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Antidiarrhoeal activity of *Psidium guajava* bark extracts

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Abstract

Objective: To evaluate the different extracts of *Psidium guajava* bark for its antidiarrhoeal potential against several experimental models of diarrhoea in rats. Materials and methods: Antidiarrhoeal activity of methanolic and aqueous extracts of *Psidium guajava* bark was studied at dose of 50 mg/kg and 100 mg/kg body weight against gastrointestinal motility, castor oil-induced diarrhoea and prostaglandin E_2-inducead enteropooling (PGE_2) in rats. Results: The methanolic and aqueous extracts of *P. guajava* bark showed significant antidiarrhoeal activity in a dose dependent manner against all the experimental diarrhoea models. The potent antidiarrhoeal activity may be attributed to the presence of tannin and quercetin. Conclusion: The results indicated that both the extracts of *P. guajava* bark at dose of 100 mg/kg were found to be more potent than the reference standard drugs, loperamid and atropine.

Key words: *Psidium guajava*, antidiarrhoeal, PGE_2, bark.

1. Introduction

Psidium guajava (Myrtaceae) is a widely cultivated shrub in India and neighboring Asian countries for its edible fruits. The plant is indigenously known as Amrud in Hind, Perukah in Sanskrit, Peyara in Bengali and Perala-hannu in Kannada. The various parts of the tree are widely used in Ayurveda, Siddha and Unani systems of medicine for a variety of diseases like diarrhoea, dysentery, ulcers, cholera, haemorrhages, gingivitis, vomiting etc [1]. Leaf and unripe fruit extracts of *P. guajava* has been reported to possess antidiarrhoeal activity [2,3]. The plant is reported to contain catechol, tannins, wax, resins, quercetin, β-sitosterol, sugars, carotene, vitamins B_1, B_2, B_6 and niacin [4]. However, the literature survey afforded no scientific claim on antidiarrhoeal activity of *P. guajava* bark. In view of this, the present study was taken up.

2. Materials and methods

2.1 Plant material

Fresh bark of *P. guajava* was collected from Tripura state in the month of October 2004. Prof K.Prabhu authenticated the plant and a

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voucher specimen (No: SCSCP/PG/27/2004) was deposited in the herbarium of S.C.S. College of Pharmacy, Harapanahalli.

2.2 Preparation of extracts

The shade dried and powdered bark of *P. guajava* was subjected to successive extraction using methanol and distilled water in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get a semisolid mass (yield 18.43% and 33.60%). For antidiarrhoeal study, methanolic extract was suspended in a 2% (w/v) aqueous gum acacia.

2.3 Phytochemical studies

The methanolic and aqueous extracts of *P. guajava* were subjected to preliminary phytochemical investigation, which showed the presence of carbohydrates, flavonoids, tannins, terpenoids and saponins [5].

2.4 Animals used

Albino rats weighing between 150 - 200 g of either sex were used. The animals were housed in standard environmental conditions and provided with food and water *ad libitum*.

2.5 Acute toxicity study

The acute toxicity for the methanolic and aqueous extracts of *P. guajava* bark was carried out for the determination of LD_{50} in female albino mice (20-25 g) by adopting fixed dose method of Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA) [6].

2.6 Gastrointestinal motility test

The method described by Pazhani G.P *et al.* [7] was used in this study. The animals were starved for 18h prior to the experiment with free access to water and placed in six cages containing six animals each. Each rat was administered orally with 1 ml of charcoal meal (3% deactivated charcoal in 2% aqueous tragacanth) followed by oral administration of methanolic extract of *P. guajava* to group III and IV and aqueous extract to group V and VI at dose of 50mg/kg and 100 mg/kg respectively. Animals of group I received vehicle (0.2ml, 2% w/v aqueous gum acacia) and group II treated with atropine (5 mg/kg i.m.) the standard drug for comparison. Thirty min later, each rat was sacrificed and intestinal distance moved by the charcoal meal from pylorus was measured and expressed as a percentage of distance from pylorus to caecum.

2.7 Castor oil-induced diarrhoea

The method of Awouters *et al.* [8] was adopted with minor modifications. Albino rats of either sex fasted for 18 h and divided into six groups of six each. Vehicle (0.2ml, 2% aqueous gum acacia) was given to the group I orally while the group II received loperamide (1 mg/kg i.p.). Methanolic extract of *P. guajava* was administered to group III and IV and aqueous extract was administered to group V and VI at dose of 50 mg/kg and 100 mg/kg p.o. respectively. One hour after treatment, each rat received 1 ml of castor oil orally and then observed for defecation. Up to 4 h after castor oil challenge the presence of characteristic diarrhoeal droppings were noted in transparent plastic dishes placed beneath individual cages.

2.8 PGE_2- induced enteropooling [9]

In this method, rats were deprived of food and water for 18h and placed in six cages, with six animals per cage. The first group received only 1ml of a 5% v/v ethanol in normal saline (i.p.) and then it was treated with vehicle (0.2ml, 2% aqueous gum acacia) orally, which served as vehicle control. The second group was treated with PGE_2 (100 μg/kg, p.o) and served as PGE_2 control. Group III, IV and V, VI were treated with methanolic and aqueous extracts of *P. guajava* at dose of 50 mg/kg and

Table 1. Effect of *P. guajava* barks extracts on gastrointestinal motility after Charcoal meal in rats.

Treatment	Dose (mg/kg)	Mean length of intestine ± SEM (cm)	Mean distance traveled by charcoal ± SEM	Mean percentage Movement of charcoal (cm)	Percentage Inhibition
Control	-	82.67 ± 2.99	65.75 ± 2.65	80.08	-
Atropine	5	76.92 ± 2.6	13.91 ± 1.80	18.08*	81.91
Methanolic extract	50	79.83 ± 2.90	31.64 ± 1.66	39.64*	60.36
Methanolic extract	100	71.50 ± 3.82	7.83 ± 1.37	10.85*	89.04
Aqueous extract	50	80.17 ± 1.95	33.84 ± 1.57	42.21*	57.79
Aqueous extract	100	67.67 ± 1.43	7.92 ± 1.55	11.75*	88.30

Values are mean ± S.E. (n=6) *P<0.001 compared with control.

Table 2. Effect of *P. guajava* bark extracts on Castor oil induced diarrhoea in rats.

Treatment	Dose (mg/kg)	Mean weight of stool ± S.E. after 4 hrs (gm)	Percentage of inhibition
Control	-	2.42 ± 0.12*	-
Loperamide	1	0.37 ± 0.02*	84.71
Methanolic extract	50	0.91 ± 0.019*	62.39
Methanolic extract	100	0.21 ± 0.01*	91.35
Aqueous extract	50	0.97 ± 0.014*	59.91
Aqueous extract	100	0.30 ± 0.01*	87.49

Values are mean ± S.E. (n=6) *P<0.001 compared with control.

Table 3. Effect of *P. guajava* bark extracts on PGE_2 - induced enteropooling in rats.

Treatment	Dose (mg/kg)	Mean volume of intestinal fluid ± S.E (ml)	Percentage of inhibition
Control	-	0.78 ± 0.03*	-
PGE_2-Control	100µg/kg	2.86 ± 0.05*	-
Methanolic extract	50	1.68 ± 0.04*	41.25
Methanolic extract	100	1.10 ± 0.05*	61.53
Aqueous extract	50	1.80 ± 0.04*	37.06
Aqueous extract	100	1.24 ± 0.03*	56.64

Values are mean ± S.E. (n=6) *P<0.001 compared with PGE_2-Control

100 mg/kg, p.o. respectively. Immediately after the extracts administration, PGE_2 (Astra Zenica India) was administered orally to each rat (100 μg/kg) in the Groups III-VI. After 30min, each rat was sacrificed and the whole length of the intestine from the pylorus to the caecum was dissected out and its contents were collected in a test tube and the volume was measured.

2.9 Statistical analysis

The results expressed as mean ± SEM were calculated using Kruskal-Wallis (non-parametric) test. Values $P < 0.001$ were considered statistically significant.

3. Results

In the acute toxicity study, methanolic and aqueous bark extracts of *P. guajava* were found to be toxic (2/3 mice died) at dose of 2000 mg/kg but, the extracts were found to be safe (non lethal) at dose of 300 mg/kg. Hence, the LD_{50} cutoff value for both the extracts was fixed as 1000 mg/kg. So that 1/20th and 1/10th of LD_{50} cutoff value i.e. 50 mg/kg and 100 mg/kg of methanolic and aqueous extracts were selected as screening dose for antidiarrhoeal activity.

The methanolic and aqueous bark extracts significantly decreased propulsion of the charcoal meal through the gastrointestinal tract when compared with the control group. Atropine reduced the motility of the intestine significantly (Table 1).

In castor oil induced diarrhoea, the methanolic extract, aqueous extract and loperamide showed significant reduction in diarrhoeal episodes (Table 2).

In PGE_2 - induced enteropooling significant increases in the fluid volume of rat's intestine were observed when compared with control animals receiving only ethanol in normal saline. Both the extracts of *P. guajava* significantly inhibited PGE_2 - induced enteropooling (Table 3).

4. Discussion

The methanolic and aqueous extracts of bark of *P. guajava* when administered orally to rats, showed a significant and dose - dependent antidiarrhoeal activity. The bark extracts of *P. guajava* decreased significantly the propulsion of charcoal meal through the gastrointestinal tract and the frequency of defecation, faecal droppings, when compared with untreated control rats. Similarly *P. guajava* bark inhibits significantly PGE_2 - induced enteropooling. Both the extracts of *P. guajava* at a dose of 100 mg/kg were found to be more potent than the reference standard drugs, loperamide and atropine. Many plants that have tannins as their constituents exhibit significant anti-diarrhoeal activity. In fact tannins are responsible for the denaturation of proteins and form a complex (protein tannate), which makes the intestinal mucosa more resistant and reduces the secretion by virtue of which is said to be an excellent remedy for diarrhoea [10]. Earlier report reveals that quercetin, a flavonoid exhibits inhibitory action on intestinal transit [11]. In the present study also, tannins and quercetin present in the title plant [4] may be responsible for potent antidiarrhoeal activity.

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