



Assessment of antidiarrhoeal activity of *Desmostachya bipinnata* L. (Poaceae) root extracts

[Evaluación de la actividad de antidiarreica de extractos de la raíz de *Desmostachya bipinnata* L. (Poaceae)]

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Abstract

Desmostachya bipinnata root has been used in the Indian traditional system of medicine for treatment of diarrhoea and dysentery. The antidiarrhoeal effect of both alcoholic and aqueous extracts of the roots of *Desmostachya bipinnata* were studied in rats against castor oil induced diarrhoea and charcoal meal test at the doses of 200 and 400 mg/kg body weight. The alcoholic extract and to a lesser extent aqueous extract significantly reduced the weight of the faeces and decreased the propulsion of charcoal meal through the gastrointestinal tract. The phytochemical screening of the extracts showed the presence of alkaloids, glycosides, flavonoids, tannins, phytosterol, terpenoids, polyphenolics, protein and carbohydrates. These results may support the fact that this plant is used traditionally to cure diarrhoea.

Keywords: *Desmostachya bipinnata* Antidiarrhoeal activity, Castor oil, Charcoal meal.

Resumen

La raíz de *Desmostachya bipinnata* ha sido utilizada en el sistema tradicional de medicina Hindú para el tratamiento de diarrea y disentería. El efecto antidiarreico de los extractos alcohólicos y acuosos de los extractos de la raíz de *Desmostachya bipinnata* fueron estudiados en ratas, utilizando la diarrea inducida por aceite de castor y el ensayo de la prueba por carbón en dosis de 200 y 400 mg/kg de peso corporal. El extracto alcohólico y en menor grado, el extracto acuoso, redujeron significativamente la propulsión de carbón a través del tracto gastrointestinal. El análisis de los extractos mostraron la presencia de alcaloides, glicósidos, flavonoides, taninos, fitoesterol, terpenoides, polifenoles, proteínas y carbohidratos. Estos resultados pueden apoyar el hecho de que esta planta sea usada tradicionalmente para curar la diarrea.

Palabras Clave: *Desmostachya bipinnata*, actividad antidiarreica, aceite de castor, Charcoal meal.

List of abbreviations: OECD- Organisation for economic cooperation and development guidelines for acute oral toxicity; AOT-Acute oral toxicity.

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INTRODUCTION

Diarrhoeal disease has long been recognized as a leading cause of morbidity and mortality (Snyder and Merson, 1982) and major cause of sickness and death among young children in developing countries (Feachem *et al.*, 1983).

Medicinal herbs constitute an indispensable component of the traditional medicine practised worldwide due to the economical viability, accessibility and ancestral experience. Despite the availability of a vast spectrum of approaches for diarrhoeal management, a vast majority of the people of the developing countries relies on herbal drugs for the management of diarrhoea (Afroz *et al.*, 2006). WHO has encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medical practices (Atta and Mouneir, 2004).

Desmostachya bipinnata belonging to family Poaceae (India locally called as Darbha or darbhapullu), is commonly known as sacrificial grass, as it is being used in Yagnas and religious rites (Sivaranjan and Indira, 1994). *Desmostachya bipinnata* is a tufted perennial grass with thick scaly root stocks, which sends out creeping rhizomes in all directions. Leaves many up to 50 cm long and 1 cm broad at the base (Prajapati *et al.*, 2003). It is distributed throughout India in hot and dry places and also found in Nubia, Egypt and Syria (Kirtikar and Basu, 1918).

Roots of *Desmostachya bipinnata* are used in the Indian traditional system of medicine as cooling, sweet, astringent, diuretic and galactogogue and also useful in dysentery, diarrhoea, urinary calculi, dysuria, other diseases of bladder and skin diseases (Joshi, 2003). The culms are said to possess diuretic and stimulant properties. In the Kokan they are prescribed in compound decoctions with more active drugs for the cure of dysentery, menorrhagia, etc. (Kirtikar and Basu, 1918).

Previous chemical work on the plant resulted in the isolation of some known coumarins (scopoletine and umbeliferone), sugars, amino acids and carbohydrates (Hifnawy *et al.*, 1999). Pharmacological studies on plant of *Desmostachya bipinnata* established its antiulcerogenic, analgesic, antipyretic and anti-inflammatory activities (Amani *et al.*, 2008; Panda *et al.*, 2009). Five main flavanoid glycosides were isolated from ethanolic extract of *Desmostachya bipinnata* and two of the isolated compounds (trycin and trycin-7-glucoside) show very promising

antiulcerogenic activity (Amani *et al.*, 2008). Furthermore, another flavanoid compound 4-methoxy quercetin-7-o-glucoside isolated from whole plant of *Desmostachya bipinnata* showed good in vitro antihelicobacter activity (Mohammed *et al.*, 2009).

The dried tender leaves of the plants are very active as antimicrobial and previous studies on the roots showed considerable antibacterial activity against *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* (Hashmi and Rashid, 2001). This may support the traditional use of this plant as the antidiarrhoeal agent.

The aim of the present study was to evaluate the antidiarrhoeal activity of alcoholic and aqueous extracts of roots of *Desmostachya bipinnata*.

MATERIALS AND METHODS

Plant material

Desmostachya bipinnata roots were collected from Chenagaalpattu (Tamilnadu), in August 2009 and identified by Dr. A. Saravana Ganthi (PhD), Lecturer, Department of Botany, Rani Anna Govt. College for Women Tirunelveli, Tamilnadu. A voucher specimen with an identification number (D.B-10-04) was deposited in the herbarium, Department of Pharmacognosy, PES College of Pharmacy, Bangalore.

Preparation of extracts

The roots of *Desmostachya bipinnata* were washed with distilled water to remove dirt and soil. It was further shade dried and coarsely powdered. The powdered roots weighing about 100 g were extracted in a Soxhlet extractor with 90% ethanol and aqueous extract was prepared by macerating about 100 g with water at room temp for 24 h and filtered. These extracts were concentrated at a reduced temperature (50 °C) and pressure using rotary evaporator. Yields for alcoholic and aqueous extracts were 9.3% and 12.30% (w/w) respectively. The extracts were stored in a desiccator. Prior to use for the pharmacological experiment, the dried extracts were suspended in 2% (w/v) aqueous tween 80 suspension and administered to animal orally for evaluation of antidiarrhoeal activity.

Phytochemical screening

Phytochemical analysis of alcoholic and aqueous extracts of roots of *Desmostachya bipinnata* were carried out using a standard procedure (Kokate, 1994).

Experimental animals

Swiss albino mice (20 - 25 g) and Wistar albino rats (150 - 200 g) of either sex were acclimated for 7 days under standard husbandry conditions. i.e. room temperature 26 ± 2 °C, relative humidity 45 - 55% and light: dark cycle 12:12 h. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of PES College of Pharmacy, Bangalore and conducted according to the guidelines of the Committee for the Purpose of the Control and Supervision on Experiments on Animals (CPCSEA).

Acute oral toxicity studies

The acute toxicity of alcoholic and aqueous extracts of *Desmostachya bipinnata* was determined in female albino mice (20 - 30 g). After administration of extracts up to 2000 mg/kg doses neither showed any mortality nor any visible clinical signs of toxicity in the animals. LD50 was calculated as per OECD guidelines 425 using AOT 425 software.

Antidiarrhoeal activity test by castor oil-induced diarrhoea in rats

The method of Awouters *et al.*, (1978) was followed. Albino rats of either sex weighing 150 - 200 g were used. They were divided into 6 groups containing six animals each. Rats were fasted 24 h before the test with free access to water. Rats were treated orally. Group 1 (control) received vehicle, group 2 received loperamide (3 mg/kg, p.o) and group 3 - 6 treated with alcoholic and aqueous extracts at doses of 200 and 400 mg/kg (one dose of extract per group). One hour later all groups received castor oil. Each rat was then housed separately in a cage over clean filter paper. Then diarrhoea episodes were observed for a period of 5 h. During this period, cumulative wet faecal mass were recorded. Antidiarrhoeal activity was determined in terms of percentage reduction in cumulative faecal mass with respect to the vehicle treated group.

Gastrointestinal motility test with charcoal meal test

Albino mice of either sex weighing 20 - 25 g were used. Mice were fasted for 24 h before commencing the experiment with free access to water. They were divided into 6 groups containing six animals each. First and second groups were treated with vehicle (2% tween 80 p.o.) and standard atropine sulphate (1 mg/kg, i.p) respectively. The test groups received the alcoholic and aqueous extracts at doses of 200 and 400 mg/kg body weight (one dose of extract per group). After 5 min of extract treatment, 1 ml of a charcoal meal [3% deactivated charcoal in 2% aqueous tween 80 orally] was administered by oral route to all the animals in each group. After 30 min of charcoal treatment, each mouse was sacrificed and distance moved by the charcoal meal from the pylorus to the caecum was measured to express as a percentage of distance travelled by the charcoal meal in a ratio to the intestinal length. Percentage inhibition produced by extracts was calculated (Offiah and Chikwendu, 1999; Biswas *et al.*, 2001).

Statistical analysis

Values are expressed as mean \pm SEM from 6 animals. Statistical difference in the mean were analyzed using one way ANOVA (analysis of variance) followed by Dunnett's, multiple comparison test. Results were considered significantly when $p < 0.05$.

RESULTS

Phytochemical screening

Phytochemical analysis of the alcoholic and aqueous root extracts of *desmostachya bipinnata* revealed the presence of alkaloids, glycosides, saponins, flavonoids, terpenoids, tannins, phytosterol, polyphenolics, proteins and carbohydrates (Table 1).

Acute oral toxicity study

The acute LD50 value for *Desmostachya bipinnata* root extracts was found to be safe up to 2000 mg/kg p.o. So we have selected 200 and 400 mg/kg as the study doses for antidiarrhoeal activity.

Table 1. Phytochemical screening.

Tests	Extracts	
	Alcoholic	Aqueous
Alkaloids	+	+
Glycosides	+	+
Saponins	+	+
Flavanoids	+	+
Terpenoids	+	-
Tannins	+	+
Phytosterol	+	-
Polyphenolics	+	+
Proteins	+	+
Carbohydrates	+	+

Acute oral toxicity study

The acute LD50 value for *Desmostachya bipinnata* root extracts was found to be safe up to 2000 mg/kg p.o. So we have selected 200 and 400 mg /kg as the study doses for antidiarrhoeal activity.

Table 2. Effect of root extracts of *Desmostachya bipinnata* on castor oil-induced diarrhoea in rats.

Treatment	Dose (mg/kg)	Mean weight of faeces after 5hrs (g)	% Reduction of faeces
Control	---	5.15±0.39	---
Loperamide	3	0***	100
Alcoholic extract	200	2.75±0.36*	46.57
	400	0.82±0.21***	84.07
Aqueous extract	200	3.057±0.68*	40.72
	400	2.17±0.95**	57.88

Values are Mean ± S.E.M. (n=6); Significance vs. Control group: ***P < 0.001, **P < 0.01 and *P < 0.05. By using one- way ANOVA followed by Dunnett's multiple comparison test.

Castor oil-induced diarrhoea

Alcoholic and aqueous extracts of *Desmostachya bipinnata* extracts significantly reduced the mean weight of the faeces when compared to control (Table 2). Alcoholic extract at 400 mg/kg decreased the mean weight of the faeces more significantly ($P < 0.001$) compared to the aqueous extract ($P < 0.01$) at 400 mg/kg.

Effects on gastrointestinal motility

Results of the present study revealed that the both the extracts significantly inhibited the gastrointestinal transit of charcoal in mice (Table 3). This activity was significant ($P < 0.01$) at 400 mg/kg of alcoholic extract compared to aqueous extract ($P < 0.05$) at 400 mg/kg.

Table 3. Effect of root extracts of *Desmostachya bipinnata* on charcoal meal test in mice.

Treatment	Dose (mg/kg)	%Movement of charcoal meal	%Inhibition
Control	---	86.27±3.7	-----
Atropine sulphate	1	31.56±2.6***	63.41
Alcoholic extract	200	60.95±5.2**	29.34
	400	56.80±6.03**	34.16
Aqueous extract	200	64.97±5.07*	24.68
	400	62.48±6.82*	27.57

Values are mean ± s.e.m. (n=6); significance vs. control group: *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ by using one- way anova followed by dunnett's multiple comparison test.

DISCUSSION

It is known that the active component of castor oil is the ricinoleic acid, which is liberated from the action of lipases on castor oil. The ricinoleic acid produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins (Yoshio *et al.*, 1999). This condition induces an increase in the permeability of the mucosal cells and changes in electrolyte transport, which results in a hyper-secretory response (decreasing Na^+ and K^+ absorption), stimulating peristaltic activity and diarrhoea (Zavala *et al.*, 1998). Inhibitors of prostaglandin synthesis are known to delay diarrhoea induced with castor oil (Sunil *et al.*, 2001). These results suggest that the antidiarrhoeal effect of both alcohol and aqueous extracts may be due to the inhibition of prostaglandin biosynthesis.

In the evaluation of intestinal transit, atropine sulphate was used as a standard drug. Atropine is

known to inhibit intestinal transit probably due to its anticholinergic effect (Izzo *et al.*, 1999). Studies showed that activated charcoal readily adsorbs drugs and chemicals on the surface of the charcoal particles and their by preventing absorption (Venkatesan *et al.*, 2005). Hence, gastrointestinal motility test with deactivated charcoal was carried out to find out the effect of alcohol and aqueous extract on peristaltic movement. The extracts appear to act on all parts of the intestine. The results showed that these extracts of *Desmostachya bipinnata* suppressed the propulsion of the charcoal meal which there by increases the time for absorption of water and electrolytes. The alcohol extract showed more significant anti-motility activity than aqueous extract.

From this study, we can suggest that alcoholic and aqueous extract of *Desmostachya bipinnata* inhibited diarrhoea either by decreasing intestinal motility or by decreasing the prostaglandin biosynthesis.

Anti-dysenteric and antidiarrhoeal properties of medicinal plants were found to be due to tannins, alkaloids, saponins, flavonoids, sterols and/or triterpenes and reducing sugars (Havagiray *et al.*, 2004). These phytochemical constituents have been detected in alcoholic and aqueous extracts of *Desmostachya bipinnata*. These constituents may be responsible for antidiarrhoeal activity.

CONCLUSIONS

In conclusion, the results of the present study showed antidiarrhoeal activity of alcoholic and aqueous extracts of roots of *Desmostachya bipinnata*. Alcoholic extract showed more significant antidiarrhoeal activity compared to aqueous extract. It can be suggested that these results provide pharmacological evidence for its folklore claim as an antidiarrhoeal agent. However, further studies are required to identify the active principle(s) and exact mechanism(s) of action.

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