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Mechanism of antidiarrhoeal effect of ethanolic extract of *Psidium guajava* leaves

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ABSTRACT: The mechanisms by which the ethanolic extract of the leaves of *Psidium guajava* exerts its antidiarrhoeal effect were investigated. Antimicrobial analyses of the extracts were carried out using standard cultures of *Escherichia coli* (ATCC 15597) and *Staphylococcus aureus* (ATCC 6538). There was a concentration dependent inhibition of growth that was less significant for S. aureus ($p < 001$). Zones of inhibition remained clear without bacterial regrowth even after 72 hours. Diarrhea was induced with castor oil (10ml/kg body weight) in different groups of Wister albino rats (A - E) and treated per *os* with normal saline for the positive control (1ml/kg body weight); extract (40 mg/kg body weight); extract (80 mg/kg body weight), loperamide as standard drug (10 mg/kg body weight); gum acacia (10 mg/kg body weight) respectively. Group F which received no treatment served as the negative control. Results showed that the inhibition of diarrhea, measured as percentage faecal output relative to the positive control, was dose - dependent and comparable to the standard drug. The plant extract also showed a reduction in gastrointestinal motility, measured as the distance traveled by the charcoal plug in the small intestine. This was non – significantly different from the standard drug, loperamide ($p < 0.05$). The leaf of *P. guajava* therefore exerted its antidiarrhoeal effect by a dual action of its antimicrobial effect and reduction in gastrointestinal motility.

Key words: *Psidium guajava,* antidiarrhoeal, feacal output, gastrointestinal motility

Introduction

Diarrhea can be defined as the increased frequency of bowel movements, accompanied by a loose consistency of stools[1]. Diarrhoea results from hyper peristalsis of the small intestine or colon. Large amounts of Na^+ and K^+ and water are washed out of the colon and small intestine in diarrhoeal stools, causing dehydration, hypovolaemia and eventually shock and cardiovascular collapse. A more insidious complication of chronic diarrhea, if fluid balance is maintained, is severe hypokalaemia[2]. Diarrheal patients may report frequent loose or watery stool, defaecation usually more than three times per day, often accompanied by pain and abdominal cramping. The condition of diarrhea is particularly dangerous in infants and young children because of the rapidity with which serious dehydration may occur. In recent years, emphasis on the treatment of diarrhea has focused on oral dehydration therapy. However, there is still need for a continuing search for effective antidiarrhoeal drugs without side effects and as adjunct to ORT[3].

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P. guajava Linn. (Family: Myrtaceae) has a rich ethnobotanical history. In many parts of Africa, the leaf, stem-bark and roots are used traditionally for the management, control, and/or treatment of an array of human disorders. The leaf and bark extracts have been used for ages to fight diarrhoea and dysentery. The plant is indigenous to tropical America, widely distributed from Mexico to Brazil. It is also found all over Africa and Asia in semi cultivation. Other major ethnotherapeutic uses of the plant include the treatment of malaria with the leaves as an ingredient in the preparation of fever 'teas' or as part of the pot herbs used in steam treatment, and as mouth rinses and gargles in the treatment of stomatitis and phengingivitis. A weak decoction of the leaves and tender branches is also used as a tonic in psychiatric management[4], and has also been reported to have anti inflammatory and analgesic properties[5]. The fruits are rich in vitamins A and C, mucilage, pectin, small amounts of protein, fat, minerals (mainly potassium, iron, calcium, phosphorous), with a mild laxative effect. It is also recommended for physical exhaustion, malnutrition or weakness[6]. The whole fruit is used to make a refreshing drink while the seedy pulp is used to make guava jelly as well as a blend in ice creams.

The present study was undertaken to determine the mechanism and extent of the antidiarrhoeal effect in comparison with loperamide, a known antidiarrhoeal drug in some experimental animal paradigms.

Materials and Methods

Animals

The experimental animals used in this work were Wister albino rats (average weight 80 -180g) of either sex. They were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Plant Materials

The leaves of *P. guajava* were collected in October, around the Nnamdi Azikiwe University, Awka environment in Anambra State, Nigeria.

Preparation of extract

The leaves were dried in a shady, well ventilated, dust free area, ground to coarse powder with electric grinder before extraction. The powdered leaves were exhaustively extracted with 70% ethanol (v/v) using soxhlet method. The extract was concentrated to dryness using a water bath at 40^{o}C and stored till use at 4^{o}C in a refrigerator.

Micro-organisms

Standard cultures of *Escherichia coli* (ATCC15597) and *Staphylococcus aureus* (ATCC 6538) used for this work were obtained from the Department of Applied Microbiology, Nnamdi Azikiwe University, Awka.

Phytochemical Analysis

The extracts were tested for the presence or absence of tannins, flavonoids, alkaloids, saponins and glycosides according to Harborne [7].

Antimicrobial Tests

The antimicrobial activity/screening of the extract was tested using the streak plate bore hole method. The organisms were grown in nutrient agar and plant extract with concentration ranging from 1.0% to 10.0% (w/v) with sterile distilled water were used for the antimicrobial analyses. A sterile inoculating loop was used to streak the organisms over the surface of the medium. Different concentrations of plant extract were impregnated into wells using sterile pipettes. The plates were incubated for 24 hours and the zones of inhibition around the wells were measured.

Antidiarrhoeal Tests[8,9]

Six groups (A-F) albino rats were used (n = 8 each). Diarrhoea was induced orally in each rat using castor oil (1ml/kg.b.wt.), except for the control group (F). After one hour of castor oil treatment, two different doses of plant extract (40mg and 80mg/kg body weight) were administered orally to groups B and C respectively. Group A received normal saline while Loperamide (10mg/kg body weight.) and gum acacia (2% w/v) were administered to groups D and E respectively, and F was left untreated. The animals were housed in individual metal cages lined with white non absorbent paper. Faecal output was assessed by collecting the faecal material for 8 hours after drug administration, dried at 50^{o}C for 2 hours then weighed. The percentage feacal output (% FOP) was calculated as follows:

$$\% \text{ FOP} = \frac{\text{Ft x 100}}{\text{Fc}}$$

Where Ft = mean faecal weight of each group
Fc = faecal weight of control

Gastrointestinal Motility Test

The gastrointestinal motility test was carried out according to Akah et al[10]. Three groups of albino rats, G – I were used for the experiment (n = 6 each). The animals were starved for 24 hours before the experiment. Group G received 80mg/kg b. wt.) of extract orally, group H received 10mg/kg b. wt. of loperamide while group I which received normal saline (5mls/kg body. weight.) served as control. After 5 minutes of drug administration 0.5 ml of 5.0 % charcoal suspension in 10.0% aqueous solution of tragacanth powder was administered orally to each animal. The animals were sacrificed 30 minutes later, their abdomens opened and the distance traveled by the charcoal plug, from pylorus to the caecum was measured.

Results and Discussion

Antibacterial screening of the ethanolic extract (70%, v/v) of the leaves of *P. guajava* showed a concentration dependent growth inhibition for both *E. coli* and *Staph. aureus*, though non – significantly less for the latter (table 1). The zones of inhibition remained clear without bacterial regrowth after 72 hours. Leaf extract of *P. guajava* has also been reported to be inhibitory against *Entamoeba histolytica, Salmonella, Shigella* and Enterohepatic *E. coli*, and has as well been found to be as effective as tetracycline in the treatment of acute infectious diarrhoea1[1, 12, 13, 14]. One suggested mechanism of action was that lectins found in guajava bind on *E. coli* thereby preventing adhesion to intestinal walls.

Induction of diarrhoea by castor oil increased peristaltic activity and induced permeability changes in the mucosal membrane to electrolyte and water. There was, however, absence of diarrhoea in those animals treated with extract and loperamide respectively (table 2). There was also reduction in percentage faecal output relative to control (group A), which ranged from 68.18% of control for group B (40mg/kg b. wt) to 46.97% of control for group C (80mg/kg body weight) as shown in table 3.These results showed significant differences ($p < 0.01$) between the test groups B and C and the control group A and also between the test groups and the standard loperamide group, D and gum acacia group E ($p < 0.01$).

Results from the gastrointestinal motility tests (table 4) showed that the average distance moved by the charcoal plug was greatest for the control (group G). The distance was least for the test group (group I), which received 80mg/kg body weight of extract, indicating a non-significantly higher inhibition than the group treated with loperamide (group H) at $p < 0.05$. These results agree with similar reports which have established reduction in gastric motility as being the mechanism by which many antidiarrhoeal agents act[15, 9, 10, 16]. Phytochemical analysis from this work revealed the presence of saponins, glycosides, tannins and alkaloids (table 5). Other investigations have reported additional presence of anthocyanins, essential oils, phenols, triterpenes, quercetin and vitamin C[17]. Quercetin has been reported as the main active constituent of *P. guajava* and has been attributed to be responsible for the spasmolytic and antidiarrhoeal effects of the leaf extract [18, 19].

Quercetin relaxes smooth muscles and inhibits bowel contraction, probably by inhibiting intracellular calcium release from the sarcoplasmic reticulum[20]. Triterpenes have also been shown to have antispasmodic activities, as well as aid in the reabsorption of water in the intestine [21, 11].

It is therefore concluded that the mechanisms by which the leaf extract of *P. guajava* exerts its antidiarrhoeal effect are by antimicrobial activities and the reduction of gastrointestinal motility. The numerous tannins, polyphenolic compounds, flavonoids, ellagic acid, triterpenoids, guiajaverin, quercetin, and other chemical compounds reportedly present in the plant[5] are speculated to account for the observed effects. These results therefore confirm the basis for the ethnobotanical use of the plant for the treatment of diarrhoea.

Table 1: Antimicrobial screening of plant extract

CONC OF EXTRACT (%)	ZONE OF INHIBITION	
	E. coli	*Staph. aureus*
1.00	6.00 ± 1.41	5.50 ± 0.71
2.50	8.50 ± 2.12	7.50 ± 2.23
5.00	10.50 ± 0.71	9.590 ± 0.95
10.00	14.00 ± 1.20	11.50 ± 2.12

Table 2: Effect of *P. guajava* leaf extract on castor oil – induced diarrhea in albino rats

GROUP	DOSE	DIARRHOEA INDUCTION
A	Normal saline (5ml/kg b. wt)	+++
B	Extract (40mg/kg b. wt)	-
C	Extract (80mg/kg b. wt)	-
D	Loperamide (10mg/kg b. wt.)	-
E	Acacia (10mg/kg b. wt)	++
F	(No treatment)	- (no induction)

Table 3: Effect of *P. guajava* leaf extract on faecal output in albino rats

GROUP	DOSE	FOP(g dry wt)	% CONTROL
A	Normal saline (5ml/kg b. wt)	0.66 ± 0.34	100%
B	Extract (40mg/kg b. wt)	0.45 ± 0.16*	68.18%
C	Extract (80mg/kg b. wt)	0.31 ± 0.09*	46.97%
D	Loperamide (10mg/kg b. wt.)	0.24 ± 0.05*	36.34
E	Acacia (10mg/kg b. wt)	0.18 ± 0.09*	27.28
F	(No treatment)	0.26 ± 0.03*	39.40

* Values significant when compared with control at $p < 0.01$

Table 4: Effect of *P. guajava* leaf extract on intestinal motility

GROUP	DOSE	AVERAGE DISTANCE ± SEM (CM)
G	Normal saline(5ml/kgb. wt)	75.70 ± 2.30
H	Loperamide (10mg/kg b. wt.)	68.50 ± 16.30
I	Extract (80mg/kg b. wt)	67.70 ± 1.80

Not significantly different at $p < 0.05$

Table 5: Phytochemical analysis of extract

TEST	RESULTS
Alkaloids	++
Saponins	++
Flavonoids	++
Tannins	++
Glycosides	++

Key: - absent
+ trace
++ present

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