin equivalents) as measured by total phenol and total flavonoid content determination assays respectively (Table 4).

Table 1: Effect of hydromethanol extract of the leaves of *C. alismatifolia* on castor oil-induced diarrhoea in mice.

Groups	Treatment	Dose (p.o.)	No. of faecal droppings in 4 h	% Inhibition of defaecation
Group-I	1% Tween 80 in water	0.4 ml/mouse	21 \pm 3.416	-
Group-II	Loperamide	10 mg/kg	5.8 \pm 2.029**	72.38**
Group-III	HME of <i>C. alismatifolia</i>	250 mg/kg	13.8 \pm 2.620	34.29
Group-IV	HME of <i>C. alismatifolia</i>	500 mg/kg	8.8 \pm 2.668**	58.10**

Values are presented as mean \pm SEM, (n = 5); ** $p < 0.05$, Dunnet test as compared to control. HME: Hydromethanolic extract.

Table 2: Effect of hydromethanol extract of the leaves of *C. alismatifolia* on MgSO₄-induced diarrhoea in mice.

Groups	Treatment	Dose (p.o.)	No. of faecal droppings in 4 h	% Inhibition of defaecation
Group-I	1% Tween 80 in water	0.4 ml/mouse	19.4 \pm 1.245	-
Group-II	Loperamide	3 mg/kg	4.6 \pm 1.597**	76.29**
Group-III	HME of <i>C. alismatifolia</i>	250 mg/kg	13.8 \pm 2.244	28.87
Group-IV	HME of <i>C. alismatifolia</i>	500 mg/kg.	8.8 \pm 2.070**	54.64**

Values are presented as mean \pm SEM, (n = 5); ** $p < 0.05$, Dunnet test as compared to control. HME: Hydromethanolic extract.

Table 3: Effect of hydromethanol extract of the leaves of *C. alismatifolia* on charcoal meal-stimulated gastrointestinal transit

Treatment	Dose (p.o.)	Intestinal length (cm)	Distance traveled by charcoal (cm)	% GI transit
1% Tween 80 in water	0.4 ml/mouse	64 \pm 2.769	49.2 \pm 2.636	77.40 \pm 4.555
Atropine	0.1 mg/kg	62.8 \pm 3.141	20.6 \pm 2.033**	32.75 \pm 2.581**
HME of <i>C. alismatifolia</i>	250 mg/kg	60.6 \pm 2.320	39.0 \pm 2.843	64.54 \pm 4.777
HME of <i>C. alismatifolia</i>	500 mg/kg.	58.8 \pm 3.128	29.8 \pm 3.128**	51.93 \pm 6.731**

Values are presented as mean \pm SEM, (n = 5); ** $p < 0.05$, Dunnet test as compared to control. HME: Hydromethanolic extract.

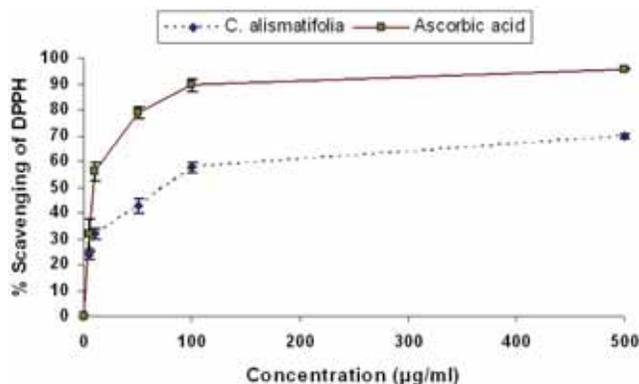


Fig. 1: DPPH radical scavenging activity of hydromethanol extract of the leaves of *C. alismatifolia*. Values are the average of duplicate experiments and represented as mean SD.

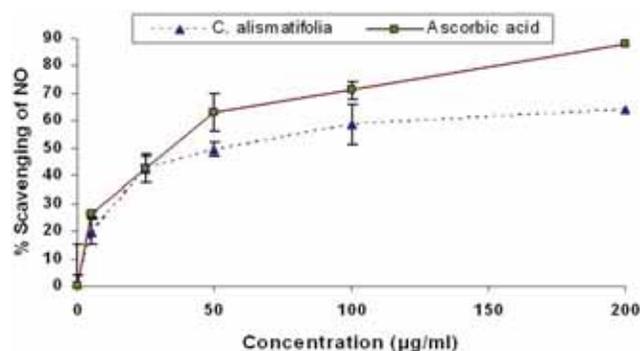


Fig. 2: Nitric oxide scavenging activity of the hydromethanol extract of the leaves of *C. alismatifolia*. Values are the average of duplicate experiments and represented as mean SD.

Table 4: Total phenol and total flavonoid contents of methanol extract of the leaves of *C. alismatifolia*

Extract	Total phenol (in mg/g, Gallic acid equivalents)	Total flavonoid (in mg/g, Quercetin equivalents)
Hydroethanol extract of <i>C. alismatifolia</i> leaves	374.09±1.537	888.72±11.18

Values are the mean of duplicate experiments and represented as mean ± SD.

Discussion:

Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied by hurry resulting in an excess loss of fluid in the faeces. In some diarrhoea the secretory component predominates while other diarrhoea is characterized by hypermotility (Chitme *et al.*, 2004). Castor oil causes diarrhoea due to its active metabolite, ricinoleic acid (Ammon *et al.*, 1974; Watson and Gordon, 1963) which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Again, castor oil is reported to induce diarrhoea by increasing the volume of intestinal contents by preventing the reabsorption of water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin (Galvez *et al.*, 1993; Pierce *et al.*, 1971).

Earlier reports suggest that antidiarrhoeal properties of medicinal plants might be ascribed to tannins, alkaloids, saponins, flavonoids, sterols and reducing sugars (Longanga *et al.*, 2000). As the previous phytochemical screening of extract showed the presence of alkaloids, flavonoids, tannins and gums (Akter *et al.*, 2008), these constituents may mediate the antidiarrhoeal property of the extract. Flavonoids, present in the plant extract, are reported to inhibit release of autacoids and prostaglandins, thereby may inhibit motility and secretion induced by castor oil (Veiga *et al.*, 2001). The antidiarrhoeal activity of the extract may also be due to denature proteins forming protein tannates which make intestinal mucosa more resistant and reduce secretion. In addition to other previous mechanisms to explain the diarrhoeal effect of castor oil, recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil (Mascolo *et al.*, 1996). Antidiarrhoeal effect may be attributed, at least in part, to nitric oxide scavenging activity of the extract. 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 reported 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 (Galvez *et al.*, 1993; Zavala *et al.*, 1998). The methanol extract was found to improve 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. In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease (Liao and Yin, 2000). Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals. Polyphenolic compounds, like flavonoids, tannins and phenolic acids,

commonly found in plants have been reported to have multiple biological effects, including antioxidant activity. Flavonoids and tannins present in the plant extract, as evident from phytochemical screening (Akter *et al.*, 2008), may be responsible for the antioxidant action in the tested models. Moreover, nitric oxide is implicated for inflammation, cancer and other pathological conditions. Hence, nitric oxide scavenging capacity of the extract may help to arrest the chain of reactions initiated by excess generation of nitric oxide that are detrimental to the human health (Moncada *et al.*, 1991).

Conclusion:

On the basis of the results of the present study, it can be inferred that the extract contains pharmacologically active substance(s) with antidiarrhoeal and antioxidant properties. These results suggest the use of *C. alismatifolia* leaves as an antidiarrhoeal drug and antioxidant. Further research is to be carried out to fractionate and purify the extract, in order to find out the molecules responsible for the activity observed.

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