Evaluation of anti-diarrhoeal activity of Curcuma zedoaria rhizome

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Abstract
Curcuma zedoaria (family: Zingiberaceae) is broadly used as a folk medicine in dyspepsia, cough, dermatosis, inflammations, diarrhoea and other diseases. In the present study, ethanol extracts of Curcuma zedoaria rhizomes was evaluated for phytochemical screening and antidiarrhoeal activity. The results showed that Curcuma zedoaria extract dose-dependently reduced the severity & frequency of diarrhoea in mice compared with the standard antimotility drug loperamide. Preliminary phytochemical screening of ethanol extract showed the presence of tannins, flavonoids, saponins, alkaloids, terpinoids, carbohydrates and steroids as major constituents, some of which possess antidiarrhoeal activity and justify the traditional use of the plant extract in the treatment of diarrhea.

Keywords: Curcuma zedoaria, phytochemical screening, antidiarrhoeal

1. Introduction
Curcuma zedoaria or white Turmeric is a herbaceous and rhizomatous perennial plant belonging to the family Zingiberaceae. Some common names of the plant are kachur (Hindi), karchur (Sanskrit), shatkachuro (Gujarati), Meitei Yaingang (Manipur) and shoti (Bangla) [1]. The plant is native to Bangladesh, India, Sri Lanka and Indonesia and cultivated in Brazil, China, Japan, Nepal, Malaysia, and Thailand [2]. The perennial herb has a woody, warm-spicy and camphoraceous cineolic odor and bears shiny yellow flowers, with green and red bracts. The ovate leaves are 1 to 2 feet long, narrowing at the base and possess purple-colored spots. Fruits are triangular and ovate in shape. Seeds are spear or oval shaped. The rhizome of C. zedoaria is reported to possess anti-allergic [3], hepatoprotective [4], antimicrobial [5-6], anti-zedoaronediol [7], analgesic [8], antipyretic [9], antioxidant [11] and anti-hyperlipidemic [12] activities. The major active constituents of the plant are terpenoids, specially sesquiterpenoids like furanodienone and furanodiene, sesquiterpenes like procurcumenol and epiprocurcumenol [13], zederone, curzerenone, curzeone, germacrone, 13-hydroxygermacrone, dehydrocucumenone, zedoaronediol [7], cucumenone, cucumanolide A, cucumanolide B [14], isocucumenol, beta-turmerone and ar-turmerone [15].

The rhizome of C. zedoaria has been used traditionally for the treatment of menstrual disorders, dyspepsia, cough, vomiting [16], inflammations, hepatitis and diarrhoea [17]. However, no scientific work has been reported yet in support of the anti-diarrhoeal activity of C. zedoaria. Henceforth, the current study was carried out to investigate the anti-diarrhoeal activity of C. zedoaria.

Materials and Methods
Plant material
We collected the plant Curcuma zedoaria from Savar, Bangladesh. Experts of Bangladesh National Herbarium (BNH) Mirpur, Dhaka identified the plant (accession number 36098) and preserved the specimen.

Preparation of plant extract
The rhizomes of the collected plant were washed with water and separated from unwanted materials. They were partially air dried by the use of a fan. Afterward, we heated the rhizomes in the oven at below 40ºc for two days. The dried rhizomes were then grinded to make powder with a grinder. The powder was stored in a zipper bag in the refrigerator at +4ºc. About 500 gm of powdered material was taken in a clean, flat-bottomed glass container and soaked in 1500 ml of 80% ethanol. The container with the contents was sealed and kept for seven days and was subjected to occasional shaking and stirring. The entire mixture went through a coarse filtration by a piece of clean cotton cloth. Then we filtered the mixture through a cotton plug followed by Whatman filter paper number 1.
At the end, we concentrated the extract with a rotary evaporator at low temperature and reduced pressure and preserved that at +4°C until further investigation.

**Phytochemical screening**

10% (w/v) solution of ethanolic extract of *Curcuma zedoaria* was subjected to preliminary phytochemical screening to detect the presence of various classes of phytoconstituents using the following reagents and chemicals: tannins with ferric chloride, flavonoids and terpinoids with concentrated HCl, saponins with ability to produce stable foam, alkaloids with Wagner’s reagent, gums with Molisch reagent and sulphuric acid, reducing sugar by ring test and steroids by acetic anhydride and sulphuric acid. We followed standard procedures to identify the phytoconstituents by characteristic color changes [18].

**Anti-diarrhoeal test**

The anti-diarrhoeal activity was performed by the reported method [19]. Male albino mice (average weight: 20 - 25g) were kept in well cross ventilated room. We provided them commercial rodent pellet diet and water ad libitum. 20 animals were divided into four groups of five animals in each group. Group I (control) received distilled water and Groups II (positive control) received standard drug, loperamide at the dose of 2 mg/kg body weight. Groups III, IV and V (test groups) were treated with suspension of *Curcuma zedoaria* extract at the oral doses of 250, 500 and 750 mg/kg, respectively. After one hour treatment with distilled water, standard drug or plant extract, 1 ml of castor oil was orally given to all the overnight-fasted animals to induce diarrhoea. Each mouse was housed separately and observed for diarrheal episode for 4 hours. During this time, number and weight of diarrheal feces were taken and noted after every thirty minutes. We calculated the mean diarrheal episodes and percent protection of diarrhoea in mice.

**Statistical analysis**

The data of stool weight was expressed as mean ± standard error of mean (SEM). We performed one-way ANOVA test followed by Dunnet’s multiple comparisons. We compared the results with the control group. P values <0.05 were considered to be statistically significant.

**Results and discussion**

**Phytochemical screening**

Phytochemical studies showed the presence of tannins, flavonoids, saponins, alkaloids, terpinoids, carbohydrates and steroids in the ethanolic extract of *Curcuma zedoaria* rhizomes (Table 1).

**Table 1: Phytochemical constituents of Curcuma zedoaria rhizomes.**

<table>
<thead>
<tr>
<th>Plant Extract</th>
<th>Tannins</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Alkaloids</th>
<th>Terpinoids</th>
<th>Gums carbohydrates</th>
<th>Reducing sugars</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
</tbody>
</table>

Symbol (+) indicates presence in trace concentration, symbol (++) indicates presence in moderate concentration and symbol (+++) indicates presence in high concentration of the respective phytochemicals.

**Antidiarrheal activity**

Diarrhoea was induced by castor oil in about one hour in the mice. The onset of diarrhoea was significantly prolonged by administration of ethanol extract of leaves of *Curcuma zedoaria* at in dose-dependent manner (Table 2). During three hours’ study, the total number of feces was significantly reduced by the plant extract in comparison to control animals. The amount of stool (g) was also significantly decreased by ethanolic extract of *Curcuma zedoaria* compared to control animals. The plant extract protected 52.84 %, 71.55 % and 78.39 % diarrhoea at the dose of 250, 500 and 750 mg/kg respectively, in comparison to 90.12% protection of diarrhoea in animals treated with loperamide (2 mg/kg). So this study reveals that alcohol extracts of *Curcuma zedoaria* have significant antidiarrheal activity.

**Table 2: Antidiarrheal activity of Curcuma zedoaria**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Onset of diarrhea (min.)</th>
<th>Total no. of feces in 3 hr</th>
<th>Weight of stools (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Water 10 ml/kg)</td>
<td>164.4 ± 6.256**</td>
<td>26.2±1.15</td>
<td>1.184±0.029</td>
</tr>
<tr>
<td>Standard (Loperamide 2 mg/kg)</td>
<td>98.4 ± 2.638*</td>
<td>19.7±0.67</td>
<td>0.558±0.0962**</td>
</tr>
<tr>
<td><em>C. Zedoaria</em> (250 mg/kg)</td>
<td>116.4 ± 3.501*</td>
<td>16.04±1.17</td>
<td>0.337±0.022**</td>
</tr>
<tr>
<td><em>C. Zedoaria</em> (500 mg/kg)</td>
<td>123.4 ± 9.03**</td>
<td>11.08±0.88</td>
<td>0.267±0.039**</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± Standard Error of Mean (SEM), n = number of mice = 5. *P < 0.05, ** P < 0.01, *** P < 0.001 as compared with control group.

The remarkable anti-diarrheal effect (% protection) of *Curcuma zedoaria* rhizome extracts against castor oil-induced diarrhea model suggests that it is effective in an extensive range of diarrheal conditions. It is well known that castor oil produces diarrhoea due to its most active constituent ricinoleic acid which causes irritation and inflammation of the intestinal mucosa. This leads to release of prostaglandins resulting in stimulation of secretion [20]. Since the ethanol extract of *Curcuma zedoaria* inhibited castor oil-induced diarrhoea in the test groups of the experiment, the extract might have showed its antidiarrhoeal effect through antisecretory mechanism. The antidiarrhoeal activity of the plant may be due to its content of tannins [21] and/or flavonoids [22].

**Conclusions**

In the present study, we can draw a conclusion that the ethanol extracts of *Curcuma zedoaria* have significant anti-diarrheal effects, which support the traditional use of this plant in diarrhoea. Further studies will be conducted to identify the major component(s) of the plant responsible for the antidiarrheal effect.

**References**