

Antibacterial Properties of *Psidium guajava* (guava) Leaf Extract on Two Pathogenic Bacteria Strains.

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Abstract- *Psidium guajava* (Guava) is known for its food and nutritional values. This research was carried out to determine the antimicrobial activities of the different extract of the leaves of guava. The leaves was collected and shade dry for two weeks under room temperature after which it was pulverized into fine powder. 100grams of the pulverized leaves of *Psidium guajava* was cold macerated in three different solvents of increasing polarities (Hexane, ethanol and water) respectively. The results of the phytochemical screening of the different extracts reveals the presence of certain secondary metabolites tannins, saponins, steroid, flavanoids, glycosides and anthraquinones. The extracts were reconstituted by dissolving 1gram in 4 ml of 10% DMSO to produce a stock solution of 250mg/ml and double fall serial dilution was done to produce concentration of 125mg/ml, 62.5mg/ml and 31.25mg/ml. The efficacy of these crude extracts was tested against the bacteria using agar well diffusion method employing different concentrations of the reconstituted leaf-extract solution per well. According to the findings of the antibacterial assay, the ethanol, hexane and water extracts of *Psidium guajava* leaves had a broad spectrum inhibitory activity against the bacteria as compare to the control drug gentamycin. At $p < 0.05$ there was a significant difference in the antibacterial activities of the extracts on the test organism. The water extract had the highest inhibitory activity of between $15 \pm 0.24 - 25 \pm 0.29$ against *Escherichia coli*, followed by ethanol $12 \pm 0.04 - 22 \pm 0.39$ and hexane $9 \pm 0.11 - 22 \pm 0.29$, while for *Staphylococcus aureus* the ethanol extract had the highest inhibitory activity of $10 \pm 0.09 - 20 \pm 0.36$, followed by the hexane $11 \pm 0.38 - 18 \pm 0.41$ and water extracts $8 \pm 0.24 - 17 \pm 0.42$. The minimum inhibitory concentration for all of the different extracts and concentrations was between 31.25mg/ml and 62.5mg/ml. While the minimum bactericidal concentration was 250mg/ml for the ethanolic extract against *Escherichia coli*. The result of this findings support the use of this plants in folk medicine.

Index Terms- Bacteria, Extract, Antimicrobial, phytochemical, Concentration.

I. INTRODUCTION

Psidium guajava L. (guava), a fruit belonging to the family Myrtaceae, is found all over the world. It is a phytotherapeutic plant used in folk medicine that has been used for the management of various disease conditions and is believed to act. Various parts of this plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, dysentery,

wounds, ulcers, toothaches, coughs, sore throat, inflamed gums, etc (Abdelrahim *et al.*, 2015). Thus its uses in traditional medicine are well established against enteric human bacteria. This plant has also been used for controlling life-changing conditions such as diabetes, hypertension, and obesity (Begum *et al.*, 2004). Leaves and bark of *Psidium guajava* plant have a long history of medicinal uses that are employed today (Kumar, 2012).

Numerous plants contain natural preservatives which exist either as anti-microbial or anti-oxidant, some of these plant compounds can inhibit the growth of microorganisms and still have anti-microbial activity. It is true that guava leaves can cure many disease symptoms, and they contain some active compounds like saponins, flavonoids, tannins, eugenol and terpenoids. The compounds which dominate guava leaves are: Flavonoids and tannins and can inhibit the growth of bacteria (Badan, 2004).

Escherichia coli is a type of bacteria that normally lives in the intestines of people and animals. However, some types of *Escherichia coli*, particularly O157:H7 can cause intestinal infection. This strain of bacteria and other strains that cause intestinal sickness are called Shiga-toxin producing *Escherichia coli* (STEC) after the toxin that they produce. Severe cases can lead to bloody diarrhea, dehydration, or even kidney failure. Symptoms include abdominal cramping, gas, loss of appetite, vomiting, fatigue, fever, etc. Most intestinal infections are caused by contaminated food or water. Proper food preparation and good hygiene can greatly decrease any chances of developing an intestinal infection.

Staphylococcus aureus is the most dangerous of all of the many common staphylococcal bacteria. This is present in the nose of about 30% of healthy adults and on the skin of about 20%. The percentages are higher for people who are patients in a hospital or who work there. These bacteria are common and can be spread through the bloodstream and infect distant organs. Skin infections are spread by having direct contact with an infected person, the use of contaminated objects, or by inhaling infected droplets dispersed by sneezing or coughing. Some symptoms are: Swollen, red, tender or painful skin, fevers, chills, low blood pressure, boils, etc. Thorough washing of hands can help prevent the spread of the infection; use of antibiotics can also be effective against the strain causing the infection (Larry, 2011). Therefore this research was aim at evaluating the antimicrobial activities of the different extract of *Psidium guajava* of *E.coli* and *S.aureus*

II. MATERIALS AND METHODS

STUDY AREA

This research was conducted in the Chemistry and Biology laboratories of the Federal College of Forestry, Jos, Plateau State, Nigeria. Jos, Plateau state is located in the middle zone of Nigeria. It lies between latitude 45.56°-9055° north and Longitude 31.63°-8053° east. Temperature ranges between 21°C-25°C and mean and annual rainfall is about 1,400mm (55 inches). The average elevation is 1,829m above sea level.

PREPARATION OF GUAVA LEAVES

The guava leaves were collected and dried in the shade at room temperature (25°C) for two weeks and then pulverized into coarse powder. The powder was then stored in an airtight bottle at 4°C until when needed.

EXTRACTION OF PLANT MATERIALS

100gram of the pulverized powder was cold macerated in 200ml of different solvents (ethanol, hexane and water) contained in 500ml sterile conical flasks and covered with rubber cork & aluminium foil. These were placed aside with intermittent shaking 24 hours. Each of the extracts was filtered with a muslin cloth and the mache was discarded. The filtrate was subjected to evaporation in a rotary evaporator to obtain a dried extract. The dried extract was stored at 4°C until used for further study (Atata *et al.*, 2003).

PHYTOCHEMICAL ANALYSIS

The crude extracts of *Psidium guajava* were subjected to qualitative screening for identification of various classes of active chemical constituents such as tannins, saponins, cardiac glycosides, steroids, terpenoids, flavonoids, anthraquinones, and alkanoids. The phytochemical analysis was done according to standard method (Harborne, 1988).

Qualitative phytochemical analysis of the plant extracts (Harborne 1988).

The leaf extracts were analyzed for tannins, saponins, cardiac glycosides, steroids, terpenoids, flavonoids, anthraquinones, alkaloids, phenols and volatile oils.

TEST FOR TANNINS

Ferric chloride (2ml) was added to 1ml of each of the plant extracts, and the formation of a greenish black coloration indicated the presence of tannins.

TEST FOR SAPONINS

Distilled water (2ml) was added to 2ml of each of the plant extracts and shaken for 15mins lengthwise. The formation of 1cm layer of foam indicated the presence of saponins.

TEST FOR CARDIAC GLYCOSIDES

Chloroform (3ml) and ammonia solution (10%) were added to 3ml of the plant extracts. Formation of pink colour indicated the presence of glycosides.

TEST FOR STEROIDS

The extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid was added and mixed. A change of red colour through blue to green indicates the presence of steroids (Lieberman Buchard Test).

TEST FOR TERPENOIDS (SALKOWSKI'S TEST)

5ml of each extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown precipitate of the interface indicated the presence of terpenoids.

TEST FOR FLAVONOIDS

Sodium hydroxide (1ml of 2N) was added to 1ml of each of the plant extracts. A yellow colour indicated the presence of flavonoids.

TEST FOR ANTHRAQUINONES

0.5ml of the extract was boiled with 10ml of sulphuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform and observed for colour changes.

TEST FOR ALKALOIDS

Concentrated hydrochloric acid (2ml) was added to 2ml of each of the plant extracts. Then a few drops of Meyer's reagent were added. The presence of a green colour or white precipitate indicated the presence of alkaloids.

TEST FOR PHENOLS

Distilled water (2ml) followed by few drops of 10% ferric chloride was added to 1ml of the extracts. Formation of blue or green colour indicated the presence of phenol.

SOURCES OF BACTERIAL STRAINS

The bacterial strains which are *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) were obtained from Veterinary Research Institute Vom Jos plateau state.

STANDARDIZATION OF INOCULUM

Using inoculation loop, enough material from an overnight culture of the test organism was transferred into a test tube containing nutrient broth until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National committee for clinical laboratory standard (NCCLS, 2008).

PREPARATION OF NUTRIENT AGAR

Nutrient agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Immediately after autoclaving, it was allowed to cool in a 45°C to 50°C water bath. The freshly prepared and cooled medium was poured into sterile agar plates on a level, horizontal surface to give a uniform depth of approximately 2mm.

ANTIBACTERIAL ACTIVITY BY AGAR WELL DIFFUSION ASSAY METHOD

The antibacterial activity of the crude solvents (ethanol, hexane and water) leaves' extracts of *Psidium guajava* against gram-positive as well as gram-negative bacterial strains was evaluated by agar well diffusion assay (AWDA) method (Parekh *et al.*, 2007; Kumar *et al.*, 2014). The diameters of the inhibition zones were measured in millimeters. For this, a well (5mm diameter) was made with the help of a cork borer in cooled nutrient agar plate, overlaid with soft agar (5ml), seeded with a target strain

(~10⁶cfu/ml). Aliquots of the test compound (100ul) were introduced into the well and the plates were incubated overnight at 37°C. For each bacterial strain, the dissolving solvent 10% DMSO and gentamycin (50ug/ml) were used as negative and positive controls respectively.

PREPARATION OF NUTRIENT BROTH

6.5grams of nutrient broth powder was suspended in 500mls of distilled water and it was dispensed in to a 500ml conical flask. It was sterilized in an autoclave at 121°C for 15mins.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION

The MIC is the concentration giving the least inhibitory activity and below which there is no further inhibition. This was determined by the method as described by Ochei *et al* (2000). The Minimum Inhibitory Concentration of the *Psidium guajava* leaves was determined according to the broth (tube) dilution method. 13 sterile tubes were set up and labelled. 1ml of the nutrient broth was

pipetted into tube 2 to 10, 11 and 13. Tube 11 was inoculum control, 12 was broth control, 13 was antibiotic control. 1ml of the working solution (250mg/ml of the extract) was pipetted into tubes 1, 2 and 13. A double dilution was prepared from tube 2 to 10 using 1ml of the amount. 1ml of working inoculum was pipetted into tubes 1 to 1. The least tube that showed no turbidity was taken as the MIC. (Ochei and Kolhatkar, 2000)

MINIMUM BACTERICIDAL CONCENTRATION

The Minimum Bactericidal Concentration of the plant extracts on the bacteria was carried out according to National Committee for Clinical Laboratory Standard (NCCLS, 2008). A loopful of broth was collected from the determination of MIC tubes which did not show any growth and streaked on a sterile nutrient agar. All the plates were then incubated at 37°C for 24hours. The least concentration of the leaf extracts with no visible growth after incubation was taken as the minimum bactericidal concentration.

III. RESULTS AND DISCUSSION

RESULTS

Preliminary Phytochemical Screening

Table 1: Preliminary Screening for Phytochemical Constituents

S/No.	Phytochemical constituents	Ethanol extract	Hexane extract	Water extract
1.	Tannins	+	+	+
2.	Saponins	+	-	-
3.	Cardiac glycosides	-	+	+
4.	Steroids	+	+	+
5.	Terpenoids	+	+	+
6.	Flavonoids	+	+	-
7.	Anthraquinones	+	+	+
8.	Alkaloids	+	+	+
9.	Phenols	+	-	+
10.	Volatile oils	+	+	-

+ indicates the presence of the compound in each extract.

-indicates the absence of the compound in each extract.

ANTIBACTERIAL ASSAY FOR *Escherichia coli*

Table 2 Antibacterial Activities for *Escherichia coli* by the Three Extracts of *Psidium guajava*.

Extracts	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
Hexane	22±0.29	15±0.45	10±0.21	9±0.11
Aqueous	25±0.30	21±0.73	20±0.14	15± 0.24
Ethanol	22±0.39	17±0.38	14±0.35	12±0.04
LSD	0.28			

ANTIBACTERIAL ASSAY FOR *Staphylococcus aureus*

Table 3 Antibacterial Activities for *Staphylococcus aureus* by the Three Extracts of *Psidium guajava*.

Extracts	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml
Hexane	18±0.41	15±0.06	13±0.17	11±0.38
Aqueous	17±0.42	12±0.54	20±0.07	8± 0.24
Ethanol	20±0.36	17±0.02	14±0.17	10±0.09
LSD	0.24			

Antimicrobial Screening of *Escherichia coli* and *Staphylococcus aureus*.

Table 4: Antimicrobial Effects of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Ethanol, Hexane and Water Extracts of *Psidium guajava*.

TEST ORGANISM	EXTRACTS	MBC	MIC
<i>Escherichia coli</i>	Hexane	-	31.25mg/ml
	Water	-	62.5mg/ml
	Ethanol	250 mg/ml	62.5mg/ml
<i>Staphylococcus aureus</i>	Hexane	-	31.25mg/ml
	Water	-	62.5mg/ml
	Ethanol	-	62.5mg/ml

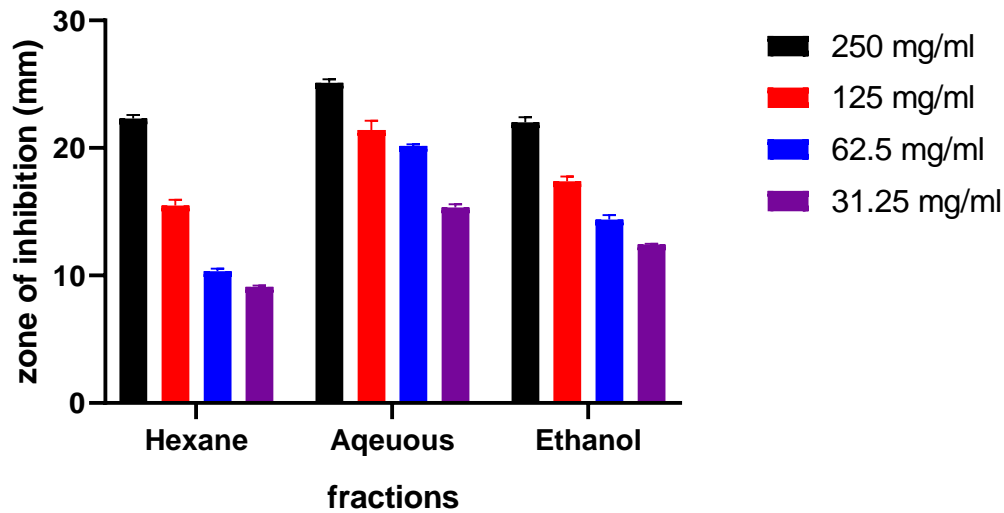


FIGURE 1 The antibacterial activities of different extract of *psidium guajava* on *E.coli*

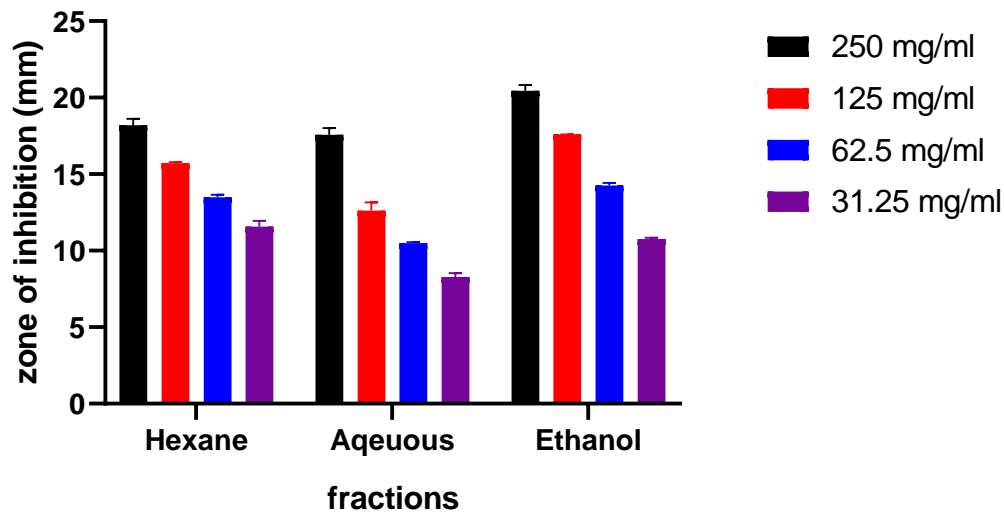


FIGURE 1 The antibacterial activities of different extract of *psidium guajava* on *S. aureus*

IV. DISCUSSION

Components of the leaf extracts: Table 1 shows the results for the phytochemical screening for components of the leaf extracts of *Psidium guajava* e.g alkaloids, tannins, steroids, etc. Tannins, saponins, steroids, terpenoids, flavonoids, anthraquinones, alkaloids, phenols and volatile oils were present in the ethanolic extracts except cardiac glycosides. Tannins, cardiac glycosides, steroids, terpenoids, flavonoids, anthraquinones, alkaloids and volatile oils were present in the hexane extracts except for saponins and phenols which were absent. Tannins, cardiac glycosides, steroids, terpenoids, anthraquinones, alkaloids and phenols were present in the water extract except for saponins, flavonoids and volatile oils which were absent. The presence of phytochemical compounds in *P.*

guajava extracts were reported to be responsible for antimicrobial activity by several authors (Singh and Bhat, 2003) particularly alkaloids and tannins are well known for their antimicrobial activity.

Antibacterial activities of the leaf extracts of *Psidium guajava*: The results of antimicrobial activities of hexane, ethanol and water extracts against the test organisms are shown in table 2. The zones of inhibition of the bacteria are a function of the antimicrobial activities of the extracts. The activities of the leaf extracts were shown to be concentration dependent. The extracts showed significant inhibition against all the test organisms. However, the water extracts showed more activity than the other extracts. Gentamycin had a zone of inhibition of 25mm against *Escherichia coli* and 10% DMSO were used as positive and negative controls respectively, which 10% DMSO was inactive against the organisms.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC): Table 3 shows the results of MIC and MBC determination on the test organisms. The test tube which showed no turbidity was recorded as the minimum inhibitory concentration (MIC). The test tubes which showed no turbidity were cultured on nutrient agar for 24hrs the concentration which showed no growth was taken as the Minimum bactericidal concentration (MBC) as shown in table 3 above. The MIC for the test organisms ranged between 31.25mg/ml and 62.5mg/ml for both organisms. The MBC was taken as 250mg/ml for the ethanolic extract against *Escherichia coli* which had no visible growth after 24hrs of incubation.

V. CONCLUSION

The results obtained in this study showed that *Psidium guajava* extracts were able to inhibit the activity of the bacteria used in the study as such *Psidium guajava* presents a potential for the production of drugs used in the treatment of bacteria infections caused by *Escherichia coli* and *Staphylococcus aureus*. The information obtained may provide validation for *Psidium guajava's* reported medicinal uses explaining why it is widely used traditionally as an alternate means of medication for certain ailments.

VI. RECOMMENDATION

In the course of this antimicrobial study only bacterial organisms were used, we recommend that other bacteria especially gastro intestinal microorganisms and fungi can be incorporated into this study in the future so as to exploit the many potentials of *Psidium guajava*.

In the course of this study, four concentrations ranging from 250mg/ml, 125mg/ml, 62.5mg/ml to 31.25mg/ml were used so we recommend that more concentrations can be used for future research to effectively monitor the best concentration for human consumption.

For future research more solvents can be introduced during the process of extraction as three solvents were used for this research.

Guava has a lot of potential as a medicinal plant we hope future research will look into other parts of the plants such as the stem bark and the roots and also the antioxidant properties of this plant and hopefully the synergistic potentials of *Psidium guajava*.

Since *Psidium guajava* is known to have many medicinal properties, we recommend that *Psidium guajava* be grown as an economic tree not only for its fruits but also for the other plant parts which can be used for medicinal purposes.

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