**ANTIMICROBIAL ACTIVITY AND ANTIDIARRHEA EVALUATION OF *PSIDIUM GUAJAVA* (LINN) STEM BARK EXTRACT ON ALBINO RATS**

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**Abstract**

 Antimicrobial activity of ethanol stem bark extract of *Psidium guajava* was evaluated against five (5) enteropathogenic organisms and antidiarrheal effect was investigated on albino rats. Minimum Inhibitory Concentration and Zone of Inhibition were evaluated by doubling dilution techniques. Varying levels of the extract solution: 1000 mg/ml, 500mg/ml, 250mg/ml, 125mg/ml and 62.5mg/ml in pre-seeded nutrient agar was used for the test. Acute toxicity test (LD50) of the extract was determined. The anti-diarrheal effect of the ethanol stem bark extract was evaluated using charcoal meal transit and castor oil induced diarrheal models in albino rats. The animals were grouped into four (1-4) having five rats each and administered treatment in the following order: Group 1 received distilled water (negative control); 2 Loperamide (standard control), while groups 3 and 4 received 500mg/kg and 1000 mg/kg of the extract respectively. Results revealed that the stem bark extract had acute toxicity value > 5000 mg/kg. The extract also significantly inhibited growth of the microbes in concentration dependent manner (p<0.05) with 1000 mg/ kg of the extract producing 39.33±1.16, 31.00±1.00, 29.00±1.00, 25.67±1.53 and 23.33±1.55mm zones of inhibition for *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Campylobacter jejuni* and *Shigella dysentery* respectively. The MIC of the test organisms are as follows: 62.5mg/ml, 62.5mg/ml, 62.5mg/ml, 125mg/ml and 125mg/ml respectively. Significant decreased (p<0.05) in the frequency and amount of wet stool output was recorded in the diarrheal-induced rats and significant inhibition of charcoal meal transit was obtained in the motility study. In both models, the activities of the guava stem bark extract compared favourably with that of Loperamide, the standard control. . Hence, the use of guava stem bark extract to manage diarrhoea by local folk is hereby scientifically justified and the need to carry out further researches on the guava plant is emphasized.

**Keywords: *Psidium guajava*, Gastrointestinal, Loperamide, Antidiarrhea**

 **Introduction**

Diarrhoea, a gastrointestinal disorder, has been recognised as the second leading cause of mortality among children under five years of age next to respiratory infections and kills more young children than AIDS, malaria, and measles combined (Liu *et al.,* 2012). The disease is characterised by a discharge of semi-solid or watery faecal matter from the bowels three or more times per day (Dosso *et al.,* 2012). It involves an increase in the fluidity, volume and frequency of bowel movements, abdominal pain accompanied by increased secretion and decreased absorption of fluid and thus the loss of water and electrolytes (Ezekwesili *et al.* 2010).

Diarrhoea is usually a symptom of disease in the intestinal tract which can be caused by a variety of bacterial (*Escherichia coli, Vibrio cholerae, Shigella* species etc.), viral (Rota virus, Norovirus, Cytomegalovirus etc.) and parasitic organisms (protozoa and helminths) (Tadesse *et al.,* 2017).

Although humans and microbes have shown a great benefit from their relationship; however, there are still several microbial diseases bedevilling man. The illnesses arise from microbes dwelling on human body and in the environment (Ayienda, 2019). The worldwide death toll due to bacterial diseases is 17 million people yearly according to World Health Organization estimates (WHO, 2015) and consistent efforts by scientists are geared toward changing the narrative. It is cheering to note that bacterial cells could be killed by the rupture of cell walls and membranes and by the irregular disruption of the intracellular matrix when treated with plant extracts (Kim and Fung, 2004).

*Psidium guajava* (Linn.), commonly known as guava, is a plant of the family Myrtaceae, native to South America, but to date widespread throughout the tropical zone especially where the climate is favorable (Sanda *et al.,* 2011). It is medium-sized plant, up to 10m tall, used as food, and all its parts have pharmacological properties (Regina and Santosh, 2017). It has a plethora of laboratory proven biological activities such as antimicrobial (Nair and Chanda, 2007), antidiarrhoea (Jaiarj *et al.,* 1999), hepatoprotective (Roy *et al.* 2006), antioxidant (Jimenez-Escig *et al.,* 2001), and anticancer (Mbaveng *et al.,* 2018).

In folk medicine, extracts of root bark and leaves of guava leaves are used to treat gastroenteritis, vomiting, diarrhoea, dysentery, wound, ulcers, toothache, cough, sore throat, inflamed gums and a number of other conditions (Esimone *et al.,* 2012).

Guava stem bark is an effective astringent, helping with acne and other skin conditions (Gutierrez *et al.,* 2008). The high Vitamin C content of guava plant is also apparent in the bark and has good antioxidant properties. These can help fight free radicals in skin, leaving the complexion refreshed and renewed (Grant, 2020). Esimone *et al.* (2012) in their study showed that the phytochemical constituents of guava stem bark are carbohydrates, glycosides, tannins, and proteins.

However, whereas several pharmacological activities such as anti-bacterial, antioxidant and anti-tumor (Braga *et al.,* 2014), anti-hyperlipidemic and anti-diabetic (Deguchi and Miyazaki, 2010), analgesic and anti-inflammatory (Ojewole, 2006), and anti-gout and anti-hypertensive (Irondi *et al.,* 2016) effects have been reported for the leaves, most studies on the stem bark focused on its antimicrobial effects (Barbalho *et al.,* 2012; Morais-braga *et al.,* 2016). Hence, to further explore the pharmacological benefits of guava stem bark, this study evaluated the anti-microbial properties as well as the anti-diarrhoea properties of guava stem bark on gastro-intestinal pathogens.

The guava stem bark was reported by Irondi (2019) to contain ellagic acid and quercetin as the most abundant phenolic acid and flavonoids. The extract from the stem bark was used as a source of nutraceuticals for suppressing the production of uric acid, ammonium hydroxide, fatty acids and cholesterol, and mitigating oxidative stress. These bioactivities, which can be attributed to the phenolic compounds, suggest anti-nephrolithiatic and anti-obesity potentials of guava stem bark phenolics-rich extract (Irondi, 2019).

Recent evidence suggests that the rapid onset of diarrhoea induced by *E. coli* could result from direct effects on intestinal ion transport processes (Elisha *et al.,* 2017). Several invasive pathogens, including *Shigella* and *Salmonella* species, cause an inflammatory diarrhoea characterized by fever (Hodges and Gill, 2010). Diarrhoea is usually a symptom of diseases in the intestinal tract which can be caused by a variety of bacteria and microorganisms. Each year there are approximately 4 billion cases of diarrhoea worldwide. In the past 2 decades, there has been a search for drugs that might inhibit the process of diarrhoea and bacterial infection development especially the secretory process. Although a number of drugs have emerged, none has found a place in the routine management of diarrhoea. Local herbalists have depended on medicinal plants such as guava stem bark as reliable means of treating diarrhoea.

Furthermore, microbial infections cause discomfort and suffering hence lowering productivity of the victims. Several gastrointestinal pathogens such as *Escherichia coli, Campylobacter spp., Salmonella typhi, Shigella dysentery* and *staphylococcus aureus* causes infections that damage the host physiology and are mostly life threatening (Hien *et al* 2007; 2008). Thus, there is great need to develop safe and cheap antimicrobial agents to combat their activities. Hence the use of medicinal plants that possess anti-diarrhoea and antimicrobial activities has been explored as a measure that could be of benefit in combating widespread diarrhoea infection.

The emergence of resistance strains of these organisms which is as a result of inappropriate and prolonged use of antibiotics, have created a need for newer drugs and development of effective and affordable drugs to treat microbial infections especially in developing countries like Nigeria where up to one half of death are due to infectious diseases (Walsh and Toleman, 2012; Awouafack *et al.,* 2013). The phytochemical and biomolecules present in plants are highly efficient in the treatment of bacterial infection (Fernebro, 2011). It is hoped that the phytochemicals in ethanol extract of *Psidium guajava* stem bark will provide the much-needed alternative, safe, cheap and quick treatment for diarrhoea and may be a lasting solution to diarrhoea related deaths among children and adults as well.

Therefore, this study examines the antimicrobial activity and evaluate the antidiarrheal effect of ethanol extract of *P. guajava* stem bark on albino rat. The definite objectives were to establish (i) the zone of inhibition of guava stem bark ethanol extract against the organisms, (ii) the minimum inhibitory concentration of the plant extract on *Escherichia coli, Campylobacter spp., Salmonella typhi, Shigella dysentery* and *staphylococcus aureus,* (iii) the effect of guava stem bark extract on characoal meal transit, (iv)the inhibitory effect of guava stem bark extract on number of wet stools and (v) the inhibitory effect of guava stem bark extract on weight of wet stool.

**MATERIALS AND METHODS**

**Collection of Plant Material**

The stem bark of *Psidium guajava* were collected locally from the premises of Mrs. Ndidi Okechukwu in Amaba Umudike, Ikwuano Local government area of Abia State, and was identified by Dr. F. N. Ugwuja of the Department of Plant science and Biotechnology in Michael Okpara University of Agriculture, the stem bark of the plant was dried and used for the antimicrobial and antidiarrhea activities.

**ANIMALS**

Adult albino rats of both sexes obtained from the Veterinary Animal Facility of the Veterinary College, Michael Okpara University of Agriculture, Umudike, were used in the study. The animals were housed under standard conditions (25 ± 2˚C and 12hour light/dark cycle). The rats were maintained on standard pellets (Livestock feed®). All animals were allowed unrestricted access to drinking water and was acclimatized for seven days. The use and care of laboratory animals in the study were in accordance with the ethical guidelines as contained in the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (EEC Directive of 1986; 86/609/EEC) and amended in the European Treaty Series (ETS No. 170) of 2005.

**OTHER MATERIALS**

The weighing balance, beakers, glass jar, filter paper were collected from the Plant Science and Biotechnology laboratory while the transparent bucket was bought from market. The loperamide and charcoal meal were bought from Grace and Mercy pharmacy, Umuahia. Pasture pipette, hand glove, cotton wool, plastic petri dishes, methylated spirit, swap stick, ethanol and castor oil were bought from Mr. Jochem Chemical Stores’ Limited.

**PREPARATION OF PLANT EXTRACT**

The identified stem barks were dried at room temperature for two weeks and was grounded into powdered form with a model ED-5 Thomas Wiley Mill machine. 300 g of the dried powdered stem bark was poured into a glass jar and soaked in 1200 ml of ethanol (in the ratio of 1:4) for three days and then filtered with using Whatsman No. 1 filter paper and funnel. The ethanol plant extract was however concentrated under reduced pressure at 400C using rotary evaporator (Buchi R110/NKE6540/2) and was poured into a beaker covered with a perforated foil and placed in the oven at 400C to dry. The yield was recorded.

**ANTIMICROBIAL SCREENING**

**Experimental microorganisms**

The pathogenic micro-organisms used were: *Salmonella typhi, Escherichia coli, Staphylococcus aureus, Shigella dysenteriae* and *Campilobacter jejuni.* The isolates were obtained from the Royal Medical Diagnostics and Research Laboratory, Olokoro Umuahia North local government area in Umuahia Abia State. An extract (1 g) was weighed and dissolved in 1 ml of sterile distilled water to obtain a concentration of 1000 mg/1ml. Then, 1000 mg/1ml was the concentration of the extract used to check the antimicrobial activities of the extracts. The nutrient agar well diffusion technique was used to screen the plant extracts for antimicrobial activities. The microorganism was placed in the petri dishes using the process called seeding and the well was cut at the centre of the plate using a cork borer. A sample of the extract solution was introduced into the nutrient agar well. The nutrient agar plate was incubated (37ºC, 24h) and observed for zone of inhibition.

**DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)**

The minimum inhibitory concentration of the extract was determined by incorporating constant volumes (0.2 ml) of each dilution of the extract into the punch-holes on pre-seeded nutrient agar. An aliquot of the extract 1g was dissolved in 1 ml of sterile distilled water to obtain1000 mg/ml. This 1000 mg/ml concentration was then doubly diluted in sterile distilled water to obtain a concentration of 1000 mg/mg, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml. Incubated at 370C for 24hrs in the incubator. Following the incubation, the diameter of the zone of inhibition was recorded. The minimum inhibitory concentration was determined by comparing the different concentrations of the extract having different zones of inhibition and then selecting the lowest concentration of the extract.

**DETERMINATION OF THE LATHER DOSAGE (LD-50)**

The proposed method was designed by Lorke. (1983) is divided into three stages, with the outcome of each stage determining whether to terminate testing of proceed to the next stage. A confirmatory (confidence) test is used to validate the final test result.

**Phase 1**

In this phase, nine animals were used. The nine animals are divided into three groups of three animals each. Each group of animals are administered different doses (10, 100 and 1000 mg/kg) of test substance. The animals are placed under observation for 24 hours to monitor their behaviour as well as if mortality will occur.

**Phase 2**

In this phase, nine animals were also used, which are distributed into three groups of three animals each. The animals were administered higher doses (1600, 2900 and 5000 mg/kg) of test substances and then observed for 24 hours for behaviour as well as mortality.

**Final confirmatory test**

5000 mg/kg was repeated on another set of three rats still no mortality was recorded but the animals were unstable and could not carry out any activity within 24 hours and a further seven days.

Accurate concentration for inducement was calculated as

Dose × body weight

Concentration × 100

Then the LD50 is calculated by the formula:



D0 = Highest dose that gave no mortality,

D100 = Lowest dose that produced mortality. (Ijioma *et al*. (2019)

**DETERMINATION OF ANTIDIARRHEA ACTIVITY**

**Effect of extract on small intestinal transit time of charcoal meal in rats**

The method used by Ijioma *et al*. (2019) was adopted. Briefly, 20 adult male albino rats assigned to 4 groups of 5 rats each were used. The rats were fasted for 18hrs prior to commencement of the experiment but were allowed free access to water. Group 1 received distilled water and served as the control, group 2 was administered Loperamide (0.5 mg/kg body weight) while groups 3 and 4 received 500 and 1000 mg/kg body weight of the extract respectively. All treatments were administered by the oral route. Thirty minutes after treatments, animals received 1 ml of charcoal meal (10% charcoal suspended in 10% gum acacia) mixed with water orally. The charcoal meal is an anti-monitoring pathway one way of inhibiting diarrhoea. In a further 30 minutes, the animals were sacrificed by cervical dislocation and the small intestine was carefully harvested and its full length measured from the pyloric sphincter to the ileocecal junction. For each animal, the distance travelled by the charcoal meal was also measured and expressed as a percentage of the full length using the relationship below:

Gastrointestinal transit (%) = $\frac{Distance moved by charcoal meal }{whole of length of small intestine}×\frac{100}{1}$

The inhibitory effect of the extract on gastrointestinal transit was calculated relative to the control as:

% inhibition = $\frac{Gastrointestinal transit of control-Gastrointestinal transit of test}{Gastrointestinal transit of control}×\frac{100}{1}$

Or

% inhibition = $\frac{Movement in control-movement in test}{Movement in control}×\frac{100}{1}$

**Effect of extract on castor oil-induced diarrhoea test**

The method described by Ezenwali *et al.* (2009) and Salazar *et al.* (2006) were adopted with little modifications.

**Principle:**

Castor oil has a laxative effect which is mediated by ricinoleic acid, a hydroxylated fatty acid released from castor oil by the intestinal lipase (Tunaru *et al.,* 2012). The liberated ricinoleic acid causes irritation and inflammation of the intestinal mucosa leading to the release of prostaglandins and nitric oxide which stimulate gastrointestinal secretion, motility, epithelial permeability and oedema of the intestinal mucosa (Ezenwali *et al.,* 2009).

Twenty adult male albino rats assigned to 4 groups of 5 rats each were used. The rats were fasted for 18hrs prior to commencement of the experiment but were allowed free access to water. Group 1 received distilled water and served as the control, group 2 was administered Loperamide (0.5 mg/kg body weight) while groups 3 and 4 received 500 and 1000 mg/kg body weight of the extract respectively. All treatments were via the oral route. Thirty minutes after treatments, animals received 1 ml of castor oil orally and were placed individually in a cage with weighed absorbent paper and diarrhoea episode was observed for a period of 3 hours. The parameters recorded included the onset of diarrhoea stool (latent period), the number of both wet and dry stools and weight of the wet stools. All these were measured every 1 hr and the paper changed after each evaluation. The percentage of rats that responded to diarrhoea in each group was calculated. The mean number of stools passed by the treated groups was compared with that of the control and the mean number of diarrhoea faeces pooled by the control group was considered as 100 %.

The percentage inhibition of wet faeces and frequency of stool caused by extract was calculated relative to the control using the relation:

Inhibition of defecation (%) = [(NC - NT)/NC] x 100

Where: NC= mean number of faeces of control group

NT- mean number of faeces of treated group.

The level of reduction (%) in defecation of watery faeces was calculated using the relation:

Inhibition of diarrhoea faeces (%) = [(Nc - NT)/NC] x 10

Where: NC= mean number of diarrhoea faeces of control group;

NT= mean number of diarrhoeic faeces of treated group

**STATISTICAL ANALYSIS**

The data obtained were subjected to both one way and two-way analysis of variance (ANOVA) using Statistical Products and Service Solutions (SPSS) version 22. The means were compared using Duncan multiple range comparison test and statistical significance were established at 95 % confidence level

**Result**

**Acute Toxicity (LD50) and Phytochemical contents of Guava Stem Bark Ethanolic Extract**

Results of the acute toxicity test of the stem bark extract presented in Table 1 showed thatacross the various groups of animals feed with the different concentrations of the stem bark extract no mortality or signs of acute intoxification was recorded except for the highest dose administered (5000 mg/kg) where the animals showed momentary signs of instability but recovered fully with 24 hours. Hence the acute toxicity value was >5000 mg/kg (Table 1). Results of phytochemical studies of *P. guajava* stem bark extract adopted from previous researchers revealed the presence many bioactive chemicals including phenol, tannins, flavonoids and saponins (Adikwu *et al.,* 2022). Other include alkaloid, saponin, flavonoids, resins, terpenoids, steroids, glycosides, anthraquinones, terpenoid. and polysaccharide (Abdullah, 2019; Abdluhamid *et al*., 2014). Phytochemical investigation of *Psidium guajava* stem bark for hematological indices showed that the methanolic extract may possibly serve as a blood booster in an anemic condition or prophylactic purpose.

**Antimicrobial Activities of *Guava* Stem Bark Ethanolic Extract.**

Results of the antimicrobial activities of guava stem bark ethanolic extract (Table 2) revealed that at the highest dose 1000 mg/ml the extract significantly inhibited the enteropathogenic microbes and was comparable to the standard drug Ciprofloxacin which gave the highest inhibition. However, the highest inhibition zone of the stem bark extract on the test organisms was against *S. aureus* which had 39.33±1.16 mm followed by *E. coli*; 31.00±1.00mm, *S. typhi*; 29.00±1.00mm, *C. jejuni;* 25.67±1.53mm and *S. dysenteriae*; 23.33±1.53mm.

 **Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration (MIC) of the guava stem bark extract on the microorganisms with 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml and 62.5 mg/ml dosages (table 2) indicated that not all doses of the extract had inhibition on all the organism. *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus* were inhibited by all the dosages of the extract while *Shigella dysenteriae* and *Campilobacter jejuni* were not affected by the extract concentration at 62.5mg/ml dose. The MIC of these organisms is therefore 125 mg/ml. All the other organisms had MIC of 62.5mg/ml.

**Table 1: Acute toxicity of *Psidium guajava* stem bark ethanolic extract to albino rats**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Group | Number of rats per test | Doses of extract (mg/kg) | Number of deaths | Survival | Mortality ratio |
| 1 |  3 |  10 |  0 |  3 |  0/3 |
| 2 |  3 |  100 |  0 |  3 |  0/3 |
| 3 |  3 |  1000 |  0 |  3 |  0/3 |
| 4 |  3 |  1600 |  0 |  3 |  0/3 |
| 5 |  3 |  2900 |  0 |  3 |  0/3 |
| 6 |  3 |  5000 |  0 |  3 |  0/3 |

**Table 2: Antimicrobial Activity (zone of inhibition) of *Psidium guajava*) stem bark ethanolic extract on some enteropathogenic microbes**

|  |  |  |
| --- | --- | --- |
| Microbes | 1000mg/ml Ciprofloxacin | 1000mg/ml stem bark extract |
| *Salmonella typhi* | 59.00±1.00c | 29.00±1.00b |
| *Escherichia coli* | 50.00±2.00b | 31.00±1.00b |
| *Staphylococcus aureus* | 49.00±1.00b | 39.33±1.16c |
| *Shigella dysenteriae* | 33.67±2.08a | 23.33±1.55a |
| *Campilobacter jejuni* | 36.00±1.00a | 25.67±1.53a |

*Values are presented as mean ± standard deviation (n = 3). Mean on the same column with different letter superscripts are significantly different (P < 0.05).*

**Table 3: Minimum inhibition concentration (MIC) of *Psidium guajava* stem bark ethanol extract on some enteropathogenic microbes**

|  |  |  |
| --- | --- | --- |
| **Microbes** |  **Concentrations** |  |
| **1000****mg/ml *P. guajava* stem bark extract** | **500****mg/ml *P. guajava* stem bark extract** | **250****mg/ml *P. guajava* stem bark extract** | **125****mg/ml *P. guajava* stem bark extract** | **62.5****mg/ml P. *guajava* stem bark extract** |  **MIC (mg/ml)** |
| ***Salmonella typhi*** | 29 | 16 | 13 | 4 | 2 | 62.5 |
| ***Escherichia coli*** | 31 | 16 | 15 | 6 | 3 | 62.5 |
| ***Staphylococcus aureus*** | 39 | 20 | 18 | 7 | 3 | 62.5 |
| ***Shigella dysenteriae*** | 23 | 12 | 6 | 3 | 0 | 125 |
| ***Campilobacter jejuni*** | 25 | 14 | 11 | 4 | 0 | 125 |

**Effects of Guava stem bark ethanol extract on Small Intestine Transit Time of Charcoal Meal in Rats:**

The effect of *P. guajava* stem bark extract on charcoal meal transit shown in Table 4 revealed significant reduction (p<0.05) in distance travelled, percentage movement, and percentage inhibition of the charcoal meal in all the groups treated with the extract (500 mg/kg and 1000 mg/kg) and Loperamide compared with the control. Percentage inhibition of charcoal movement in the control group and Loperamide (0.00±0.00 and19.39±0.96%) were significantly lower than 25.70±0.72 and 22.04±2.33% obtained with 500 mg/kg and 1000 mg/kg respectively. It is pertinent to note that the best % inhibition of charcoal meal transit in the rats’ small intestines was achieved not with the standard drug Loperamide but with 500 mg/kg extract

**Table 4: Effects of *Psidium guajava* stem bark ethanol extract on charcoal meal transit in rats**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Treatments | Length of intestine (cm) | Distance travelled (cm) | Percentage movement of charcoal meal (%) | Percentage inhibition of charcoal meal transit (%) |
| Control | 94.20±2.78ab | 78.20±2.95b | 82.99±3.13c | 0.00±0.00a |
| 0.5 mg/kgLoperamide | 88.40±4.28a | 59.60±7.30a | 73.44±8.41b | 19.36±0.96b |
| 500 mg/kg Guavastem bark extract | 105.40±7.64c | 65.70±7.60a | 62.18±3.25a | 25.70±0.72d |
| 1000 mg/kg Guava stem bark extract  | 96.40±3.21b | 63.40±5.98a | 65.88±7.91a, b | 22.04±2.33c |

*Values are presented as mean ± standard deviation (n = 5). Mean on the same column with different letter superscripts are significantly different (P < 0.05).*

**Table 5: Effect of *P. guajava* stem bark ethanol extract on percentage inhibition and total weight of weight stool from rats three hours after induction of diarrhea**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatments | Weight of wet stool 1hr | Weight of wet stool 2hr | Weight of wet stool 3hr | Total weight of wet stool | % Inhibition of weight of wet stool |
| Control | 2.40±0.16d,3 | 0.88±0.23b,1 | 1.58±0.16b,2 | 4.86±0.53d | 0.00±0.00a |
| Loperamide 0.5 mg/kg | 0.32±0.13a,1 | 0.46±0.11a,1 | 0.00±0.00a,1 | 0.78±0.18a | 67.89±1.55c |
| Guava stem bark 500 mg/kg | 1.62±0.13c,2 | 0.46±0.11a,1 | 0.00±0.00a,1 | 2.12±0.19b | 53.45±1.56b |
| Guava stem bark 1000 mg/kg | 0.82±0.08b,1 | 0.86±0.17b,1 | 2.30±0.20c,2 | 4.18±0.44c | 52.57±0.95b |

*Values are presented as mean ± standard deviation (n = 5). Mean on the same row with different number superscripts are significantly different (P < 0.05) while means on the same column with different letter superscripts are significantly different (P < 0.05).*

**DISCUSSION**

Results from the present study have demonstrated the invaluable and unique characteristics of guava stem bark ethanol extract in terms of acute toxicity, antimicrobial activity, minimum inhibitory activity and antidiarrhea activity.

The acute toxicity test (LD50) of the plant extract was found to be safe as no sign of toxicity was observed in the acute oral toxicity test at the limit dose of 5000 mg/kg in rats. In addition, during the seven days post treatment period no mortality and delayed toxicity was observed across all the tested doses. These results corroborate the findings of Manekeng *et al.* (2019) who reported that the median lethal dose (LD50) of *P. guajava* stem bark extract was greater than 5000 mg/kg body weight. Okere and Iliemene (2014) obtain similar results while working on anti-diarrhoea property of crude aqueous leaf extract of *P. guajava*. This implies that the oral acute toxicity value of the extract is greater than 5000 mg/ kg reveals and the extract can be safely administered in the management of animal or human infections without lethal effects as no toxicity sign was perceived in the rats orally administered with the extract at the limit dose of 5000 mg/kg. Jaganathan *et al.* (2012) opined that if the LD50 value of a test substance is more than 3 times its minimum effective dose such substance is considered a good candidate for further investigation. The findings of the present study showed that the LD50 of guava stem bark ethanol extract is more than three times its minimum effective (62.5 mg/ml) dose. This therefore, proves that the extract is tolerable and safe for oral administration, validating it’s use in folk medicine.

 Results also revealed significant growth inhibition of the enteropathogenic isolates by guava stem bark extract implying that the extract contained some bioactive substances (secondary metabolites which might be responsible for the antimicrobial activity as was revealed by previous researchers (Adikwu *et al*., 2022; Manekeng *et al* 2019., Abdullah *et al* 2019). Esimone *et al.,* (2012) in their study showed that the phytochemical constituents of guava stem bark are carbohydrates, glycosides, tannins, and proteins. The guava stem bark was also reported by Irondi (2019) to contain ellagic acid and quercetin as the most abundant phenolic acid and flavonoids. The extract from the stem bark was reportedly used as a source of nutraceuticals for suppressing the production of uric acid, ammonium hydroxide, fatty acids and cholesterol, and mitigating oxidative stress. Irondi, (2019) opined that these bioactivities, which can be attributed to the phenolic compounds, suggest anti-nephrolithiatic and anti-obesity potentials of guava stem bark phenolics-rich extract.

The antimicrobial activity of guava stem bark which is ultimately related to its antidiarrhea activity was previously illustrated by researcher in different studies (Nair and Chanda, 2007; Jaiarj *et al.,* 1999; Barbalho *et al.,* 2012 and Morais-braga *et al.,* 2016). In addition to the present activity, it has been proven to have a plethora of other biological activities such as hepatoprotective (Roy *et al.* 2006), antioxidant (Jimenez-Escig *et al.,* 2001), and anticancer (Mbaveng *et al.,* 2018). However, the standard antibiotics (Ciprofloxacin) showed higher antimicrobial activity against all the isolates than the guava stem bark extract. This may be due to the crude nature of the extract which may contain significant amounts of impurities capable of limiting the amount and action of the active ingredients such as ellagic acid and quercetin (Irondi, 2016) compared to the standard drug which have been processed and purified.

 The antimicrobial activity of the stem bark was in the order *S. aureus* >*E. coli* >*S. typhi* >*C. jejuni* >*S. dysenteriae* indicating highest inhibition against *Staphylococcus aureus*. These findings are in tandem with the results obtained by Adikwu *et al*.(2022) who studied the antibacterial activity of *P. guajava* stem bark on selected bacteria and reported that *S. aureus* was the most susceptible of all the tested isolates. Thus, the differential sensitivity of the stem bark extract on the isolates suggests that the organisms’ response to inhibitory substances varies based on their innate genetic make-up.

The MIC of the guava stem bark extract depicted significant inhibition on the clinical isolates at varying concentration: *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus* at minimal dosage of 62.5 mg/ml while *Shigella dysenteriae* and *Campilobacter jejuni* were affected by all the extract doses except 6.25mg/ml. This suggests that the efficacy of the extract is dose dependent and attributed to differences in concentrations of bioactive compounds in each dose (Ayienda 2019). The insignificant effect of the extract at lower dosages on the *S. dysenteriae and C. jejuni* may be attributed to differential susceptibility of the isolates to chemicals in dosage dependent pattern, genetic variation or the inherent nature of the organism which differs from one organism to another.

Significant reduction (p<0.05) in distance travelled, percentage movement, and percentage inhibition of the charcoal meal in the extract (500 mg/kg and 1000 mg/kg) and Loperamide-treated groups compared to the control reflect the ability of the extract and Loperamide to delay the onset of diarrhoea in rats. Specifically, the relative distance travelled by charcoal meal and the percentage inhibition of charcoal movement in the extract groups correlate reduction in bowel movement by the extract. It is pertinent to note that the highest inhibition of charcoal meal transit in the rats’ small intestines was achieved by the extract at 500 mg/kg but this did not differ comparably with that of the standard drug Loperamide at 0.5 mg/kg. However, the inhibitory impact of the Loperamide in the reduction of the distance travelled by the charcoal meal at a very little concentration of 0.5 mg/kg is worthy of note. Hence 500 mg/kg crude stem bark extract of *P. guajava* can be said to be equivalent to 0.5 mg/kg Loperamide in anti-diarrhoea activity.

Results revealed that a lower dosage of the guava stem bark extract (500mg/kg) showed higher inhibition compared to a higher dose 1000 mg/kg. These finding are worthy of further investigation to determine if there are ample number of inhibitors against the active constituents in the crude extract responsible for the antimotility effect in higher doses.

Diarrhoea is marked by frequent discharge of semi-solid or watery faecal matter from the bowels three or more times per day (Dosso *et al.,* 2012) involving, according to Ezekwesili *et al.* (2010), an increase in the fluidity, volume and frequency of bowel movements, abdominal pain accompanied by increased secretion and decreased absorption of fluid and thus the loss of water and electrolytes. The significant decreased (p<0.05) in the frequency and amount of wet stool output recorded in the diarrheal-induced rats furthermore demonstrate the anti-diarrheal activity of this extract. This followed the same trend as in the charcoal meal transit of the motility study as 500 mg/kg of the extract caused significant decline in frequency of wet stool output, total weight of wet stool and percentage inhibition of wet stool after three hours induction with diarrhoea. In both models, the activities of the guava stem bark extract at 500 mg/kg were higher than those of the Loperamide although they compared favourably, whereas no inhibition was recorded in the distilled water treatment.

 Thus, at high dosage the plant has a potential antidiarrheal activity, which may serve as a template in the development of a novel anti-diarrheal drug. This can be as a result of the numerous phytochemicals such as flavonoids which have the ability to inhibit intestinal motility and hydro electrolytic secretions (Venkatesan *et al.,* 2005).

**Conclusion**

 In conclusion, this study observed that the ethanolic extract of *P. guajava* at 500mg/kghas no significant (P<0.05) difference to the standard drug Loperamide in anti-diarrhoea activity. It is an indication that the extract possesses anti-diarrhoea potentials. The extract was found to be non-toxic and its median lethal dose (LD50) was greater than 5000 mg/kg. Following the above findings, the extract is safe and effective in the treatment of diarrhoea. These findings further suggest that guava stem extract may be an effective treatment for specific and non-specific diarrhoea given its ability to significantly inhibit the growth of some enteropathogenic organisms associated with diarrhoea, the motility of charcoal, delay the onset of diarrhoea and reduce the frequency and amount of weight stool in induced-diarrheal rats.

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