ANTIMICROBIAL ACTIVITY AND ANTI-DIARRHEAL POTENTIALS OF *PSIDIUM GUAJAVA* LINN LEAF EXTRACT IN EXPERIMENTAL RAT MODELS

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ABSTRACT

This study evaluated the antimicrobial activity and antidiarrheal potentials of guava leaf ethanol extract (GLEE). GLEE prepared from dried guava leaves was first subjected to acute toxicity (LD_{50}) test. Antimicrobial activity of GLEE was tested in vitro against some enteropathogenic organisms including Escherichia coli, Cympylobacter jejuni, Salmonella typhi, Shigella dysenteriae and Staphylococcus aureus. The zone of inhibition for each GLEE application was determined using Agar well diffusion techniques, while the minimum inhibitory concentration (MIC) was determined by doubling dilution technique compared with Ciprofloxacin. The anti-diarrheal effect of GLEE was evaluated using charcoal meal transit and castor oil induced diarrhea models in rats. For each model, 36 Wistar rats assigned 4 groups of 3 rats, replicated thrice and treated according to the order: group 1(control), group 2(0.5 mg/kg Loperamide), groups 3 and 4 (500 and 1000 mg/kg of GLEE respectively) were used. Results obtained indicated an LD_{50} value >5000 mg/kg for GLEE. GLEE also significantly inhibited microbial growth in concentration dependent pattern ($p<0.05$) with the highest concentration producing inhibition zones measuring 9, 13, 14, 17 and 17 mm against S. dysenteriae, S. aureus, S. typhi, E. coli and C. jejuni respectively. In the castor oil induced diarrhea model, GLEE significantly reduced frequency and weight of wet stool output in rats, and also inhibited charcoal meal transit significantly in the motility study. In both cases, the activities of GLEE compared favourably with that of Loperamide, the standard drug used. These results therefore justify the local use of guava leaf for the treatment of diarrhea.

Keywords: *Psidium guajava*, Albino rats, Antimicrobial, Antidiarrhea, Ethanol extract

INTRODUCTION

Diarrhea and its associated complications remain a major cause of morbidity and mortality in children, especially in developing countries of Sub-Saharan Africa and Southern Asia (Liu *et al.,* 2016). The fact that diarrhea is a major public health problem in Nigeria is well established (Ezekwesili *et al.,* 2010). The disease which is known to be the second most common cause of death in children under five years of age is known to be responsible for 2.4 million deaths globally each year (Forsberg *et al.*, 2007; Haque *et al.,* 2013). During diarrhea,

there is hyper peristalsis of the small intestine or colon leading to loss of large amounts of body fluid and electrolytes (Na^+ and K^+) and causing dehydration, shock and eventual cardiovascular collapse (Ezekwesili *et al.,* 2010). Diarrhea is characterized by an increased frequency and volume, and decreased consistency of stool from the norm, with higher frequency of stooling in children (Guarino *et al.,* 2008). Persistent diarrhea occurs when the duration of symptoms exceeds seven days and chronic diarrhea when it lasts more than 14 days (Guarino *et al.,* 2008).

The worldwide emergence and proliferation of human pathogenic bacteria have become a major health problem (Jones *et al.,* 2008). Virulent strains of *Escherichia coli* and *Shigella* spp. result in gastroenteritis, hemorrhagic colitis, neonatal meningitis, urogenital infections and Crohn's disease (Abernethy *et al.,* 2017; Bruyand *et al.,* 2018). *E. coli* strain produces Shiga toxin which causes inflammatory responses resulting in bloody diarrhea (Lupindua, 2018). These infections lead to urinary tract complications (Bien *et al*., 2012) which can result to severe abdominal cramps, vomiting and fever. It has been estimated that 70 % of diarrhea disease in children younger than 5 years are caused by microbial infections (Anderson *et al.,* 2019).

The causes of persistent diarrhea in populations are poorly understood, and in individuals often unknown. Some pathogens such as *Shigella dysenteriae* (Bai *et al.,* 2004), *Staphylococcus aureus* (Abba *et al.,* 2009), *E. coli* (Abba *et al.,* 2009), *Salmonella typhi* (Qu *et al.,* 2012) and *Campylobacter jejuni* (Shen *et al.,* 2016) are associated with persistent diarrhea in various locations. These enteric organisms alter the movement of ions and water which follows an osmotic gradient through transporters or the lateral spaces between cells (Hodges and Gill, 2010) resulting to diarrhea. Some of the clinically used drugs are ineffective and have led to emergence of resistant bacterial pathogens. In the view of these setbacks, there is a great need to develop antibacterial agents that are effective, affordable, and easily available with less side effects as alternative medicines. Alternative and complementary herbal medicines are safe and effective drugs useful in treating various disorders. They are thus the preferred option to replace or complement conventional synthetic drugs (Hosseinzadeh *et al.,* 2015).

Management strategies against diarrhea have not yielded desired outcome, despite efforts that have been made in terms of drug development and diverse public health campaigns (Abdela, 2019). Alternative treatment sources are needed because majority of affected individuals do not have access and cannot afford these orthodox medicines. This may be the reason for the ongoing promotion and global support for herbal medicines (Ekor, 2014).

The use of medicinal plants in Nigeria to treat diseases has been an age long practice (Sofowora *et al.,* 2013; Ekor, 2014). *Psidium guajava* Linn. (Myrtales: Myrtaceae) popularly known as common guava is only one of the numerous plants that have healing virtues and is therefore been scientifically investigated for better medicinal applications (Daswani *et al.*, 2017; Díaz-de-Cerio *et al.,* 2017; Naseer *et al.*, 2018). It is a small tree native to tropical areas from Southern Mexico to Northern South America. Guava trees have been grown by many other countries having tropical and subtropical climates, thus allowing production around the world (Salazar *et al.,* 2006). Traditionally, preparations of the leaves have been used in folk medicine in several countries, mainly as anti-diarrhea remedy (Gutiérrez *et al.,* 2008). Moreover, other several uses have been described elsewhere on all continents, with the exception of Europe (Dakappa *et al.,* 2013; Morais-Braga *et al.,* 2016).

The guava leaf in folk medicine is believed to have active components that help to treat and manage various diseases (Biswas *et al.,* 2013). Extract from the leaves have been used for the controlling of life-changing conditions such as diabetes (Abdelrahim *et al.,* 2002; Sunagawa *et al.,* 2004), hypertension (Babatola and Oboh, 2021) and obesity (Sunagawa *et al.,* 2004). The consumption of decoction, infusion, and boiled preparations of guava leaves is the most common way to overcome several disorders, such as rheumatism,

diarrhea, diabetes mellitus, and cough (Dakappa *et al.,* 2013), while the decoction is used as gargle for mouth ulcers (Salazar *et al.,* 2006) and as anti-bacteria (Sanda *et al.,* 2011). These activities are due to groups of secondary metabolites including but not limited to tannins, alkaloids, flavonoids, saponins and glycosides present in the extract as has been reported by many authors (Gutiérrez *et al*., 2008; Metwally *et al.,* 2010; Okere and Iliemene, 2014; Naseer *et al*., 2018).

Owing to the claims of efficacy of the leaves of *P. guajava*, this study aimed at scientifically evaluating the antimicrobial activity and antidiarrheal effect of GLEE on albino rat. The specific objectives of the study were to determine: (i) the zone of inhibition of GLEE against the organisms, (ii) the minimum inhibitory concentration (MIC) of GLEE on *E. coli, C. jejuni, S. typhi, S. dysenteriae* and *S. aureus,* (iii) the effect of GLEE on charcoal meal transit, (iv) the inhibitory effect of GLEE on number of wet stool and (v) the inhibitory effect of GLEE on weight of wet stool.

MATERIALS AND METHODS

Collection of Materials: The leaves of *P. guajava* were collected locally from an orchard in Amaba Umudike, Ikwuano Local Government Area of Abia State, and were identified at the taxonomy unit of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, where voucher specimen (MOH0050) was deposited at the Departmental herbarium.

Preparation of Plant Extract: The plant materials were shade air-dried to a constant weight and pulverized into powdered in a model ED-5 Thomas Wiley Mill machine. 150 g of the dried powdered leaves was soaked in 450 ml of 96 % ethanol for 48 hours and then filtered with Whatman No. 1 filter paper. The extract in solution was then concentrated under reduced pressure at 40^0C using rotary evaporator and allowed to finally dry in a hot air oven maintained at 40^0C to dry. A crude extract which weighed 6.98 g and represented 8.73 % extract yield was obtained. The extract was preserved under refrigeration (4 ± 2^{o}C) pending use.

Animals: Forty two (42) adult male albino rats obtained from the Veterinary Animal Facility of the Veterinary College, Michael Okpara University of Agriculture, Umudike, were used for the study. The animals were housed under standard conditions (25 ± 2˚C and 12 hour light/dark cycle). The rats were maintained on standard pellets (Finisher mash, Chikkun Feeds, Nigeria, with crude protein of 19.90 % and metabolizable energy of 3209.64 Kcal). All animals were allowed unrestricted access to drinking water. Guidelines for laboratory animal use and care as prescribed by 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 were strictly followed (Council of Europe, 1986).

Microbial Cultures: The entheropathogenic micro-organisms used in the study were: *S. typhi, E. coli, S. aureus, S. dysenteriae* and *C. jejuni*. The isolates were obtained from the Royal Medical Diagnostics and Research Laboratory, Olokoro, Umuahia North Local Government Area, Abia State, Nigeria.

Antimicrobial Screening of Guava Leaf Ethanolic Extract: The nutrient agar well diffusion technique was used to screen GLEE for antimicrobial activities. Wells were created on solidified media in plates using cork borer and the microorganisms were placed in the Petri dishes using the process called seeding (Linthoingambi and Singh, 2013). Sample of given concentration of GLEE was introduced into the nutrient agar well. The nutrient agar plate was incubated (37ºC, 24 hours) and observed for zone of inhibition (Omodamiro and Ajar, 2017).

Determination of Minimum Inhibitory Concentration: To determine the MIC, an aliquot of GLEE (1 g) was dissolved in 1 ml of sterile distilled water to obtain 1000 mg/ml. This 1000 mg/ml concentration was then doubly

diluted in sterile distilled water to obtain a concentration of 500, 250, 125 and 62.25 mg/ml and constant volumes (0.2 ml) of each dilution of GLEE was incorporated into the punch-holes on pre-seeded nutrient agar and incubated at 370°C for 24 hours. Following the incubation, the diameter of the zone of inhibition was recorded. The MIC was determined by comparing the different concentrations of GLEE having different zones of inhibition and then selecting the lowest concentration of GLEE.

Determination of Lethal Dose (LD_{50}): The method used by Lorke (1983) was adopted with little modification. Briefly, three stages of tests were involved with the outcome of each stage determining whether to terminate testing of proceed to the next stage. A confirmatory (confidence) test was used to validate the final test result. In the first stage, nine albino rats divided into three groups of three animals each were administered 10, 100 and 1000 mg/kg of GLEE respectively via the oral route. The animals were thereafter placed under observation for 24 hours to monitor their behavior as well as to record mortalities. The test proceeded to the second stage when no mortality was observed after 24 hours. In the second stage, another set of nine rats assigned to three groups of three rats were administered 1600, 2900 and 5000 mg/kg of GLEE respectively and were also observed for toxicity signs within 24 hours. When no mortality was recorded, 5000 mg/kg was repeated on another set of three rats at the confirmatory test stage. These rats were also observed for 24 hours and a further seven days. LD_{50} value for GLEE was calculated using the formula: $LD_{50} = \sqrt{(D_0 \times D_{100})}$, where D_0 = highest dose that gave no mortality and D_{100} = Lowest dose that produced 100 % mortality (Lorke, 1983).

Phytochemical Content of Guava Leaf Ethanolic Extract: The phytochemical contents of guava leaf ethanolic extract were adopted from the studies of Metwally *et al.* (2010), Okere and Illiemene (2014) and Naseer *et al*. (2018).

Effect of Guava Leaf Ethanolic Extract on Small Intestinal Transit Time of Charcoal Meal in Rats: The methods used by Mascolo *et al*. (1994) and Rahman *et al.* (2012) were adopted. Briefly, 36 adult male albino rats assigned to four treatment groups, with each group replicated thrice and each replicate having three rats in a completely randomized design was employed. The rats were fasted for 18 hours 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 GLEE 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 GLEE is an anti-monitoring pathway (one way of inhibiting diarrhea). In a further 30 minutes, the animals were humanely sacrificed 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 (%) = Distance moved by charcoal meal ÷ Total length of the intestine x 100. The inhibitory effect of GLEE on gastrointestinal transit was calculated relative to the control as: % inhibition = Gastrointestinal transit of control – Gastrointestinal transit of test ÷ Gastrointestinal transit of control x 100 (Mascolo *et al*., 1994; Rahman *et al.*, 2012).

Effect of Guava Leaf Ethanolic Extract on Castor Oil-Induced Diarrhea Test: 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.,* 2010).

The methods described by Ezenwali *et al.* (2010) and Saralaya *et al.* (2010) were adopted with little modifications. Thirty six adult male albino rats assigned to four treatment groups with three replicates each having three rats per replicate in a completely randomized design were used. The rats were fasted for 18 hours 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 GLEE 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 diarrhea episode was observed for a period of 3 hours. The parameters recorded included the onset of diarrhea stool (latent period), the number of both wet and dry stools and weight of the wet stools. All these were measured every 1 hour and the paper changed after each evaluation. The percentage of rats that responded to diarrhea 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 diarrhea faeces pooled by the control group was considered as 100 %.

The percentage inhibition of wet faeces and frequency of stool caused by GLEE was calculated relative to the control using the relation: Inhibition of defecation (%) = $[(N_C - N_T)/N_C] \times 100$, where: N_C= mean number of faeces of control group, N_T- mean number of faeces of treated group. The level of reduction (%) in defecation of watery faeces was calculated using the relation: Inhibition of diarrhea faeces (%) = $[(N_c - N_T)/N_C] \times 10$, Where: N_C= mean number of watery stools in control group and N_T= mean number of watery stools in treated group (Mascolo *et al.*, 1994; Rahman *et al.*, 2012).

Statistical Analysis: The data obtained were subjected to 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.

RESULTS

Phytochemical Contents and Acute Toxicity (LD_{50}) of Guava Leaf Ethanolic Extract: Antioxidants and secondary metabolites including essential oils, polysaccharides, minerals, vitamins, enzymes, terpinene, triterpenoid acids, alkaloids, steroids, glycosides, tannins, flavonoids, saponins, carbohydrate, cardiac glycosides, alpha-pinene, beta-pinene, limonene, quercetin, quercetin-3-O-∞-L-arabinofuranoside, and quercetin-3-O-β-D-arabinofuranoside are present in guava leaf ethanolic extract.

No mortality was recorded across the groups and all stages of the test, even at the highest dose administered (5000 mg/kg). The animals were physically stable and did not show any obvious sign of toxicity except animals administered 5000 mg/kg which were physically unstable for a moment but recovered fully within 24 hours. Acute toxicity value was therefore found to be >5000 mg/kg (Table 1).

Antimicrobial Activities of Guava Leaf Ethanolic Extract: The antimicrobial activities of 1000 mg/ml of GLEE and standard drug are presented in Table 2. GLEE significantly inhibited microbial growths *in vitro* although effect observed against each microbial agent was lower than the effect produced by corresponding dose of the standard drug (ciprofloxacin).

Minimum Inhibitory Concentration of Guava Leaf Ethanolic Extract: The MIC of GLEE showed that the MIC of GLEE on *S. typhi* was 250 mg/ml which was obtained as 3 mm. There was no inhibition when 125 and 62.5 mg/ml were administered. The MIC on *E. coli* was also 250 mg/ml, while the MIC on *S. aureus* was 125 mg/ml. The MIC on *S. dysenteriae* and *C. jejeuni* were 250 mg/ml respectively (Table 3).

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

Group	Number of rats per test	Doses of extract (mg/kg)	Number of death	Survival	Mortality ratio
1	3	10	0	3	0/3
2	3	100	0	3	0/3
3	3	100	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 activities (zone of inhibition) of *Psidium guajava* ethanol extract on enteropathogenic microorganisms

Microorganisms	1000 mg/ml ciprofloxacin	1000 mg/ml of *P. guajava* leaf extract
Salmonella typhi	59.00 ± 1.00^{c}	$14.67 \pm 0.58^{b,c}$
Escherichia coli	36.00 ± 1.00^{a}	$17.00 \pm 0.58^{b,c}$
Staphylococcus aureus	33.67 ± 2.08^{a}	13.67 ± 3.22^{b}
Shigella dysenteriae	49.00 ± 1.00^{b}	9.00 ± 1.00^{a}
Cympylobacter jejuni	50.00 ± 2.00^{b}	17.00 ± 1.00^{c}

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

Table 3: Minimum inhibitory concentration (MIC) of *Psidium guajava* ethanol extract on some enteropathogenic microorganisms

Microorganisms	1000 mg/ml of *P. guajava* leaf extract	500 mg/ml of *P. guajava* leaf extract	250 mg/ml of *P. guajava* leaf extract	125 mg/ml of *P. guajava* leaf extract	62.25 mg/ml of *P. guajava* leaf extract	MIC (mg/ml)
Salmonella typhi	14.6	7	3	0	0	250
Escherichia coli	17	7.6	4.3	0	0	250
Staphylococcus aureus	13.6	5.6	5	2	0	125
Shigella dysenteriae	9	10	5.6	0	0	250
Cympylobacter jejuni	17	7.3	2.6	0	0	250

The Effect of Guava Leaf Ethanolic Extract on Small Intestine Transit Time of Charcoal Meal in Rats: The effect of GLEE on small intestinal charcoal transit presented in Table 4 indicated a significant reduction ($p<0.05$) in charcoal meal transit in all groups treated with GLEE when compared with control. Percentage inhibition of movement of charcoal meal in control was 0.00 ± 0.00 but for the groups treated with 500 and 1000 mg/kg of GLEE, percentage inhibition of movements were 22.37 ± 1.02 and 20.57 ± 2.69 % respectively. The observed anti-motility effect of GLEE also compared favorably with that of the standard drug (Loperamide) used.

Effect of Castor Oil-Induced Diarrhea on the Weight of Wet Stool: GLEE also significantly lowered ($p<0.05$) the weight of wet stools in GLEE treated groups when compared with the control. Percentage inhibitions of 31.71 ± 1.03 and 77.37 ± 2.15 % were obtained for 500 and 1000 mg/kg respectively, and this compared favourably with Loperamide (0.5 mg/kg) which had a percentage inhibition of 82.38 ± 1.15 % (Table 5).

Effect of Guava Leaf Ethanolic Extract on Castor Oil-Induced Diarrhea on the Frequency of Wet Stool: Pattern of results obtained for the frequency of wet stool was similar to that which was obtained for wet stool weights.

Table 4: Effect of *Psidium guajava* ethanol extract on charcoal meal transit in rats

Treatment	Length of intestine (cm)	Distance travelled (cm)	% Movement of charcoal meal	% Inhibition of charcoal movement
Control	94.20 ± 2.78^{b,c}	78.20 ± 2.95^{b}	82.99 ± 3.13^{b}	0.00 ± 0.00^{a}
0.5 mg/kg Loperamide	88.4 ± 4.28^{a}	57.60 ± 5.60^{a}	67.44 ± 8.36^{a}	19.36 ± 0.96^{b}
500 mg/kg of Guava leaf extract	95.00 ± 3.54^{b}	61.80 ± 6.14^{a}	64.99 ± 5.35^{a}	22.37 ± 1.02^{c}
1000 mg/kg of Guava leaf extract	90.20 ± 2.17^{a,b}	62.60 ± 6.88^{a}	69.38 ± 7.51^{a}	20.57 ± 2.69^{b,c}

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

Table 5: Effect of *Psidium guajava* ethanol extract on percentage inhibition and total weight of wet stool from rats three hours after induction of diarrhea

Treatments	Weight of wet stool			Total weight of wet stool	% inhibition of weight of wet stool
	After 1 hour	After 2 hours	After 3 hours		
Control	2.40 ± 0.16^{c3}	0.88 ± 0.23^{b1}	1.58 ± 0.16^{c2}	4.86 ± 0.53^{c}	0.00 ± 0.00^{a}
0.5 mg/kg Loperamide	0.32 ± 0.13^{a2}	0.46 ± 0.11^{a3}	0.00 ± 0.00^{a1}	0.78 ± 0.11^{a}	82.38 ± 1.15^{d}
500 mg/kg Guava leaf extract	2.04 ± 0.11^{b3}	0.44 ± 0.10^{a1}	0.98 ± 0.38^{b2}	3.26 ± 0.18^{b}	31.71 ± 1.03^{b}
1000 mg/kg Guava leaf extract	0.34 ± 0.11^{a12}	0.48 ± 0.11^{a2}	0.26 ± 0.09^{a1}	1.08 ± 0.18^{a}	77.37 ± 2.15^{c}

Values are presented as mean ± standard error (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)

GLEE significantly lowered (p<0.05) the frequency of wet stools in GLEE treated groups when compared with control causing percentage inhibitory activities of 54.33 ± 2.63 and 63.92 ± 1.07 % for 500 and 1000 mg/kg respectively, and compared favourably with Loperamide (0.5 mg/kg) which had a percentage inhibitory activity of 72.28 ± 2.97 % (Table 6).

DISCUSSION

GLEE has shown significant inhibitory activity against the enteropathogenic organisms in this study implying that some phytochemicals present in the extract may be responsible for the antimicrobial effects as was revealed by earlier studies (Okere and Iliemene, 2014; Naseer *et al.*, 2018). The authors demonstrated that the leaf extract of *P. guajava* contained tannins, saponins, carbohydrates and cardiac glycosides among others which might have conferred antidiarrheal effect on it. Another remarkable implication of the present results is that guava leaf extract has the potential to reverse diarrhoea caused by bacterial pathogens especially the ones used in this study. Naseer *et al.*(2018) in their study on phytochemistry and medicinal value of *P. guajava* reported the presence of antioxidants and secondary metabolites including essential oils, polysaccharides, minerals, vitamins, enzymes, triterpenoid acids, alkaloids, steroids, glycosides, tannins, flavonoids and saponins. Similarly, the phytochemical analysis of aqueous extract of *P. guajava* leaves (Okere and Illiemene, 2014) revealed the presence of high concentration of tannins, carbohydrate, cardiac glycosides and flavonoids as well as moderate concentration of alkaloids. Naseer *et al.* (2018) attributed fungicidal and bactericidal activities of leaf extract of *P. guajava* to the presence of specific compounds like quercetin, alpha-pinene, beta-pinene and limonene, among others.

Table 6: Effect of *Psidium guajava* ethanol extract on percentage inhibition and total number of wet stool from rats three hours after induction of diarrhea

Treatments	Number of wet stool			Total number of wet stool	% Inhibition of number of wet stool
	After 1 hour	After 2 hours	After 3 hours		
Control	2.60 ± 0.55^{c1}	3.60 ± 1.14^{b1}	5.40 ± 0.41^{c2}	11.60 ± 0.89^{c}	0.00 ± 0.00^{a}
0.5 mg/kg Loperamide	1.00 ± 0.00^{a2}	2.40 ± 0.05^{a3}	0.00 ± 0.00^{a1}	3.40 ± 0.55^{a}	72.28 ± 2.97^{d}
500 mg/kg Guava leaf extract	1.80 ± 0.84^{b12}	2.20 ± 0.37^{a2}	1.00 ± 0.00^{b1}	5.00 ± 0.71^{b}	54.33 ± 2.63^{b}
1000 mg/kg Guava leaf extract	1.00 ± 0.00$_{a1}$	1.80 ± 0.22^{a1}	1.40 ± 0.55^{b1}	4.20 ± 0.10^{ab}	63.92 ± 1.07^{c}

Values are presented as mean ± standard error (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)

Metwally *et al.* (2010) also ascribed the antimicrobial activities of this extract to the presence of quercetin, quercetin-3-O-∞-L-arabinofuranoside, and quercetin-3-O-β-D-arabinofuranoside among others.

In this study the standard antibiotics (Ciprofloxacin) significantly showed higher antimicrobial activity against all the isolates than GLEE. The low antimicrobial activities of GLEE on the isolates compared to Ciprofloxacin can be as a result of the unpurified nature of GLEE, thus the presence of impurities can hinder the full action of the active ingredients like quercetin, alpha-pinenes, beta-pinenes, limonene and menthol which are reported to be presence in leaf extract (Metwally *et al.*, 2010; Naseer *et al.*, 2018). The antimicrobial activities of GLEE on *S. typhi, E. coli, S. aureus, S. dysenteriae* and *C. jejuni* showed that GLEE had significant inhibitory activity on the organisms. This may attributed to the presence of essential phytochemicals such as alkaloids, flavonoids, terpenoids and saponins in the guava leaves (Farag *et al.,* 2020). Similar findings have been earlier reported by Metwally *et al.* (2010) on the antimicrobial activity of guava leaves on *S. aureus, E. coli* and *P. aeruginosa*.

GLEE showed the highest activity on *E. coli* and *C. jejuni* and the lowest inhibition on *S. dysenteriae*. The antimicrobial activity of GLEE was effective against the isolates in order *E. coli* > *C. jejuni* > *S. typhi* > *S. aureus* > *S. dysenteriae*. The highest activity shown by GLEE on *E. coli* corroborates the findings of Metwally *et al.* (2010), while the differential sensitivity of the other organisms may be attributed to the varied presence and quantities of the different phytochemicals extracted by the solvent (ethanol) used in this study. Naseer *et al.* (2018) reported that methanol extract of leaves of *P. guajava* produced high antimicrobial activity against *S. aureus*, *Bacillus* and *Salmonella* bacteria, while aqueous and ethanol extracts had low antimicrobial activity. These authors opined that the high activity shown by methanol extract of *P. guajava* leaves was due to the presence of active flavonoid compounds (terpinene and pinene). The antimicrobial activity of GLEE showed that GLEE may be a better antimicrobial agent in cases of emergency treatment, where the cause of the infection is not known and in cases of resistance to conventional antibiotics.

The MIC of GLEE showed that GLEE had significant effects on all the clinical isolates at 1000 mg/ml, 500 mg/ml and 250 mg/ml. At 125 mg/ml, the GLEE was slightly effective on *S. aureus.* The difference in MIC among the concentrations of GLEE tested may be due to the differences in concentrations of the active phytochemicals constituents that made up each concentration of GLEE, the susceptibility of the isolates to GLEE' constituent in a concentration dependent manner and on the inherent nature of the organisms as previously reported by Oncho *et al.* (2021). The insignificant effect of GLEE on the isolates at lower dosages may be attributed to the insignificant level of the active components at such doses; hence the impurities

can prevent the active inhibition of GLEE in small quantity.

With regard to acute toxicity test, the plant GLEE 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. At the test dose, mortality and delayed toxicity were not observed in the 7 days post treatment period. Based on the findings of the oral acute toxicity test, the LD_{50} value of GLEE is above 5000 mg/kg. Generally, if the LD_{50} value of the test chemical is more than 3 times of its minimum effective dose, the substance is considered as a good candidate for further investigation (Jaganathan *et al.,* 2012). Therefore, the present finding implies that the LD_{50} value of GLEE was more than three times of its minimum effective (100 mg/kg) dose, and the plant is a good candidate for further investigation. Overall, the finding of oral acute toxicity test indicated that GLEE is tolerable and safe after oral administration which validates the safe use of the plant in traditional settings.

In the present study, the inhibitory impact of the Loperamide was reflected in the reduction of the length of the intestine and the distance travelled by the charcoal meal. GLEE caused a significant delay in the onset of the diarrhea through reduction in length of intestine and distance travelled compared to the control group. Higher dosage of the guava leaves GLEE (1000 mg/kg) showed higher inhibition. This could imply that the lower dose may have an insufficient concentration of active constituents responsible for the antimotility effect (Abdela, 2019). This finding suggested that GLEE has the antimotility effect at higher doses, in a similar manner to Loperamide (Chen *et al.,* 2012).

Diarrhea is characterized by frequent defecation of faeces, which may be due to a disturbance in the transport of water and electrolytes in the intestines. The findings of the study showed that the frequency of the wet stool output decline in a dose dependent manner at several time intervals, in which the highest inhibition was observed at 1000 mg/kg, second to the standard antidiarrheal drug, Loperamide. This indicated that the 1000 mg/kg of GLEE is associated with a better antidiarrheal effect which is comparable with the standard Loperamide. This could imply that the constituents of GLEE, which are responsible for antidiarrheal activities, are more likely to be concentrated in the higher doses of GLEE or this may indicate that a relatively high dose of GLEE was needed to produce a pronounced antidiarrheal effect (Abdela, 2019). These findings are in agreement with reports from Tadesse *et al.* (2014) and Sisay *et al.* (2017) conducted on other species of plants.

The antidiarrheal activity of GLEE was further confirmed by the reduction in number of wet stool of the rats treated with different dosages of GLEE. The result showed that the number of wet stool of rats administered with 1000 mg/ml of GLEE reduced drastically compared to the control and was comparable to the antidiarrheal drug (Loperamide). This could indicate that the plant has a potential antidiarrheal activity, which may serve as a template in the development of a novel antidiarrheal drug. Similar results were obtained by Okere and Iliemene (2014) who linked the antidiarrheal action to direct inhibitory effect of the extract on propulsive movement of the gastrointestinal tract smooth muscles.

Conclusion: GLEE demonstrated a significant delay in the onset of diarrhea, reduced the frequency of wet feces and also endowed with significant antidiarrheal effects at all doses evaluated experimentally. In addition, GLEE also indicated the antimotility effect at its higher doses. Moreover, the study also evaluated the acute toxicity of GLEE in which the plant is found to be nontoxic and its LD_{50} was greater than 5000 mg/kg, which ensures the safe use of GLEE in folk medicine.

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