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# *In vitro* Antioxidant and Antidiarrhoeal Activities of Hydro-ethanol Stem Bark Extract of Guava (*Psidium guajava*) in Castor oil-induced Diarrhoeal Albino Rats

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## Abstract

Diarrhoea has been rampaging with high mortality, among children of five years and below most especially, in developing countries. Although, orthodox drugs for diarrhoea are available but with side effects and not within the reach of poor, hence, the use of medicinal plants such as guava (*Psidium guajava*) for its management. *In vitro* antioxidative effects and antidiarrhoeal potential of hydro-ethanolic extract of stem bark of guava (*Psidium guajava*) in castor oil-induced diarrhoeal albino rats were investigated in this study. Bioactive compositions, its effects on oxidation together with diarrhoea in Abino rats were also investigated. The extract contained phytochemicals such as phenols, tannins and flavonoids in relatively large quantity while alkaloids were completely not detected. HPLC analysis further revealed bioactive compounds such as kaempferol, chlorogenic acid, apigenin and guajiverin among others. The extract also showed high antioxidant potentials *in vivo*. Furthermore, the frequency of stooling, intestinal contents movement and enteropooling were reduced significantly ($P < 0.05$) in Albino rats treated with aqueous-ethanol stem bark extract of guava (*Psidium guajava*). In addition, the there was a significant ($P < 0.05$) reduction in $N^+$-$K^+$ ATPase activity while an increase in the concentration of nitric oxide in the blood of diarrhoeal albino rats was also observed. However, these pharmacological properties of the extract are concentration-dependent with the highest dose having the best results.

**Keywords:** Albino rats, Antidiarrhoea, Antioxidants, Phytochemicals, *Psidium guajava*

## 1.0 Introduction

Diarrhoeal is an irregular bowel movements that is characterised by an increased in the rate of watery stool (Caramia *et al*., 2015). It has led to an increase in the mortality, accounting for between five to eight millions death yearly, most especially in infants, in the developing countries (Mohd *et al*., 2004, Mujumdar *et al*., 2005). Thus, diarrhoea is an impending potential danger hovering the developing countries (Yongs and Dovie, 2007). Viruses such as norovirus, adenovirus and astroviruses have been known to

cause diarrhea in adults (Okolo *et al*., 2013). In addition, bacteria such as *E. coli, Salmonella* and some others are well known organisms that cause diarrhoea. In addition, parasites like Giardia can also cause diarrhea (Shemsu *et al*., 2013). The activities of these organisms lead to an increase in the intestinal movement due to influx of water and ions. This then leads to watery stool. This will further leads to excessive loss of water, leading to dehydration of the body and imbalance of electrolytes/ions. This can then cause fatality (Shah *et al*., 2007).

Although orthodox drugs such as Loperamide, Diphenoxylate, Racecadotril and some other known antidiarrhoeal drugs are available. However, these drugs are not without side effects, expensive and not readily available, most especially in the rural areas and villages. There is therefore the need for another means of diarhoea treatment. Fortunately, larger population in sub-Saharan Africa now rely on medicinal plants to treat their ailments (Hostettmann *et al*., 2000). These plants' uses have been encouraged by the World Health Organisation (WHO) for diarrhoea (Snyder and Merson, 1982). Therefore, these plants are potential sources for the development of drugs for diarrhoea (Aniagu *et al*., 2005; Suleiman *et al*., 2008). Some of such antidiarrhoeal medicinal plants that have been studied include *Alchornea cordifolia, Euphorbia hirta, Adansonia digitata* and *Morinda morindoides* (Gabriel *et al*., 2004; Mueller and Mechler, 2005; Meir *et al*., 2009).

Guava (*Psidium guajava*) is a phylum *Magnoliophyta,* belonging to class *Magnoliopsida* and family *Myrtaceae* (Gutirrez *et al*., 2008).

All parts of *Psidium guajava* have an old history of medicinal value (Gutirrez *et al*., 2008; Gutirrez-Montiel *et al*., 2023). However, little or no information has been found on the antidiarrhoeal effect of *Psidium guajava*. Therefore, this research aims to find out the antidiarrhoeal potential of aqueous-ethanol extract of stem bark of guava (*Psidium guajava*).

**Figure 1:** *Psidium guajava* tree

## 2.0 Materials and Methods

### 2.1 Materials

Guava (*Psidium guajava)* was harvested at Ikole-Ekiti town in Ikole Local Government Area of Ekiti state, Nigeria. It was authenticated at the Department of plant Biology, University of Ilorin, Kwara state, Nigeria and a voucher number: ULCH/001/827/2022 was assigned.

Castor oil and Loperamide were bought from Bell, Sons & co. Ltd. and Flamingo pharma, Ltd, respectively, both in UK. All other reagents are of analytical grades.

Seventy (70) male albino rats (average weight: 170 g) were bought from Animal facility centre, Redeemers University, Ede, Osun State, Nigeria and kept in a cage at the Department of Biochemistry laboratory, Federal University Oye Ekiti for two weeks to acclimatize prior to commencement of the experiment.

### 2.2 Methods

**2.2.1 Extract preparation:** Stem of *Psidium guajava* was cleaned of dirt, peeled, cut into pieces, air dried at room temperature until constant weight was obtained. The dried stem bark was then milled to into powder form with an electric blender. Five (5) Litres each of ethanol and distilled water (ratio 1:1) were used to dissolve 2 kg of the powder for 24 hrs. After 24hrs, the extract was filtered with Muslin cloth. The filtrate was evaporated with a rotary evaporator to reduce its moisture content after which it was lyophilized.

### 2.2.2 Qualitative analysis of phytochemicals in aqueous-ethanol (1:1) extract of *Psidium guajava* stem bark*:*

i. **Flavonoids:** The test adopted for flavonoid was as reported by Harborne (1973) and Sofowara (1993). The sample (0.30 g) weighed into a beaker was extracted with 30 $cm^3$ of distilled water for 2 hours and filtered with Whatman filter paper number 42 (125 mm). To 10 $cm^3$ of the aqueous-ethanol filtrate of extract was added 5 $cm^3$ of 1.0 M dilute ammonia solution followed by the addition of 5 $cm^3$ of concentrated tetraoxosulphate (VI) acid. Appearance of yellow coloration which disappeared on standing showed the presence of flavonoids.

ii. **Tannins:** Analysis used was the method reported by Ejikeme *et al*. (2014). The sample (0.30 g) was weighed into a test tube and boiled for 10 minutes in a water bath containing 30 $cm^3$ of water. Filtration was carried out after boiling using number 42(125 mm) Whatman filter paper. To 5 $cm^3$ of the filtrate was added 3 drops of 0.1% ferric chloride. A brownish green or a blue-black coloration showed positive test.

iii. **Phenols:** To 0.5g of the sample was added 3 drops of 1% solution of ferric chloride followed by 1% gelatin in sodium chloride of the same concentration. The formation of a precipitate indicated the presence of phenols (Trease and Evans, 1989).

iv. **Alkaloids:** Test for flavonoid used was as reported by Hikino *et al*. (1984). Extraction of component from 2 grams of the powder sample was carried out using 5% $H_2SO_4$) (20 $cm^3$) in 50% ethanol by boiling for 2 minutes and filtered through Whatman filter paper number 42 (125 mm). The filtrate was made alkaline with 5 $cm^3$ of 28% ammonia solution ($NH_3$) in a separating funnel. Equal volume of chloroform (5.0 $cm^3$) was used in further solution extraction in which chloroform solution was extracted with two 5 $cm^3$ portions of 1.0 M dilute $H_2SO_4$. This final acid extract was then used to carry out the following test: 0.5 $cm^3$ of Dragendorff's reagent (Bismuth potassium iodide solution) mixed with 2 $cm^3$ of acid extract and precipitated orange color infers the presence of alkaloid.

v. **Terpenoids:** Methodology was as reported by Ejikeme *et al*. (2014). The powder sample (0.30 g) was weighed into a beaker and extracted with 30 $cm^3$ and component extracted for 2 hours. A mixture of chloroform (2 $cm^3$) and concentrated $H_2SO_4$ (3 $cm^3$) was gently added to 5 $cm^3$ of extract to form a layer. The presence of a reddish brown coloration at the interface showed positive result for the presence of terpenoids.

vi. **Phlobatannins:** Analytical method was according to Ejikeme *et al.* (2014). The sample (0.30 g) weighed into a beaker was added 30 $cm^3$ of distilled water. After 24 hours of extraction, aqueous extract (10 $cm^3$) of sample was boiled with 5 $cm^3$ of 1% aqueous hydrochloric acid. Deposit of red precipitate showed positive test.

vii. **Saponins:** Methodology used was as reported by Ejikeme *et al*. (2014). Distilled water (30 $cm^3$) was added to sample (0.30 g) and boiled for 10 minutes in water bath and filtered using Whatman filter paper number 42 (125 mm). A mixture of distilled water (5 $cm^3$) and filtrate (10 $cm^3$) was agitated vigorously for a stable persistent froth. The formation of emulsion on addition of three drops of olive oil showed positive result.

### 2.2.3 HPLC Characterisation:

This was done with the method previously used by Mingtao *et al* (2015).

### 2.2.4 Antioxidant determination:

i. **Free radical scavenging ability determination of 1, 1-diphenyl-2-picryhydrazyl (DPPH):** The free radical scavenging ability of the extract against DPPH (1, 1- diphenyl-2-picryhydrazyl) using Gyamfi *et. al.*, (1999) method was determined. One (1) ml of the extract was mixed with 1ml of the 0.4mM methanolic solution of the DPPH and the mixture was left in the dark for 30 mins before measuring the absorbance at 516 nm.

$$\text{DPPH Inhibition} = \frac{(\text{Abs. of standard} - \text{Abs. of sample})}{\text{Abs. of standard}} \times 100$$

ii. **Ferric reducing antioxidant power (FRAP) determination:** The reducing property of the extract was determined by the method of Pulido *et al.*, (2000), where 0.25 ml of the extract was mixed with 0.25 ml of 200 mM of Sodium phosphate buffer pH 6.6 and 0.25 ml of 1% KFC. The mixture was incubated at 50° C for 20 mins. Thereafter 0.25 ml of 10% TCA was also added and centrifuge at 2000 rpm for 10 mins. One (1) ml of the supernatant was mixed with 1 ml of distilled water and 0.1% of $FeCl_3$. The absorbance was measured at 700 nm.

Note: A higher absorbance indicates a higher reducing power.

iii. **Assay of Superoxide Radical Scavenging activity (SOD):** The activity of SOD in the homogenates was determined according to the method described by Misra and Fridovich, (1972). A dilution of 1 ml of the sample was made with 9ml of distilled water to make a 1 in10 dilution. An aliquot of the diluted sample was added to 2.5 ml of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3 ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference curvette contained 2.5 ml buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of water. The increase in absorbance at 480 nm was monitored every 30 secs for 150 secs.

$$\text{Increase in absorbance per minute} = \frac{(A3 - A0)}{2.5}$$

Where, $A_0$= absorbance after secs and
$A_3$ = absorbance after 150 secs

$$\%\ \text{inhibition} = 100 - \frac{\text{Increase in absorbance for substrate}}{\text{Increase in absorbance of standard}} \times 100$$

Note: 1 unit of SOD activity was defined as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adrenochrome during 1 minute.

iv. **Determination of nitric oxide (NO) radical scavenging ability:** Briefly 5mM sodium nitroprusside in phosphate- saline was mixed with the extract, before incubation at 25° C for 150 min. Thereafter, the reaction mixture was added to Greiss reagent. Before measuring the absorbance at 546 nm, relative to the absorbance of standard solution of potassium nitrate treated in the same way with Greiss reagent (Jagetia and Baliga, 2004).

v. **Determination of Hydroxyl (OH) Radical Scavenging ability:** The ability of the extract to prevent $Fe^{2+}/H_2O_2$ induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge (1981). Briefly, freshly prepared extract (0-100 µl) was added to a reaction mixture containing 120 µl, 20 mM, deoxyribose, 400 µl, 0.1M phosphate buffer pH 7.4, 40 µl, 20mM hydrogen peroxide and 40 µl, 500 µl $MFeSO_4$ and the volume was made to 800 µl with distilled water. The reaction mixture was incubated at 37° C for 30 mins and the reaction was stopped by the addition of 0.5 ml of 2.8% TCA. This was followed by the addition of 0.4 ml of 0.6% TBA solution. The tubes were subsequently incubated in boiling water for 20 mins. The absorbance was measured at 532 nm in spectrophotometer. Hydroxyl radical was detected by monitoring absorbance at 532 nm.

Scavenging activity was then estimated as follows:

$$\text{Radical scavenging effect (\%)} = \frac{\text{Ao} - \text{As}}{\text{Ao}} \times 100$$

Where $A_o$: absorbance of control at 532 nm
$A_s$: absorbance of sample at 532 nm

**2.2.5 Treatment of experimental animals:** Seventy adult rats with weighing about 170 g, were divided into two equal halves. The first thirty five (35) rats used to investigate the frequency of diarrhoeal stooling, were grouped into seven consisting of 5 rats each. Group 1 representing normal control and was administered 1 ml of distilled water orally. The remaining groups 2-7 were administered 1 ml of castor oil to induce diarrhoea. Groups 3 - 6 were orally administered 1 ml of aqueous-ethanol stem bark extract of *Psidium guajava* containing 100, 200, 400, 600 mg/Kg body weight respectively. Group 7 was orally administered 1ml of standard drug, Lopermide containing 2 mg/Kg body weight of the drug.

The above procedure was also repeated with the remaining thirty-five rats for the gastro-intestinal motility and enteropooling experiments.

**Induction of diarrhoea:** Castor oil-induced diarrhoea model was done with the method earlier described by Bamisaye *et al*., (2013). The test animals were fasted for 18 hours prior to the induction but were allowed free access to water. After 30 minutes of treatment (with drug and extract), each animal was orally administered 1 ml of castor oil. The time between oil administration and appearance of the first diarrhoeal drops was noted.

**Determination of gastro-intestinal motility:** The method earlier used by Gerald *et al.,* (2007) was employed. After 30 minutes of diarrhoea induction, all groups were administered with 1ml of 10% barium sulphate solution. Thirty (30) minutes later, all the rats were sacrificed. Then, the distanced travelled by barium sulphate milk was measured and expressed as a percentage of the total length of the small intestine (Gerald *et al.,* 2007).

$$\%\ \text{Inhibition in intestinal propulsion} = \frac{\text{Positive control group} - \text{Treated group}}{\text{Positive control group}} \times 100$$

**Anti-enteropooling determination:** Accumulation of intraluminal fluid was determined with the method of Havagiray *et al.* (2004). The test animals were fasted for 18 hours prior to the experiment and were allowed free access to water. After diarrhoea induction, the extract and standard drug (loperamide) were administered accordingly. After 30 minutes, the rats were sacrificed according to the method by Yakubu *et al*. (2005). The small intestine was removed and the intestinal content was collected by squeezing into a measuring cylinder. The volume and the weight of the intestinal content were measured and weighed respectively.

Percentage volume and weight of the intestinal fluid were then determined using the following formula:

$$\%\ \textbf{Reduction in Volume of intestinal fluid} = \frac{\text{Average volume of Positive control group} - \text{Average volume of Treated group} \times 100}{\text{Average Volume of Positive control group}}$$

$$\%\ \textbf{Reduction in weight of intestinal fluid} = \frac{\text{Average weight of Positive control group} - \text{Average weight of Treated group} \times 100}{\text{Average Volume of Positive control group}}$$

**2.2.6 Biochemical assays**

**$Na^+$-$K^+$ ATPase activity:** The method of Bewaji *et al.,* (1985) was used. For the test, 400 µl of 200 mM NaCl/40 mMKCl/60 mMTris (pH 7.4) was pipetted into test tube. Thereafter, 20 µl of $MgCl_2.6H_2O$ (80 mM), 20 µl of EGTA (20 mM), 240 µl of distilled water and 20 µl of appropriately diluted tissue supernatant was added. This was mixed and incubated at $37^0$ C for

5 minutes. Then, 100 µl of ATP (8 mM) was added. This was mixed and incubated at $37^0$ C for 30 minutes. Thereafter, 200 µl of SDS (5%) and 2,000 µl of reagent C were added. The mixture was allowed to stand at room temperature for 30 minutes for color development. The blank was similarly prepared but 20 µl of distilled water was used instead of the 20 µl of tissue supernatant. The absorbance of the test was read against the blank at 820 nm. The absorbances obtained were then extrapolated from the calibration curve for phosphate to obtain concentration of inorganic phosphate.

$$\text{Specific activity (µmole Pi/mg Prot./hr)} = \frac{[\text{Pi}] \times 2 \times D.F}{1000 \times Protein\ Conc\ (mg/ml)}$$

Where;

$[P_i]$ = Concentration of inorganic phosphate in nmoles (obtain from calibration Curve).

2 = Factor introduced to obtain the amount of $P_i$ released per hour

D.F = Dilution Factor

1000 = factor introduced to convert the $P_i$ release to µmoles

**NOTE**: The actual specific activity of $Na^+,K^+$-ATPase was obtained by the subtraction of the specific activity of $Mg^{2+}$-ATPase from that of $Na^+K^+$ATPase (obtained from calculation above).

**Nitric oxide Concentration:** The NO level was determined with Miranda *et al* (2001) method. In brief, 100 µL of supernatant was applied to a micotiter plate well, 100 µL vanadium (III) chloride (8mg/ml) was added to each well (for reduction of nitrate to nitrite) and this was followed by addition of the Griess reagents, 50 µLsulfanilamide (2%) and 50 µL N-(1-Naphthyl) ethylendiaminedihydrochloride (0.1%). After 30 min of incubation at 37 º C, absorbance was read at 540 nm using the ELISA reader (Sunrise, Tecan, Austria). Concentration of NO in serum sample was determined from linear standard curve established by 0-150 µmol/l sodium nitrite.

### 2.2.7 Statistical Analysis

Data are expressed as ± standard error of mean (SEM) of five (5) determinations. Data were subjected to error bar graphs and analysis of variance (ANOVA) using SPSS statistical software for Microsoft windows operating system version 20.0. Significant differences were defined as $P < 0.05$.

## 3.0 RESULTS

Qualitative phytochemicals analysis of aqueous-ethanol (1:1) stem bark extract of *Psidium guajava* contained flavonoids, tannins, phenols, terpenes, phlobatanin and saponins while alkaloids was not detected (Table 1).

HPLC characterisation revealed the percentage composition of the phytochemical component of aqueous-ethanol (1:1) stem bark extract of *Psidium guajava* with Kaempferol having the highest % composition (29.72%) while gallic acid and eugenol are detected in traces (Table 2).

The *in-vitro* antioxidant analysis showed that DPPH has the highest activity (94.41 ±2.45%) while FRAP has the least (18.62 ±0.71%) (Table 3).

**Table 1:** Qualitative analysis of phytochemicals in aqueous-ethanol extract of Guava (*Psidium guajava*) stem bark

| Phytochemicals | Status |
|---|---|
| Flavonoids | +++ |
| Tannins | ++ |
| Phenols | +++ |
| Alkaloids | - |
| Terpenes | + |
| Phlobatanin | + |
| Saponins | + |

**Key:** +++ = Abundant
++ = Less abundant
+ = Present in minute quantity

**Table 2:** HPLC characterisation of aqueous-ethanol extract of *Psidium guajava* stem bark

| Phytochemicals | Area | Concentration | % composition |
|---|---|---|---|
| Chlorogenic acid | 2621.31 | 0.240 | 24.24 |
| Guajiverin | 767.62 | 0.071 | 7.10 |
| Gallic acid | 76.45 | 0.007 | 0.71 |
| Apigenin | 927.86 | 0.086 | 8.58 |
| Caffeic acid | 117.14 | 0.011 | 1.08 |
| Saponin | 98.43 | 0.009 | 0.91 |
| Catechin | 201.42 | 0.019 | 1.86 |
| Epicatechin | 122.87 | 0.011 | 1.14 |
| Epigallcatechin | 180.49 | 0.017 | 1.67 |
| Avicularin | 103.70 | 0.010 | 0.96 |
| Beta-caryphyllene | 101.32 | 0.010 | 0.94 |
| Isoquercitrin | 109.39 | 0.010 | 1.01 |
| Quercitrin | 103.90 | 0.010 | 0.96 |
| Kaempferol | 312.62 | 0.300 | 29.72 |
| Rutin | 234.30 | 0.022 | 2.17 |
| Limonene | 101.38 | 0.010 | 0.94 |
| 1,8-cineole | 197.38 | 0.018 | 1.83 |
| Beta-bisabolene | 191.05 | 0.018 | 1.77 |
| Eugenol | 77.09 | 0.007 | 0.71 |
| Eucar | 105.50 | 0.010 | 0.98 |
| Quercetin | 1078 | 0.100 | 0.97 |
| Eucalyptol | 81.39 | 0.008 | 0.75 |

Diarrhoea was clinically apparent in most animals in each group thirty minutes after its induction. This continued for the next six hours. Defaecation was inhibited by 100% in positive group. This defaecation in extract-treated groups was dose dependent with the highest dose having the highest inhibition/reduction in defaecation and watery stools over six hours. The least and highest doses (100 and 600 mg/Kg body weight of the extract) inhibited defaecation and watery stools by 21.10% and 59.47% respectively (Table 4).

The movement of barium sulphate was inhibited in normal gastro-intestinal motility and castor oil induced diarrhoea at all doses when compared with the control group 2 (Table 5). The maximum effect of the extract was observed at 600 mg/Kg body weight. However, standard drug, loperamide has the highest inhibition compared with the extract-treated groups (Table 5).

**Table 3:** *In-vitro* antioxidative potentials of aqueous-ethanol extract of *Psidium guajava* stem bark

| Antioxidants | Activity (%) |
|---|---|
| DPPH | $94.41^{a} \pm 2.45$ |
| NO | $34.02^{d} \pm 3.41$ |
| SOD | $80.56^{c} \pm 4.46$ |
| OH | $88.75^{b} \pm 0.61$ |
| FRAP | $18.62^{e} \pm 0.71$ |

Values are mean ±SEM, n =3

Enteropooling induced in animals was reduced in the standard drug and extract-treated groups in a dose dependent manner. The group treated with 600 mg/Kg had highest inhibition compared with other doses and the standard drug-treated groups (Table 6).

There was a significant reduction ($P < 0.05$) in the activity of $Na^{+}$-$K^{+}$ ATPase in the blood of standard drug- and extract-treated groups when compared with positive control. Group 4, which was treated with 200 mg/Kg body weight of the extract has the least activity of $Na^{+}$-$K^{+}$ ATPase.

**Table 4:** Inhibitory effect of aqueous-ethanol extract of *Psidium guajava* stem bark on frequency of stooling in diarrhoeal Albino rats

| Groups | Total number of faeces | Number of wet faeces | % Inhibition of defaecation |
|---|---|---|---|
| 1 | 2.82 ±0.02 | 0.00 ±0.00 | $100^{a}$ |
| 2 | 9.35 ±0.17 | 7.34 ±0.11 | $21.50^{e}$ |
| 3 | 9.29 ±0.02 | 7.33 ±0.09 | $21.10^{e}$ |
| 4 | 7.82 ±0.01 | 6.28 ±0.11 | $19.69^{f}$ |
| 5 | 10.62 ±0.01 | 4.34 ±0.05 | $59.13^{d}$ |
| 6 | 9.45 ±0.02 | 3.83 ±0.09 | $59.47^{c}$ |
| 7 | 7.24 ±0.013 | 2.85 ±0.09 | $60.64^{b}$ |

Values are given as Mean ±SEM. Superscripts that are not the same are significantly ($P < 0.05$) different from each other.

**Key:**

Group 1 = Normal control group, treated with 1 ml of distilled water only.

Group 2 = Positive control group, treated with 1 ml of distilled water only.

Group 3 = Treated with 1 ml of 100 mg/Kg body weight of aqueous-ethanol extract of *P. guajava* stem bark.
Group 4 = Treated with 1 ml of 200 mg/Kg body weight of aqueous-ethanol extract *P. guajava* stem bark.
Group 5 = Treated with 1 ml of 400 mg/Kg body weight of aqueous-ethanol extract *P. guajava* stem bark.
Group 6 = Treated with 1 ml of 600 mg/Kg body weight of aqueous-ethanol extract *P. guajava* stem bark.
Group 7 = Treated with 1 ml of 2 mg/Kg body weight of standard drug, loperamide.

**Table 5:** Inhibitory effect of aqueous-ethanol extract of *Psidium guajava* stem bark on mean distance travelled by barium sulphate meal in diarrhoeal albino rats.

| Groups | Mean distance travelled (mm) | % Inhibition |
|---|---|---|
| 1 | 49.00 ±6.16 | 40.82[a] |
| 2 | 82.80 ±3.92 | 0.00[f] |
| 3 | 82.00 ±4.83 | 0.97[e] |
| 4 | 80.40 ±3.16 | 2.90[d] |
| 5 | 77.50 ±4.36 | 6.40[c] |
| 6 | 77.40 ±4.93 | 6.52[c] |
| 7 | 74.80 ±6.42 | 9.66[b] |

Values are given as mean ± SEM. Superscripts that are not same are significantly ($P < 0.05$) different from each other.

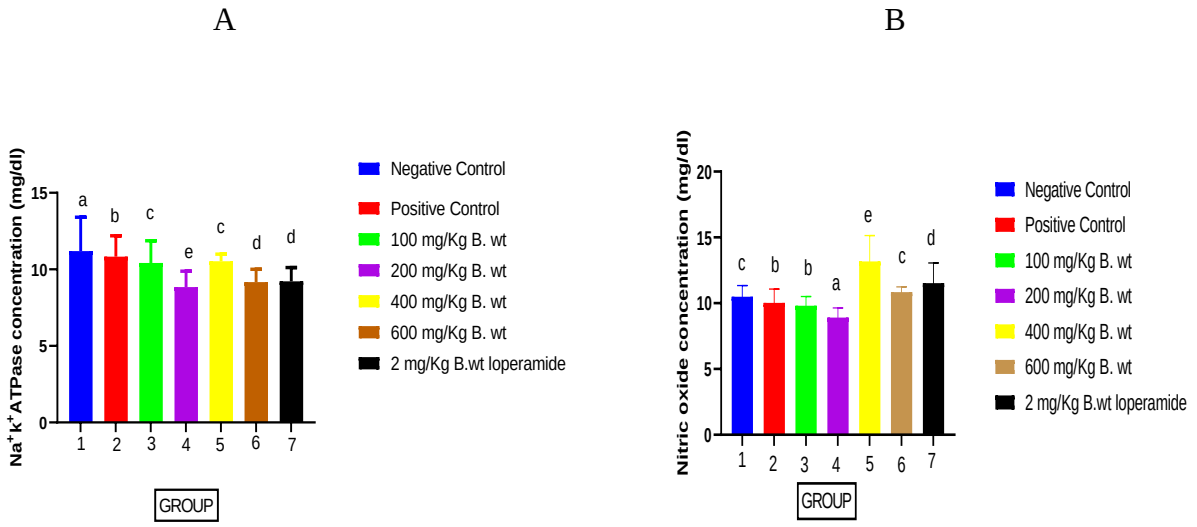


**Figure 1:** $Na^+$-$K^+$ ATPase activity (**A**) and Nitric oxide concentration (**B**) in the blood of castor oil-induced diarrhoeal albino rats treated with aqueous-ethanol (1:1) stem bark extract of guava (*Psidium guajava*).

However, there was no significant difference in the activities of $Na^+$-$K^+$ ATPase in group treated with 600 mg/Kg body weight of the extract and that of standard drug (Fig. 1A).

A significant ($P < 0.05$) increase in the nitric oxide concentration of rats treated with standard drug and those treated with 400 mg/Kg body weight of the extract was observed when compared with the positive group. However, the group that was treated with 400 mg/Kg body weight of the extract has higher nitric oxide accumulation when compared with the standard drug, loperamide (Fig. 1B).

## 5.0 DISCUSSION

Phytochemicals are non-nutritive plant chemicals that have protective or disease-preventive properties. It is well known that plant produce these chemicals to protect themselves but researches have demonstrated that they can also protect humans against diseases (Rabizadeh *et al*., 2022). Pharmacological important phytochemicals such as flavonoids, tannins, phenols, saponin, phlobatanin which are present in the aqueous-ethanol (1:1) stem bark extract of guava *(Psidium guajava)* may be responsible for the observed anti-diarrhoeal effect.

Evaluation of antioxidant potentials of agents can be investigated using *in vitro* methods to actually prone into their antioxidant efficiencies either as natural/pure compounds or plant extracts. DPPH is a widely used free radical. It is a stable radical used to access the radical scavenging activity of antioxidant compounds (Matthew and Abraham, 2006). The NO scavenging activity is also a free radical. It is obtained from sodium nitroprusside, which reacts with oxygen to give nitrite (Vidova *et al*., 2022).

FRAP assay is a process that study the ability of antioxidant to reduced iron III to iron II when 2,4,6–tri (2-pyridyl)-s-triazine (TPTZ) is present. This process formed an intense blue $Fe^{2+}$-TPT, whict minimize tissue damage through oxidation (Naji *et al*., 2020). Superoxide dismutase is an important enzyme in an antioxidant defense system (Saxena *et al*., 2022). SOD converts the superoxide anion into hydrogen peroxide and thus reduces the toxic effect. The percentage inhibition of superoxide by SOD may reduce the cellular damage (Fujii *et al*., 2022). One of ROS that can react with polyunsaturated fatty acids in biological systems is hydroxyl (OH) radical. It interact with the fatty acid moieties of phospholipids located in cell membrane, thereby damaging the cell (Michael *et al*., 2022). Therefore, the observed antioxidant activities in this extract may be due to the bioactives present in the extract. For examples, the FRAP activity is correlated to total phenols which are present in this extract. Also, NO scavenging activity is associated with total flavonoids which are found in the extract. The ability of *Psidium guajava* aqueous-ethanol stem bark to inhibit the activity of hydroxyl radicals prevents further damage to the cell.

An imbalance between the secretory and absorptive mechanisms in the gastro-intestinal pipe leads to diarrheoa. This then causes hypermotility, which lead to the faeces of the individuals to contain excess fluid (Wibowo *et al*., 2021). The presence of ricinoleic acid in castor oil causes diarrhoea in the organism. The upper part of small intestine contains lipases which can act on castor oil to liberate this acid (Tunaru *et al*., 2012). This acid then binds with prostaglandin receptor located on smooth muscle. The binding then facilitates the intestine to accumulate fluid through inhibition of absorption. This process also enhances secretion of fluid and intestinal electrolytes (Bakare *et al*., 2011). Therefore, the extract may be responsible for the observed significant ($P < 0.05$) reduction of watery stools due to diarrhoea induced by castor oil in this study. This observed action may be due to the presence of phytochemicals such as flavonoids, phenols and tannins in the extract. Earlier researches carried out by Ashok and Kumud, (2012) and Bamisaye *et al* (2013) claimed that flavonoids, phenols and tannins are antidiarrhoeal in nature.

Many antidiarrheal agents act by reducing gastrointestinal motility (Ezekwesili *et al*., 2010). Therfore, the observed inhibition of intestinal motility as the extract doses increased may be due to one or more bioactives found in the extract. This finding might suggest the action of the extract on more than one part of the intestine. When there is a reduction in the intestinal muscle motility, there will be a corresponding increase of the time a substance stays in the intestine thereby creating a greater time for absorption (Silva *et al*., 2012). The observed reduction in intestinal propulsive movement of barium sulphate meal model may be as a result of its inhibitory action on intestinal motility of the extract. The extract may have constipating potentials. Furthermore, earlier researchers have reported flavonoids and tannins as antidiarrheal metabolites because they have the inhibitory potentials for intestinal motility (Ashok and

Kumud, 2012). The production of cyclooxygenase 1 and 2 (COX-1, COX-2) and lipo-oxygenase (LOX) is modified by flavonoids. This process inhibits the production of prostaglandin (Ashok and Kumud, 2012). In another study, tannins has been reported to have precipitated proteins due to the formation of protein tannates. This product creates resistance to alteration by chemicals in intestinal mucosa leading to a reduction in peristaltic movement and intestinal secretion (Ashok and Kumud, 2012; Bamisaye *et al*., 2013). Hence, the observed anti-diarrhoeal activity of aqueous-ethanol stem bark extract of guava (*Psidium guajava*) may be as a result flavonoids, tannins and phenols present in it. However, this effect may be synergistic.

Ricinoleic acid is the active metabolite in castor oil that causes intestinal mucosa to be irritated and inflamed. This leads to the release of prostaglandins. This then leads to cascade of reactions that prevent reabsorption of sodium chloride and water (Tunaru *et al*., 2012). The observed inhibition of hypersecretion in the gastrointestinal contents as well as the intestinal accumulation of fluid (enteropooling) with increases in the reabsorption of water and electrolytes of the extract-treated animals may be due to the flavonoid and tannins in the aqueous-ethanol stem bark extract of *Psidium guajava*. Flavonoids has been implicated to have inhibited prostaglandins release (Hamalainen *et al*., 2012); this then lead to secretion inhibition but facilitate electrolytes reabsorption. Furthermore, the observed decrease in the secretion of fluid in these extract-treated animals may be associated with tannins because it has been known to cause precipitation reactions in small intestine of animals (Wang *et al*., 2010).

An important enzyme located in the intestinal membrane is $Na^+$-$K^+$ ATPase. This enzyme regulates and maintains the influx of $K^+$ and $Na^+$ ions across the membrane of small intestine. It causes high and low intracellular $K^+$ and $Na^+$ concentrations respectively, which creates intracellular gradient (Karlish *et al*., 2008; Prosenjit *et al*., 2015). Ricinoleic acid from castor oil is known to form salts of ricinoleates with potassium and sodium in the intestine. These salts have the ability to inhibit the activity of $Na^+$-$K^+$ ATPase. This then may lead to an increase in the permeability of intestinal epithelium through its impairment, thereby causing an intestinal damage and its absorptive ability (Komal and Rana, 2013). The observed improvement found in $Na^+$-$K^+$ ATPase activities of extract-treated animals may be as a result of $Na^+$/glucose co-transporter. The major route for dietary glucose transportation in the small intestine to enterocytes is through $Na^+$/glucose co-transporter. The extract may have caused an enhancement in the enzyme's activity, which in turn may have prevent the excessive loss of water and electrolytes from small intestine. This may be achieved by regulating the permeability of the epithelial cell membrane of the animals (Chen *et al*., 2016).

One of the signaling substances in the cells is Nitric oxide, NO. This chemical primarily activates soluble guanylate cyclase, which then activates cyclic guanosine monophosphate dependent kinases that is responsible for the net secretion of fluid over reabsorption (Arthur *et al*., 2014). The observed increased in concentration of nitric oxide in the extract-treated rats may imply that the extract contain phytochemicals that are responsible for this act.

## 6.0 CONCLUSION

Aqueous-ethanol (1:1) stem bark extract of guava (*Psidium guajava*) demonstrates an inhibition in frequency of diarrhoea, gastrointestinal movement and the fluid accumulation in the small intestine of castor oil-induced diarrhoeal albino rats. The extract contains reasonable concentrations of tannins and flavonoids which may have anti-diarrhoeal activities. This may justify its use for the treatment of diarrhoea by herbal practitioners. However, the anti-diarrhoeal activity of aqueous-ethanol stem bark of *Psidium guajava* is dose dependent.

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**Credit authorship contribution statement**

**Fisayo Abraham Bamisaye:** Conceived and designed the study, performed the experiment, data analysis and proofread the manuscript. **Olawale Razaq Ajuwon:** co-performed the experiment, wrote the first draft of the manuscript. **Basiru Olaitan Ajiboye:** co-performed the experiment. **Ayoola Olusegun Oluwajobi:** Methodology support, co-supervision, review, and editing. **Adedeji Aderinola Adejumo:** Methodology support, co-supervision, review, and editing. **Oladimeji Samuel Tugbobo:** Methodology support, co-supervision, review, and editing.

**Declaration of Competing Interest**

All authors confirm that there are no potential conflicts of interest.

**Data availability**

All data associated with article are available on request from the corresponding author.

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