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Antidiarrheal Potential of Ethanol Extract of *Psidium guajava* Leaves

Chigbo, C. M., Nwobodo, V. O. G., Orji, E. C., Okeke, O. D., Nworji, O. F., Ogana, J. and Iloanya, E. L.

Department of Applied Biochemistry, Faculty of Biosciences Nnamdi Azikiwe University, P.M.B. 5025 Awka, Anambra State, Nigeria.
Corresponding Author's e-mail: vo.nwobodo@unizik.edu.ng.
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ABSTRACT

Diarrhea is a common gastrointestinal disorder characterized by excessive fluid secretion and disruption of normal bowel movements. In African folk medicine, the leaf of *Psidium guajava* Linn. (Family: Myrtaceae) is used traditionally to manage, control, and/or treat diarrhea. This study aimed to comparatively evaluate the effects of the ethanol extract of guava leaves on castor oil-induced diarrhea in rats. Three models of determination of antidiarrheal properties were adopted and determined. The first model involved the determination of antidiarrheal activities of the castor oil-induced diarrhea model; results showed that *Psidium guajava* leaf extract (PGLE) of 150, 300, and 600 mg/kg groups dose-dependently and significantly ($p < 0.05$) inhibited all the diarrheal parameters tested, such as onset of diarrhea, total number of stools, number of wet stools, and weight of wet stools measured. The second model is Castor oil-induced gastrointestinal motility, in which increased doses of the extract reduced the distance travelled by charcoal meal in 300 and 600 mg/kg *Psidium guajava,* respectively, but it was not as effective as 5 mg/kg Loperamide. In the third model: castor oil-induced enteropooling. Results showed the effect of the ethanol leaf extract of *P. guajava* on intestinal fluid content demonstrated a significant dose-dependent reduction in both mean weight and mean volume of the small intestinal content at 150, 300, and 600 mg/kg, respectively. These findings suggest that the ethanol extract of *P. guajava* leaves may possess significant anti-diarrheal activity, as evidenced by its ability to reduce intestinal fluid accumulation, improve stool consistency, and ameliorate intestinal motility in castor oil-induced diarrhea rats.

Keywords: *Psidium guajava*, Diarrhea, Loperamide, and Castor oil.

Introduction

Natural products have long served as primary therapeutic remedies for preventing, managing, and curing various illnesses within Traditional Systems of Medicine (TSMs). Over the past decade, therapeutically active plants utilized in TSMs have been extensively explored to validate their clinical integration into modern herbal health supplements and functional foods (Díaz-de-Cerio *et al.*, 2017). Globally, there is a resurgent interest in profiling the bioactive chemical constituents of these plants to establish their physiological benefits, confirm their therapeutic efficacy, and expand their clinical applications in human health (Naseer *et al*., 2018).

Among these valuable medicinal resources is *Psidium guajava* Linn., popularly known as guava, which is a small-to-medium-sized evergreen tree belonging to the myrtle family (Myrtaceae). The Myrtaceae family is highly diverse, encompassing approximately 130 accepted genera and over 6,500 distinct species globally (Plants of the World Online [POWO], 2024). *P. guajava* adapts easily to a wide range of soil profiles, facilitating its extensive naturalization and commercial cultivation across tropical and subtropical zones worldwide (Díaz-de-Cerio *et al*., 2017). Reflecting its deep ethnobotanical roots in West Africa, the plant is known by several regional vernacular names: "Guava" in English, "gwaabaa" in Hausa, "ugwoba" in Igbo, "guafa" in Yoruba, and "woba" in Efik

Morphologically, the tree yields highly fragrant, white flowers featuring four to six incurved petals and numerous yellow-colored central anthers, with pollination primarily mediated by insects. The resulting fruit size ranges from small to medium (3–6 cm in length), exhibits a pyriform (pear-like) shape, and transitions to a distinctive red or yellow color when ripe (Naseer *et al*., 2018). The ripe fruit gives off a powerful yet pleasant musky odor, containing a darker central pulp embedded with a variable number (ranging from 112 to 535) of small, chewable, and regularly patterned yellowish seeds (Naseer *et al*., 2018).

Beyond the nutritional value of its fruit, the leaves of *P. guajava* possess remarkable biochemical properties, yielding approximately 9.73% crude protein on a dry weight basis (Rahman *et al.,* 2013). Historically, ethnobotanical preparations of guava leaves have served as foundational remedies in folk medicine, most notably as an anti-diarrheal treatment across several continents (Gutiérrez *et al*., 2008; Kumar *et al.,* 2021).

Diarrhea is clinically defined as an abnormal increase in the frequency, fluidity, or volume of bowel movements. It is characterized by heightened bowel sounds, intestinal hypermotility, wet or watery stools, and severe abdominal discomfort (Sharma *et al.,* 2010). If left unchecked, acute diarrheal

episodes cause rapid fluid loss, leading to severe dehydration, metabolic acidosis, critical electrolyte imbalances, and hypovolemia (Thapar and Sanderson, 2004).

Despite decades of global public health interventions, diarrheal diseases remain a leading cause of child mortality. Modern epidemiological data reveal that childhood diarrhea is responsible for roughly 444,000 deaths annually among children under five years of age, with the highest burden concentrated in developing economies within sub-Saharan Africa and South Asia (UNICEF, 2024; World Health Organization [WHO], 2024).

Conventional clinical management of diarrhea is often non-specific, primarily targeting symptom reduction and fluid replacement. When infectious agents are suspected, the systemic use of standard synthetic antibiotics presents notable clinical limitations. These include severe adverse drug events, disruptions to the intestinal microbiota, and the rapid, global escalation of antimicrobial resistance among enteric pathogens (Kouitcheu-Mabeku *et al.,* 2006).

To address this healthcare bottleneck in developing nations, the WHO has championed diarrheal disease control programs that formally advocate for the evaluation and integration of validated medicinal plants (Suleiman *et al.,* 2008). Crude plant extracts present an attractive, sustainable therapeutic alternative because they are highly cost-effective, cause fewer side effects, and contain multi-target bioactive complexes capable of mitigating multidrug-resistant pathogens (Abebe, 2014).

Recent pharmacological evaluations indicate that the rich array of tannins, essential oils, and secondary metabolites, particularly flavonoids like quercetin in guava leaves exert powerful anti-motility, anti-secretory, and smooth muscle-relaxing effects within the gastrointestinal tract (Garrido, 2024). Given this therapeutic promise, further empirical research is required to standardize its extracts and confirm its pharmacological efficacy. Therefore, the present study aimed to determine the in vivo antidiarrheal potential of the ethanolic extract of *Psidium guajava* leaves.

Materials and Method

Experimental animals

A total of 75 Wistar albino rats of both sexes, weighing 120-150 kg were used for the experiment, excluding the 13 Wistar albino rats used in the LD_{50} determination. The rats were obtained at Chris Experimental Animal Farm and Research Laboratory at Mgbakwu, Awka. The animals were housed in aluminum aerated cages and maintained under room temperature for one week with free access to food and water *ad libitum.*

Experimental Design

This is an experimental study with three different models, each laid out in a completely randomized design. The 75 Wistar albino rats were separated into groups of five (each animal serving as a replicate) and placed in aluminum cages, and the cages were labeled A, B, C, D, and E in each of the three methods.

Group A: Diarrhea untreated

Group B: 5 mg/kg Loperamide

Group C: 150 mg/kg *P. guajava*

Group D: 300 mg/kg *P. guajava*

Group E: 600 mg/kg *P. guajava*

Ethical approval

All experimental procedures involving animals received approval from Nnamdi Azikiwe University Animal Research Ethics Committee with reference number NAU/AREC/2022/00016

Plant material collection

The leaves of *Psidium guajava* were collected from Amenyi village, Awka, Awka South L.G.A., Anambra State, Nigeria. The sample was identified by a taxonomist, Dr. Iroka Finian of the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number, as deposited in the herbarium of NnamdiAzikiwe University, Awka, is NAUH-03[A].

Preparation of Plant Material

Fresh leaves of *Psidium guajava* were meticulously washed with distilled water to remove surface debris and subsequently air-dried at ambient room temperature (25 ± 2°C) for two weeks. The completely dried leaves were mechanically pulverized into a fine powder using a Corona manual grinding mill. The resulting leaf powder was stored in an airtight container until further use, following standard botanical preparation protocols (Trease and Evans, 2009).

Preparation of Hydroethanolic Extract (Maceration)

The crude plant extract was prepared via a cold maceration technique as described by Handa *et al*. (2008), with slight modifications. Exactly 500 g of the pulverized *P. guajava* leaf powder was submerged in 2 L of 70% (v/v) ethanol (ratio 1:4 w/v) within a sterile glass vessel. The mixture was allowed to stand at room temperature for 24 h to facilitate optimal dissolution of bioactive phytochemicals. To maintain a high concentration gradient and maximize extraction yield, the mixture was manually agitated at 2 h intervals throughout the maceration period.

Filtration and Concentration

Following the 24 h extraction period, the mixture was sequentially filtered to ensure maximum clarity. Initial separation of the coarse plant residue was achieved using a clean muslin cloth, followed by fine clarification through Whatman No. 1 filter paper. The resulting clear filtrate was concentrated to dryness under controlled thermal conditions using a digital water bath maintained at 50°C (Azwanida, 2015). This temperature threshold was selected to prevent the thermal degradation of heat-sensitive secondary metabolites. The final concentrated crude extract was weighed, recorded, and stored at 4°C for subsequent experimental analyses.

Acute toxicity (LD_{50}) evaluation

The acute oral toxicity profile of the *Psidium guajava* ethanolic leaf extract was evaluated using the classical Lorke's method (Lorke, 1983). A total of 13 healthy, adult Wistar rats were utilized for the study. The experiment was conducted sequentially in two distinct phases (Phase 1 and Phase 2) to minimize animal usage in alignment with the 3Rs principles (Replacement, Reduction, and Refinement).

Phase 1: Range-Finding Stage

In the first phase, nine (9) Wistar rats were randomly allocated into three groups of three animals each (n = 3). Following an overnight fast, the animals were treated orally via gastric gavage with escalating geometric doses of the *P. guajava* extract to establish a broad toxicological range:

Group 1: Received a low dose of 10 mg/kg body weight.

Group 2: Received a medium dose of 100 mg/kg body weight.

Group 3: Received a high dose of 1000 mg/kg body weight.

The animals were observed continuously for the first 4 hours post-administration for signs of acute behavioral toxicity, clinical symptoms (e.g., changes in skin, fur, eyes, respiratory patterns, or somnolence), and mortality. Observation was maintained daily for up to 14 days.

Phase 2: Determination Stage

Based on the mortality data obtained from Phase 1, the remaining four (4) Wistar rats were deployed in Phase 2 to precisely pinpoint the toxicity threshold. In this stage, the animals were not grouped together; instead, they were assigned to four individual groups containing exactly one rat each (n = 1). Each animal received a specific, single escalated doses (1600, 2900 and 5000mg/kg) of the extract determined by the Phase 1 upper survival boundary. The fourth rat was used to measure the actual concentration between 2900 and 5000 mg/kg, as part of the procedure in Lorke's method. This single-animal design allowed for the precise identification of the maximum tolerated dose and the minimum lethal dose while strictly minimizing animal sacrifice.

Once Phase 2 concluded, the exact LD_{50} value was calculated mathematically using the geometric mean of the critical pivot points:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where D_0 is the highest dose that yielded 0% mortality (all animals survived).

Where D_{100} is the lowest dose that yielded 100% mortality (the animal died).

Models of anti-diarrhea determination

1st Model: Determination of Antidiarrheal Activity of *P. guajava* leaf Extract using Castor Oil-induced Model (Frequency of Defecation Monitoring)

The model and formula described by Nwodo and Alumanah (1991) were used in this study. Twenty-five Wistar albino rats of either sex were subjected to fasting for 24 hours, with free access to water and treated with *P. guajava* leaf extract exactly when their fasting reached 24 hours (Pretreatment method). One hour later, all the rats except group A (Normal control) received 0.5ml of castor oil by oral gavage, and then, they were individually placed on the floor of

separate cages which were covered with non-wetting transport paper. During the observation period of 4 to 5 hours, the time of onset of diarrhea, the weight of wet stools, the time when they started defecating, the total number and weight of fecal output (both diarrheal and non-diarrheal) excreted by rats were recorded and compared with the control group.

Finally, the percentage of diarrhea inhibition as well as the weight of wet and total fecal output were determined according to the formula below:

$$\% \text{ inhibition of Diarrhea onset} = \frac{T_{test} - T_{control}}{T_{control}} \times 100$$

Key = T_{test} = Time for Onset of Diarrhea for different test groups

$T_{control}$ = Time for Onset of Diarrhea for the control group

$$\% \text{ Inhibition of diarrhea} = \frac{\text{Mean weight of wet defecation (negative control} - \text{test)}}{\text{Mean number of wet defecation in negative control}} \times \frac{100}{1}$$

$$\% \text{ of wet fecal output} = \frac{\text{Mean weight of wet feaces of each treatment group}}{\text{Mean weight of wet feaces of control}} \times \frac{100}{1}$$

2nd Model: Castor Oil-Induced Gastrointestinal Motility Monitoring of Anti-Diarrheal Activity of *P. guajava* leaf Extract

A gastrointestinal motility test was evaluated according to the method and formula described by Tan Method (1989). Another set of 25 rats were subjected to fasting for 24 hours with free access to water and were randomly assigned to 5 groups and treated with *P. guajava* leaf extract as described in the study design. An hour later, 0.5ml of castor oil was administered. At 60 minutes after the administration of castor oil, each rat received 1ml of 5% charcoal suspension in distilled water to serve as a marker for observing motility. Then 30 minutes later, each rat was sacrificed by cervical dislocation, the abdomen was opened, and the small intestine was immediately dissected out from pylons to caecum and placed lengthwise on white paper. Therefore, the distance travelled by the marker (charcoal) from the pylons and the total length of the intestine were measured with a calibrated ruler. The peristaltic index and total length of the small intestine as well as the percentage of movement as a function of the control, were calculated.

$$\text{Peristaltic Index (PI)} = \frac{\text{Mean distance travelled by charcoal meal}}{\text{Mean length of small intestine}} \times \frac{100}{1}$$

$$\% \text{ Inhibition of motility} = \frac{\text{Mean \% distance travelled by charcoal meal (negative control} - \text{test)}}{\text{Mean \% distance travelled by the charcoal meal of negative control}} \times \frac{100}{1}$$

3RD Model: Castor Oil-Induced Enteropooling Monitoring of Antidiarrheal Activity of *P. guajava* leaf Extract

Intestinal fluid accumulation was evaluated according to the method described by OECD/OCDE (2008). Another different set of Twenty–five (25) rats were fasted for 24 hours and treated with ***P. guajava*** **leaf extract** as described in the grouping and stated in the experimental design, just one hour before oral administration of 0.5ml castor oil. After 1 hour, the rats were sacrificed, the pyloric and caecum ends of the small intestine were tied, and the intestine was removed. The dissected small intestine was weighed, and intestinal contents were expelled into a measuring cylinder and then the volume was measured. Weight of the intestine after removal of its content was taken and the difference between full and empty intestines was calculated. Finally, the percentage of inhibition of intestinal secretion was calculated according to Suleiman *et al*. (2008).

$$\% \text{ Inhibition of MVIC} = \frac{\text{Mean volume of intestinal fluid (negative control} - \text{test)}}{\text{Mean volume of intestinal fluid (negative control)}} \times \frac{100}{1}$$

Where; MVIC is mean volume of the intestinal content.

Statistical Analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 21 (SPSS Inc., Chicago, Illinois, USA). Results are presented as mean ± SEM. Differences among groups were evaluated using one-way ANOVA, with significant difference accepted at $p < 0.05$.

Results

The administration of low doses (10, 100 and 1000 mg/kg body weight) of the extract did not show any visible signs of toxicity in the experimental animals within 2 hrs and 24 hrs of administration. As seen in the result obtained (Table 1), no mortality was recorded. Thus, the *Psidium guajava* leaves extract is relatively nontoxic at doses lower than or equal to 1000 mg/kg.

Table 1: First phase of the acute toxicity **(LD_{50})** study of the ethanol extract of *Psidium guajava* leaves.

Dose (mg/kg body weight)	Mortality
10	0/3
100	0/3
1000	0/3

Number of deaths per group = 0, Number of rats per group = 3.

In Table 2, Number of deaths per group = 0, Number of rats per group = 1, when these high doses were administered, the rat administered with 1600mg/kg and 2900 mg/kg body weight showed no sign of weakness or death. It was noticed that at 5000 mg/kg body weight, the rat was slightly weak but no mortality was recorded. As a result, test doses of 150, 300, and 600 mg/kg for the PGLE were used in the main experiment.

Table 2: Second phase of the acute toxicity (LD_{50}) of the ethanol extract of *Psidium Guajava* leaves.

Extract Dose (mg/kg weight)	Mortality
1600	0/1
2900	0/1
5000	0/1

In the course of observation for 4 h after castor oil administration in the 1st model, all animals in the control group showed either wet stool or watery diarrhea. The PGLE produced a significant increase in the onset of diarrhea, and a decrease in the number and weight of wet and total stools at the doses of 300 and 600 mg/kg (p <0.05) when compared with untreated diarrhea. At the dose of 150 mg/kg PGLE, there was a significant decrease in the number and weight of wet and total feces (p <0.05), although the delay in the onset of diarrhea was also able to achieve a significant level as present on the Table 3.

Table 3: Effect of administration of ethanol extract of *P. guajava* on diarrhea parameters

Groups	Numbers of Wet Feaces	Total Weight of Fecal Output (gm)	Onset of Diarrhea (mins)	Weight of Wet Feaces (gm)
Group A: Diarrhea untreated	6.60 ± 0.51^{c}	2.56 ± 0.33^{c}	53.20 ± 13.63^{d}	2.06 ± 0.16^{c}
Group B: 5mg/kg Loperamide	0.00 ± 0.00^{b}	0.28 ± 0.09^{b}	300.00 ± 0.000^{a}	0.19 ± 0.08^{b}
Group C: 150mg/kg *P. guajava*	3.80 ± 0.49^{bc}	1.94 ± 0.23^{bc}	141.60 ± 64.76^{a}	1.63 ± 0.31^{c}
Group D: 300mg/kg *P. guajava*	2.60 ± 0.68^{bc}	1.54 ± 0.36^{bc}	161.00 ± 8.11^{a}	1.35 ± 0.43^{bc}
Group E: 600mg/kg *P. guajava*	1.60 ± 0.06^{bc}	1.06 ± 0.45^{bc}	191.60 ± 66.89^{a}	0.79 ± 0.25^{bc}

Values are expressed as Mean ± SEM (n = 5 mice in each group) and analyzed by one way ANOVA, different superscripts down a given column indicate that mean differences are statistically significant ($p < 0.05$)

The percentage inhibition by the highest dose of PGLE (600 mg/kg) used, was lower than dose of the standard antidiarrheal drugs, loperamide (5 mg/kg) employed. Furthermore, dose-dependent percentage of inhibition of diarrhea was observed by PGE 42.42 %, 61.00 % (p <0.05), and 76.00 % (p <0.05) for 150, 300, and 600 mg/kg, respectively, for the onset of diarrhea. The similar dose-dependent percentage inhibition was observed in the following diarrheal parameters: wet fecal output and total fecal output, as presented in Table 4.

Table 4: Effect of administration of ethanol extract of *P. guajava* leaves on percentage inhibition of diarrhea Onset, percentage of wet output, and percentage of total output.

Groups	**% Inhibition of Onset of Diarrhea**	**% Inhibition of Wet Fecal Output**	**% Inhibition of Total Fecal Output**
Group A: Diarrhea untreated	-	-	-
Group B: 5 mg/kg Loperamide	100.00	89.06	90.78
Group C: 150 mg/kg *P. guajava*	42.42	24.00	26.38
Group D: 300 mg/kg *P. guajava*	61.00	40.00	34.47
Group E: 600 mg/kg *P. guajava*	76.00	59.00	61.65

The results of the effect of the ethanol leaf extract of *P. guajava* on the length of the intestine via distance travelled by charcoal. The result showed that the extract caused a significant decrease (p <0.05) in 300 mg/kg *Psidium guajava* (Group C) and 600 mg/kg *Psidium guajava* (Group E) with respect to Diarrhea untreated and there is a significant increase (p <0.05) with respect to 5mg/kg Loperamide (standard drug) when the experimental animals were fed with PGLE at the concentration of 150 mg/kg (Group C) and 300 mg/kg (Group E) on the distance travelled by charcoal in the small intestine. But increased dose of the extract showed reduction in distance travelled by charcoal meal in 300 mg/kg *Psidium guajava* (Group D) and 600 mg/kg *Psidium guajava* (Group E), but it was not as effective as 5 mg/kg Loperamide (Group B).

As depicted in Table 5, the *PGLE* showed a significant percentage reduction in the intestinal motility and the effect was dose-dependent (p < 0.05) at all the tested doses. The percentages of inhibition of intestinal motility were 18.35%, 40.97 %, and 50.00 % at the concentration of 150, 300, and 600 mg/kg respectively when compared with the negative control. The dose of 600 mg/kg of the PGLE showed a significant anti-motility effect (50.00 %, p < 0.05) as compared with 150 mg/kg, which is comparable with loperamide 5 mg/kg (58.68 %, p < 0.05) (Table 5).

Table 5: Effect of ethanol extract of *P. guajava* on length of full intestine, distance travelled by charcoal, Peristaltic Index and Percentage Inhibition of motility of castor oil-induced diarrhea in rats expressed as mean ± SEM.

GROUPS	Length of Full Intestine	Distance Traveled by Charcoal	Peristaltic Index	Percentage Inhibition of Motility
Group A : Diarrhea untreated	84.20±4.64[a]	17.12±1.87[a]	20.33	-
Group B : 5 mg/kg Loperamide	86.10±4.24[a]	5.72±2.08[b]	8.40	58.68
Group C : 150 mg/kg *P. guajava*	90.10±2.17[a]	14.60±2.24[c]	16.60	18.35
Group D : 300 mg/kg *P. guajava*	88.80±2.66[a]	10.66±2.88[bc]	12.00	40.97
Group E : 600 mg/kg *P. guajava*	85.80±2.17[a]	8.54±2.70[b]	10.00	50.00

Values are expressed as Mean ± SEM (n = 5 mice in each group) and analyzed by one way ANOVA, Mean values with different superscript alphabets down a given column are significantly different.

As presented in Table 6, the PGLE demonstrated a dose-dependent reduction in both mean weight (0.99+0.82, 0.74+0.32, and 0.65+0.11; $p < 0.05$) and mean volume of the small intestinal content (0.86+0.09, 0.70+0.25, and 0.44+0.02; $p < 0.05$) at the concentration of 150, 300, and 600 mg/kg respectively. Additionally, there was a significant difference in terms of the volume of intestinal fluid and weight of intestinal contents among the three test doses of the PGLE. For instance, the dose of 600 mg/kg showed a significant reduction in both the average weight and volume of small intestine content as compared with 150 and 300 mg/kg doses ($p < 0.05$). This dose was found to have comparable antidiarrheal activity with 5 mg/kg loperamide in both mean weight (0.54 ± 0.98^{b}; $p < 0.05$) and mean volume of the small intestinal content (0.46 ± 0.07^{b}; $p < 0.05$) respectively.

Table 6: Effect of ethanol extract of *P. guajava* on diarrhea parameters expressed as mean ± SEM and Percentage Inhibition.

Groups	Mean Volume of intestinal fluid	Inhibition (%)	Mean Weight of intestinal content	Inhibition (%)
Group A: Diarrhea untreated	1.22 ±0.17[a]	-	1.37 ±0.20[a]	-
Group B: 5 mg/kg Loperamide	0.46 ±0.07[b]	62.28	0.54 ±0.98[b]	60.54
Group C: 150 mg/kg *P. guajava*	0.86 ±0.09[a]	29.51	0.99 ±0.82[a]	27.74
Group D: 300 mg/kg *P. guajava*	0.70 ±0.25[a]	42.62	0.74 ±0.32[a]	45.99
Group E: 600 mg/kg *P. guajava*	0.44 ± 0.02[b]	63.93	0.65 ± 0.11[b]	52.56

Values are expressed as Mean ± SEM (n = 5 mice in each group) and analyzed by one way ANOVA, Mean values with different superscript alphabets down a given column are significantly different.

Table 7 shows that in all diarrhea models used, the antidiarrheal activity of *P.guajava* crude leaf extract was increased in a dose-dependent manner. However, the ADI value of *P.guajava* crude leaf extract at the tested dose of 600 mg/kg was higher than all tested doses

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Table 7: Antidiarrheal Index (ADI)

Treatment (mg/kg)	Delay in diarrhea (minute)	Gut meal travel distance (Gmeq) (%)	Purging frequency (Pfreq) (%)	Antidiarrheal index (ADI)
5 mg/kg Loperamide	100.00	58.68	89.06	80.55
150 mg/kg *P. guajava*	42.42	18.35	24.00	26.53
300 mg/kg *P. guajava*	61.00	40.97	40.00	46.41
600 mg/kg *P. guajava*	76.00	50.00	59.00	60.75

Discussion

This study aimed to determine the toxicity associated with the administration of *Psidium guajava* leaf extract (PGLE) at different doses and the *in vivo* antidiarrheal activity of the crude ethanol extract of *P. guajava* leaves. Administration of the extract at low doses (10, 100, and 1000 mg/kg body weight) showed no obvious signs of toxicity in experimental animals within 2 and 24 hours after administration. The results obtained showed that no death was recorded. Therefore, guava leaf extract is relatively non-toxic at doses below1,000 mg/kg. A similar observation was made by Roy *et al.* (2006) whereby *Psidium guajava* leaf extract administered at a lower dose was not toxic to the albino rats used.

Loperamide, the standard drug used in this study, is a potent and widely used antidiarrheal drug that acts by reducing intestinal motility and/or blocking intestinal secretions (Adzu *et al*., 2003). Therefore, to confirm the antidiarrheal effect, the crude extract of *P. guajava* leaves was tested in an intestinal motility model and an intestinal secretions model. Enteropooling in rats is also a technique used to analyze the effects of experimental drugs on intestinal secretions (Shukla and Gahlot, 2020). Castor oil causes diarrhea by different mechanisms. These include blocking Na^+/K^+ -ATPase activity through a gradual reduction of normal fluid absorption (Yakubu *et al*., 2020) and triggering the generation of inflammatory mediators by prohibiting the reabsorption of NaCl and water (Iman, 2012). Abnormal excretion and motility were included in the castor oil-induced diarrhea model (Biogae *et al*., 2020). Accordingly, the use of castor oil as a diarrheal drug is a welcome development because it mimics abnormal mechanisms and allows examination of a large number of changes in stool quantity, intestinal transit, and enteropooling (Sarin, 2013). The standard medication, loperamide hydrochloride, not only controls the digestive tract but also slows small intestinal motility. Nowadays, loperamide is commonly used in several diarrhea models to study the antidiarrheal properties of various experimental plants.

In this study, the crude extract of *P. guajava* leaves showed antidiarrheal activity by reducing castor oil-induced diarrhea in all parameters used. There was a significant reduction ($p < 0.05$) in the number of feaces and weight of wet feaces and total output of stools, as well as a delay in the onset of diarrhea. The observed activity may be attributable to phytochemicals previously reported in *P. guajava* leaves. (Yestilila, 2006). For example, flavonoids and phenolic substances contain antioxidant properties, which may be responsible for antidiarrheal effects (El-Mostafa, 2004). These phytochemicals may work by inhibiting enzymes or preventing the breakdown of arachidonic acid, thereby reducing the fluid production caused by castor oil (Jalilzadeh-Amin, and Mahan, 2015). Tannins and saponins have been tested and reported to have anti-diarrheal properties (Muthukumaran *et al.,* 2016). In addition, the results of this study are comparable to previous research on several crude plant

extracts with dose-dependent antidiarrheal effects (Krueger, 2005). These may be due to the presence of many phytochemicals in these experimental plants. Among the three dose levels of *P. guajava* crude leaf extract, the highest dose (600 mg/kg) significantly reduced all parameters used. In contrast, the lowest dose of 150 mg/kg did not significantly reduce all the above-mentioned parameters of the anti-diarrheal model compared to the negative control. For example, the reduced antidiarrheal effect of a 150 mg/kg dose of crude extract of *P.guajava* leaves may be due to the low quantity and concentration of phytochemicals in the dose. The reduction in total feces, including wet and water components, suggests that the antidiarrheal activity of *P. guajava* leaves may be due to an anti-secretory mechanism. Furthermore, the antidiarrheal effect of *P. guajava* leaf extract may be due to its inhibitory effect on the production of nitric oxide and platelet-activating factors (Tarapu and Yaday, 2004). Studies performed on plants used to treat diarrhea are consistent with the results of this study. For example, *Eremomastax speciosa* and *Xylocarpus granatum* reduce the number of wet stools and bowel movements and slow the onset of diarrhea (Rouf *et al.,* 2007).

The research was also extended to evaluate the anti-enteropooling effect to further elucidate the mechanism of the anti-diarrheal effect. Compared with the negative control, crude extracts at 600 mg/kg and 300 mg/kg of *P.guajava* leaves significantly inhibited intestinal fluid collection and reduced the weight of castor oil-induced intestinal contents ($p < 0.05$). This effect may be due to secondary metabolites active in *P. guajava* leaves, a crude extract that inhibits and prevents fluid buildup in the digestive system. However, the highest inhibition rates of the crude extract of *P. guajava* leaves were recorded (52.56 and 63.93% for the average weight and volume of intestinal contents respectively at the concentration of 600 mg/kg), equivalent to that of the standard drug loperamide. Additionally, phytochemicals such as tannins, terpenoids, flavonoids, and steroids can inhibit the production of prostaglandin E2 (Maliet *et al.,* 2017) an important component in the production of intestinal secretions through the secretion of water and electrolytes solution (Pierce *et al*., 1971).Tannins reduce efflux by reducing Cystic Fibroisis transmembrane conductance and calcium-activated chloride channels, and by inducing protein precipitation in the gastrointestinal mucosa (Araj-Khodaei *et al.,* 2020), thereby reducing the ability of the mucosa to resist chemical changes (Balaji *et al.,* 2012). Phytochemicals from plants, such as flavonoids, can activate adrenergic receptors in absorptive cells in the digestive tract and improve water and electrolyte absorption in the intestine (Araj-Khodaei *et al.,* 2020). Fluid transport across the epithelium of the gastrointestinal tract is also controlled by the administration of aquaporin-like water channels (AQPs), in addition to the movement of electrolytes. Tannins have been shown to inhibit certain kinases, reducing the generation of specific AQP, which may explain their anti-secretory and thus anti diarrheal effects (Liu *et al*., 2014). Accordingly, the anti-secretory effect

of the crude extract from *P. guajava* leaves is probably related to the presence of phytochemicals.

Another strategy to stimulate diarrhea production is to increase intestinal motility. The present study used charcoal powder as a tracer to test the anti-motility activity of *P. guajava* crude leaf extract. The parasympathetic nerve is the most important regulator of gastrointestinal motility. Parasympathetic nerve activation increases intestinal transit (Rawat *et al*, 2017). According to previous research, all smooth muscle contractions are completely dependent on the presence of Ca^{2+}, which activates contractile factors and leads to their relaxation, a mechanism associated with antidiarrheal effects of many different drugs (Rawat *et al.*, 2017). Castor oil-induced gastrointestinal motility test showed that crude extract of *P. guajava* leaves at tested doses of 600 and 300 mg/kg significantly reduced intestinal propulsive motility compared to the negative control. Unlike normal intestinal transit, castor oil-induced intestinal transit was more inhibited by *P. guajava* leaf extract. The anti-motility effect of *P. guajava* crude leaf extract (PGLE) may be related to the presence of active phytochemicals responsible for the anti-motility effect demonstrated by the reduction in distance movement of coal powder. For example, flavonoids isolated from *Catha edulis* have been shown to inhibit intestinal smooth muscle contraction by blocking Ca^{2+} channels (Arslan, 2007). Active phytochemical constituents such as ternatin and quercetin have been reported to reduce castor oil-induced intestinal motility in rats (Yacob *et al*., 2016). According to another study, tannins isolated from the bark of *Ceriops decandra* have antioxidant and nitric oxide scavenging effects, and exhibit promising in vivo anti-motility activity (Hossain *et al.,* 2012). Therefore, the antidiarrheal effect of PGLE can be explained by its ability to inhibit intestinal motility, as shown in Table 6. It is important to recognize the significance of this finding because it reduces the risk of constipation, a major side effect of most common medications, including the standard drug loperamide. The anti-motility effects of *P. guajava* crude leaf extract at concentrations of 300 and 600 mg/kg were both statistically significant, with the 600 mg/k dose having the strongest activity (50.00%, $p<0.05$). Crude leaf extracts of *P. guajava* may contain a large number of phytochemical components that contribute to dose-dependent anti-motility effects. Additionally, alkaloid fractions isolated from *W. tinctoria* showed antiperistaltic and peristaltic effects in a similar antidiarrheal model (Bigoniya and Rana, 2009).

Antidiarrheal Index (ADI) is a method that calculates the cumulative effect of antidiarrheal parameters such as reduced bowel motility, defecation delay, and fluid accumulation (Yacob *et al*., 2016). Araj-Khodaei *et al.* (2020) maintained that effectiveness of using plant extract(s) in treating diarrhea increases as the extract's ADI value increases. Furthermore, the ADI value from the current study also confirmed that the antidiarrheal activity of the crude extract from *P. guajava* leaves was equivalent to that of the standard drug loperamide. On the other hand, the ADI of

the crude extract from *P. guajava* leaves at a reported dose of 150 mg/kg was low, indicating minimal antidiarrheal activity in all the models used.

Conclusion

This study revealed that *Psidium guajava* has a considerable health benefit and possesses antidiarrheal properties. High value of the ADI value represents the effectiveness of the extract in the treatment of diarrhea. The highest ADI value was observed at the highest dose of 600 mg/kg *P. guajava* extract which is directly related to its efficacy in treating diarrhea. This finding lends pharmacological credence to the anecdotal, ethno medical use of PGLE as a natural remedy for the treatment, management and/ or control of diarrhea. However, the result is not an approval to it being consumed without dosage. The study showed that PGLE demonstrated promising antidiarrheal activity and may serve as a potential source of antidiarrheal agents and that the anti-diarrheal action of the extracts was mediated by an anti-secretory mechanism and also reducing gastrointestinal motility contributed by the phytochemical agents possessed by the plant extract.

Conflict of Interest

The authors declared no conflict of interest

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Author's contribution

Chidiebere Malachy Chigbo: Supervision, Conceptualization, Writing - Original draft, Valentine Osita Nwobodo: Writing -Review and Editing, Ejike Celestine Orji: Writing-Methodology and Investigation. Okechukwu David Okeke: Methodology and Data analysis. Ogechukwu Frances Nworji: Data analysis. Joy Ogana: Investigation and Supervision. Ebele Lorreta Iloanya:Writing-Review and Editing

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