Orient Pharm Exp Med (2014) 14:363–367
DOI 10.1007/s13596-014-0167-1

RESEARCH ARTICLE

# Comparative study of antidiarrheal activity of methanol extracts from leaf and fruit of *Pandanus odoratissimus* Linn

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Received: 1 March 2014 / Accepted: 27 August 2014 / Published online: 23 September 2014


**Abstract** *Pandanus odoratissimus*, a perennial, large shrub or small tree, has multifarious medicinal uses. The purpose of the present study was to evaluate the antidiarrheal effects of methanol extracts obtained from leaf and fruit of *P. odoratissimus*. The methanol extracts from the leaf and fruit of *P. odoratissimus* were evaluated for the antidiarrheal activity in rats. The effects of the extracts on defecation, intestinal transit and intestinal fluid accumulation (enteropooling) were assessed in castor oil-induced diarrhea. Oral administration of leaf and fruit extracts at 200 and 400 mg/kg exhibited significant ($P<0.05$–$0.01$) and dose-dependent antidiarrheal potential in castor oil-induced diarrhea. The diarrheal episode was inhibited by 34.30 % and 47.60 % by leaf extract, whereas 35.11 % and 47.15 % by fruit extract at the doses of 200 and 400 mg/kg respectively. The extracts were found to possess an antienteropooling in castor oil-induced experimental animals by reducing both weight and volume of intestinal content significantly and also decrease in intestinal transit comparable to that of standard drug, loperamide (5 mg/kg). These findings revealed that methanol extracts of leaf and fruit of *P. odoratissimus* possess significant antidiarrheal activities.

**Keywords** Antidiarrheal · Castor oil · Enteropooling · Intestinal transit · *Pandanus odoratissimus*

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## Introduction

*Pandanus odoratissimus* Linn. (Pandanaceae) is a small, dioecious tree profusely branched with copious aerial roots, commonly known as Keya and found in the coastal areas including Saint Martin Island and sporadically in other districts of Bangladesh (Thompson and Islam 2010). Leaves, flowers, roots, fruits, spadices, and bracts have been used in traditional medicines to treat tumors, leprosy, smallpox, syphilis, scabies, pain, heat of body, heart and brain diseases, leucoderma and blood diseases. The roots are claimed to be useful in treating diabetes. The oil and otto obtained from the bracts are prescribed as stimulant and antispasmodic and are administered in headache and rheumatism. The root is considered diuretic, depurative and tonic (Vaidyarathnam 1997; Chatterjee and Sc 2001; Nadkarni 2002). The plant is also used to treat asthma (Thompson and Islam 2010).

It is experimentally validated that *P. odoratissimus* possess antibacterial (Wei et al. 2008; Kumar et al. 2010; El-Shaibany 2014), anti-inflammatory (Londonkar et al. 2010), antioxidant (Jong and Chau 1998; Londonkar and Kamble 2009; Sasikumar et al. 2009; El-Shaibany 2014), antitumor (Panigrahi et al. 2011), antidiabetic (Venkatesh et al. 2012), antiepileptic (Adkar et al. 2014), anti-hyperlipidemic (Zhang et al. 2013), CNS depressant (Raju et al. 2011), nocturnal enuresis (El-Shaibany 2014), hepatoprotective and hepatocurative (Ilanchezhian and Joseph 2010; Londonkar and Kamble 2011) activities. The principle constituent of *P. odoratissimus* oil, b-phenyl ethyl methyl ether, is responsible for the characteristic aroma of spadices. 2-acetyl-1-pyrroline is a major volatile component in the tender floral leaves or spathes. Essential oil obtained from blossoms (0.1–0.3 %) yield benzyl benzoate, benzyl salicylate, benzyl acetate, benzyl alcohol, geraniol, linalool, linalyl acetate, bromostyrene, guaiacol, phenyl ethyl alcohol, and aldehydes. Cirsilineol, physcion, n-triacontanol, β-sitosterol,

β-sitostenone, tigmast-4-ene-3,6 dione stigmasterol, campesterol, daucosterol, and palmitic acid, stearic acid have been reported from rhizomes (Chopra et al. 1996; Prajapati et al. 2003). Two compounds, 4-hydroxy-3–(2',3'-dihydroxy-3'-methylbutyl)-benzoic acid methyl ester and 3-hydroxy-2-isopropenyl-dihydrobenzofuran-5-carboxylic acid methyl ester, have been isolated from the roots of *P. odoratissimus* that showed significant antioxidant activity (Jong and Chau 1998). Since *P. odoratissimus* has been used in the indigenous medicine for the treatment of a number of maladies, the experiment was designed to evaluate the antidiarrheal activity of methanol extracts of *Pandanus odoratissimus* leaf and fruit against castor oil induced diarrhea in rats.

## Materials and methods

### Plant materials

*Pandanus odoratissimus* L. leaf and fruit were collected from Saint Martin Island, Cox's Bazar, Bangladesh in the month of November 2011 and authenticated by the Director, Bangladesh Forest Research Institute, Chittagong, Bangladesh. A voucher specimen (Accession No. 37091) was deposited at the Department of Pharmacy, International Islamic University Chittagong, Bangladesh for further reference.

### Preparation of extract

The leaf and fruit were washed in tap water to remove extraneous matters, then air dried at room temperature and ground into fine powder . The ground powder (186 g leaf and 210 g fruit) then soaked in sufficient amount of methanol for 1 week at room temperature with occasional shaking and stirring, then filtered through a cotton plug followed by Whitman filter paper No.1. The solvent was evaporated under vacuum at temperature 45 °C to yield semisolid (1.24 % w/w leaf and 1.48 % w/w fruit). The extracts were then preserved in a refrigerator till further use.

### Animals

Healthy long evens rats (97–100 g) of either sex obtained from the animal house of International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR, B) were used as the experimental model for investigating the antidiarrheal activity. They were kept in well cross ventilated room at 25±2 °C temperature and 55–65 % humidity, for 1 week before and during the experiment. Animal were allowed free access to drinking water and pellet diet, collected from ICDDR, B. The study protocol was approved by the P&D Committee, Department of Pharmacy, International Islamic University Chittagong, Bangladesh (Ph-P&D-29/05'11). Food was withheld 18 h before experiments but there was free access to drinking water. Rats were divided into six groups for each experiment containing four animals each.

### Drugs and chemicals

Castor oil (WELL's Heath Care, Spain), loperamide hydrochloride (Square Pharmaceuticals Ltd., Bangladesh), normal saline solution (0.9 % NaCl), charcoal meal (10 % activated charcoal in 5 % gum acacia) were used for antidiarrheal activity tests. All other reagents were of analytical grade.

## Antidiarrheal activity

### Castor oil-induced diarrhea in rats

This method was followed according to the method of Awouters et al. 1978. Group I marked as control was given normal saline 2 mL/kg body weight (b.wt) orally (p.o) while the second group received standard drug (loperamide 5 mg/kg b.wt p.o). Next third to sixth groups treated with methanol extract of leaf and fruit of *P. odoratissimus* (200 and 400 mg/kg b. wt. p.o) respectively. 1 h after the treatment, each animal received 1 mL castor oil orally through a feeding needle. Thereafter, they were placed in cages lined with adsorbent papers and observed for 4 h for the presence of characteristic diarrheal droppings. The total number of diarrheal feces of the control group was considered 100 %. The level of inhibition (%) of defecation caused by extracts was calculated relative to the control using the following relationship: Inhibition of defecation (%)=[(A - B) / A]×100; where, A=mean number of diarrheic feces of the control group and B=mean number of defecation caused by drug or extracts.

### Castor oil-induced enteropooling

Group I treated with saline (2 mL/kg), group II received loperamide (5 mg/kg) and group III-VI received crude extracts (200 and 400 mg/kg) respectively before 1 h administration of castor oil in each rat orally to induce diarrhea. Then 1 h later, the rats were sacrificed and the small intestine from the pylorus to the caecum was isolated. The intestinal contents were collected by milking into a graduated tube and their volumes were measured (Robert et al. 1976).

### Gastrointestinal motility test

1 h following the administration of castor oil, test control groups were orally administered with different extracts (200 and 400 mg/kg), respectively. The standard control group

**Table 1** Effects of methanol extracts of *Pandanus odoratissimus* leaf and fruit on castor oil induced diarrhea in rats

| Group | Treatments | Total number of feces | % Inhibition of defecation | Total number of diarrheal feces | % Inhibition of diarrhea |
|---|---|---|---|---|---|
| I | Castor oil+Saline (2 mL/kg p.o) | 18.18±1.91 | – | 11.05±1.08 | – |
| II | Castor oil+Loperamide (5 mg/kg p.o) | 7.76±0.66** | 57.32 | 5.00±0.33** | 54.75 |
| III | Castor oil+Leaf extract (200 mg/kg p.o) | 12.19±0.42 | 32.94 | 7.26±0.63* | 34.30 |
| IV | Castor oil+Leaf extract (400 mg/kg p.o) | 9.11±0.68** | 49.89 | 5.79±0.52* | 47.60 |
| V | Castor oil+Fruit extract (200 mg/kg p.o) | 11.00±0.58 | 39.49 | 7.17±0.44* | 35.11 |
| V I | Castor oil+Fruit extract (400 mg/kg p.o) | 10.67±0.67** | 41.31 | 5.84±0.51* | 47.15 |

Values are expressed as mean±SEM. ($n$=4). * $p$<0.05, ** $p$<0.01 when compared with control animals

received loperamide 5 mg/kg p.o for comparison. The control group was treated with saline water only. 1 h later, each animal received 1 mL of charcoal meal (10 % charcoal suspension in 5 % gum acacia) orally. After 1 h, each animal was sacrificed and the distance covered by the charcoal meal in the intestine, from the pylorus to the caecum was measured and expressed as percentage of distance moved (Mascolo et al. 1994).

## Statistical analysis

Experiments results were analyzed by one-way ANOVA followed by Bonferroni test using SPSS Data Editor for Windows, Version 16.0 (SPSS Inc., USA). Values are represented as mean±SEM (standard error of mean).

## Results

### Castor oil induced diarrhea

The leaf and fruit extracts of *P. odoratissimus* showed a marked antidiarrheal effect in the castor oil-induced diarrhea in the rats (Table 1). In both doses (200 mg/kg and 400 mg/kg) produced dose-dependant defecation, but higher dose exhibited significant ($p$<0.01) defecation. The total number of wet feces produced upon administration of castor oil decreased (12.19±0.42 and 9.11±0.68 at doses 200 mg/kg and 400 mg/kg by leaf extract and 11.00±0.58 and 10.67±0.67 at doses 200 mg/kg and 400 mg/kg by fruit respectively) compared to the controls (18.18±1.91) while loperamide decreased to 7.76±0.66 at the dose of 5 mg/kg.

### Castor oil induced enteropooling

Treatment with the *P. odoratissimus* leaf and fruit extracts (200 and 400 mg/kg) produced a dose-dependent and significant ($P$<0.05–0.01) reduction in intestinal weight and volume, except the leaf extract at 200 mg/kg in intestinal content (Table 2). The intestinal volume was decreased by 34.66 % and 46.93 % at doses 200 and 400 mg/kg by leaf extract and 34.30 % and 46.57 % by fruit extract respectively. The standard drug, loperamide (5 mg/kg), also significantly ($p$<0.01) inhibited intestinal fluid accumulation (54.15 %).

### Gastrointestinal motility test

The movement of the orally administered charcoal meal through the small intestine at the highest dose of 400 mg/kg was noticeably lessened from 24.38 to 42.31 % by leaf extract and 34.29 to 43.63 % by fruit extract when compared with the controls (Table 3).

**Table 2** Effects of methanol extracts of *Pandanus odoratissimus* leaf and fruit on castor oil induced enteropooling in rats

| Group | Treatment | Weight of intestinal content (g) | Volume of intestinal content (mL) | Inhibition (%) |
|---|---|---|---|---|
| I | Castor oil+Saline (2 mL/kg p.o) | 3.22±0.05** | 2.77±0.23** | – |
| II | Castor oil+Loperamide (5 mg/kg p.o) | 1.84±0.44** | 1.27±0.077** | 54.15 |
| III | Castor oil+Leaf extract (200 mg/kg p.o) | 2.66±0.032 | 1.81±0.21* | 34.66 |
| IV | Castor oil+Leaf extract (400 mg/kg p.o) | 2.163±0.06** | 1.47±0.191** | 46.93 |
| V | Castor oil+Fruit extractz (200 mg/kg p.o) | 2.17±0.065* | 1.82±0.24* | 34.30 |
| V I | Castor oil+Fruit extract (400 mg/kg p.o) | 1.99±0.08** | 1.48±0.22** | 46.57 |

Values are expressed as mean±SEM. ($n$=4). * $p$<0.05, ** $p$<0.01 when compared with control animals

**Table 3** Effects of methanol extracts of *Pandanus odoratissimus* leaf and fruit on small intestinal transit in rats

| Group | Treatment | Total length of intestine (cm) | Distance traveled by marker (cm) | Inhibition (%) |
|---|---|---|---|---|
| I | Castor oil+Saline (2 mL/kg p.o) | 107.8±2.36 | 101±2.82** | – |
| II | Castor oil+Loperamide (5 mg/kg p.o) | 103.36±1.66 | 44±.07** | 56.43 |
| III | Castor oil+Leaf extract (200 mg/kg p.o) | 100.27±0.59* | 76.37±0.62* | 24.38 |
| IV | Castor oil+Leaf extract (400 mg/kg p.o) | 104.33±0.47** | 58.27±0.65** | 42.31 |
| V | Castor oil+Fruit extract (200 mg/kg p.o) | 98.67±2.35 | 66.37±2.35** | 34.29 |
| V I | Castor oil+Fruit extract (400 mg/kg p.o) | 103.43±1.30 | 56.93±1.10* | 43.63 |

Values are expressed as mean±SEM. ($n$=4). * $p$<0.05, ** $p$<0.01 when compared with control animals

## Discussion

Diarrhea may be characterized as the abnormally frequent defecation of feces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines. Despite the availability of vast spectrum of approaches for diarrheal management, vast majority of people in developing countries rely on herbal drugs for the management of diarrhea. World Health Organization (WHO) has encouraged studies for treatment and prevention of diarrheal diseases depending on traditional medical practices (Maikere-Faniyo et al. 1989; Atta and Mouneir 2004).

It is widely known that castor oil is metabolized into ricinoleic acid in the gut which is mainly responsible for diarrhea production (Ammon et al. 1974). The peristaltic activity of small intestine is increased if ricinoleate present in small intestine, as a result $Na^+$ and $Cl^-$ permeability changed in intestinal mucosa (Palombo 2006). Secretion of endogenous prostaglandin stimulated also by ricinoleate (Beubler and Juan 1979; Zavala et al. 1998). Both leaf and fruit extract of *P. odoratissimus* at 200 and 400 mg/kg doses exhibited significant reduction of the number of diarrheal and total feces which may be due to activation of prostaglandin biosynthesis with resultant decrease in secretion of fluid into the lumen or may be due to promotion and absorption of water and electrolytes in the gut. The standard drug, loperamide (5 mg/kg) also produced statistically significant ($p$<0.01) diarrheal inhibition (54.75 %). The intestinal volume was decreased by 34.66 % and 46.93 % by leaf extract, while fruit extract exhibited 34.30 % and 46.57 % at doses of 200 and 400 mg/kg respectively. Suppression of intestinal fluid accumulation by extracts may also suggest inhibition of gastrointestinal functions (Nwafor et al. 2000; Ezeja et al. 2012). The above speculation was further supported by the inhibitory action of the extracts on intestinal charcoal meal motility. Both extracts suppressed the propulsive movement or transit of charcoal meal through the gastrointestinal tract which significantly indicates that the extracts may have potentiality to reduce the frequency of stooling in diarrheal conditions.

Preliminary phytochemical analysis of plant extracts revealed the presence of flavonoids, phenols, tannins, alkaloids, steroids, carbohydrates, proteins, saponins, triterpenes and glycosides (Chilakwad et al. 2008; Kumar et al. 2010; Kusuma et al. 2012; El-Shaibany 2014). Earlier studies have reported that components such as tannins, saponins, steroids, terpenes, alkaloids and flavonoids are responsible for anti-dysenteric and anti-diarrheal properties of different medicinal plant through different mechanisms (Longanga Otshudi et al. 2000; Ojewole et al. 2009; Awe et al. 2011). Hence, flavonoids, saponins, alkaloids, steroids, terpenes, and and/or other bioactive compound (s) may be responsible for the mechanism of action of *P. odoratissimus* antidiarrheal activity. Above observations suggest that the extracts in graded doses might reduce diarrhea by inhibiting peristalsis, intestinal motility and/or increasing colonic water and electrolyte reabsorption. Further research is needed to fractionate the extract and to isolate the molecule (s) responsible for the antidiarrheal activity observed.

**Acknowledgements** Authors are grateful to the Department of Pharmacy, International Islamic University Chittagong, Bangladesh for providing research facilities.

**Conflict of Interest statement** We declare that we have no conflict of interest.

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