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Phytochemical Screening And Antimcrobial Efficacy of Leaf Extract Of Guava (*Psidium guajava*) On Selected Bacteria

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ABSTRACT

Guava leaf extract has analgesic, anti-inflammatory, antimicrobial, hepato protective and antioxidant activities. The high resistance of bacteria to modern day drugs has led researchers in the pharmaceutical industry to look towards plant-based extracts for solutions. Hence, this study was aimed at evaluating the phytochemical and antibacterial efficacy of leaf extracts of *Psidium guajava* (guava) on some selected bacteria. Pure isolates of bacteria gotten from urine of patients in Charis Rhema Research and Diagnostic Laboratory were used in the study. Extracts from the leaves of *Psidium guajava* were tested for presence of phytochemicals. Separation of active components of the extracts was one using Chromatographic technique. The antibacterial activity of *Psidium guajava* leaves was determined using agar diffusion bioassay. Phenols were present at all concentrations (100, 80 and 60mg). The study discovered a significant relationship between extract concentration and the zones of inhibition at 60% N-hexane/40% methanol for *Klebsiella sp.,* and 0.0% N-hexane/100% methanol for *S. aureus*. Flavonoids, tannins, saponins and phenols were the active compounds in guava that helped in the inhibition of growth on the sampled isolates. Methanol and N-hexane extracts of guava can be used as potent medicines for inhibiting the growth of *Klebsiella* and *Staphylococcus aureus*.

Keywords: of *Psidium guajava* (guava), Chromatographic technique, antibacterial activity, *Klebsiella, Staphylococcus aureus*

INTRODUCTION

Over 50% of all modern clinical drugs are of natural product and natural products play important roles in drug development in the pharmaceutical industry (Preethi *et al.,* 2010). The presence of phytochemical constituents in medicinal plants made them useful for healing as well as for curing of human diseases (Muhammad and Ibrahim, 2019). Phytochemicals are naturally occurring compounds in the medicinal plants such as terpenoid, flavonoids, steroid, Alkaloids, and phenolic compound. Terpenoid exhibit various important pharmacological activities i. e. Anti-inflammatory, Anti-Cancer, Anti-malarial; Anti-Viral and Antibacterial activities (Sukanya *et al*., 2017). Currently, there is increasing interest in studying of plants regarding their chemical components of bioactive compounds, their effects on several diseases, and their use for human health as functional foods and/or nutraceuticals. In recent years, guava leaves tea and some complementary guava products are available in several shops in Japan as well as on the Internet, because guava leaf phenolic compounds have been claimed to be food for specified health use (FOSHU).

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Guava leaf is a good source of natural antioxidants. Extracts from guava leaves are beneficial as therapeutic against cancer, bacterial infections, inflammation and pain. Essential oils from guava leave display in vitro anti-cancer activity. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. The leaves of guava are rich in flavonoids and phenols. In addition, other flavonoids and triterpenes present in guava leaves show antispasmodic activity. It is also known that *P. guajava* leaves have a broad spectrum of antimicrobial action (as antigiardial and antirotaviral activity) that could be effective in controlling diarrhea due to a wide range of pathogens. The antimicrobial activity can be linked to the presence of flavonoids extracted from guava leaves. (Kumar *et al*., 2021).

Biswas et al., *2013* in their study indicated that only two of the crude solvent extracts prepared from the leaves of *Psidium guajava*, methanol and ethanol, showed inhibitory activity against bacteria Only Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, were susceptible to the two extracts, while neither of the Gram-negative bacterium showed any inhibition. At 10 mg/50 μL, the methanol extract had a slightly higher antibacterial activity with mean zones of inhibition 8.27 and 12.3 mm than ethanol extract with mean zone of inhibition 6.11 and 11.0 mm against *B. cereus* and *S. aureus*, respectively. The resistance of the Gram-negative bacteria could be attributed to its cell wall structure.

Zahidah *et al.,* 2013 in their study revealed guava leaves extract also exhibited antimicrobial effect by inhibiting the growth of *Bacillus subtilis* and *Staphylococcus aureus*. Also, that Guava leaf extract has the potential to be used as a functional food ingredient or as a bioactive ingredient in the food and pharmaceutical industry.

Statement of problem

The increasing high numbers of bacteria that are developing resistance to classical antibiotics is of great concern. Researchers are currently taking interest on natural antimicrobial molecules in hope that they will profer useful leads into anti-infective candidates. In addition, the high cost of conventional drugs particularly in resource limited communities has led to the increased use of plants such as guava *(Psidium guajava)* as an alternative for the treatment of infectious diseases.

Justification of the study

The relevant stakeholders likely to benefit from the study include students in Medicine and Pharmacy, Extension workers, Researchers in the medical field and the general populace.

For students of Medicine and Pharmacy, knowledge of the efficacy of these extracts will provide them with additional methods of treatment for bacterial infections which in some ways have become resistant to antibiotics.

This work will seek to add to the already existing literature on the efficacy of the leaf extracts of guava on the selected test organisms. It will also serve as a reference point for other researchers who may want to carry out research in future.

Consumers will also be informed through this work, on the need to embrace traditional methods of treatment which may serve as a more economic means of treating the illnesses caused by the selected test organisms.

Aim of the study

To evaluate the phytochemical and antibacterial efficacy of leaf extracts of *Psidium guajava* on selected bacteria.

Objectives of the study

i. To revalidate isolates.
ii. To evaluate the phytochemical properties of leaf extracts of guava leaves
iii. To ascertain the antibacterial efficacy of the leaf extracts of *Psidium guajava* on selected organisms.

LITERATURE REVIEW

Guava (*Psidium guajava*)

Psidium guajava L is a fruit-bearing tree commonly known as guava, which belongs to the family *Myrtaceae*. The French call it goyave or goyavier; the Dutch, guyaba, goeajaaba; the Surinamese, guave or goejaba; and the Portuguese, goiaba or goaibeira. Hawaiians call it guava or kuawa. In Guam, it is abas. In Nigeria, it is popularly called guava or “gova” by the Tiv people of Benue State (Ichoron, 2019).

Guava grows nearly throughout India up to 1500 m in height and is cultivated commercially in almost all states, the total estimated area being 50,000 hectares. The important guava growing states in India are Uttar Pradesh, Bihar, Maharashtra, Assam, West Bengal and Andhra Pradesh. Cultivated varieties grow about 10 m in height and produce fruits within 4 years. Wild trees grow up to 20 m high and are well branched. The tree can be easily identified by its distinctive thin, smooth, copper-colored bark that flakes off, showing a greenish layer beneath. Guava trees have spread widely throughout the tropics because they thrive in a variety of soils, propagate easily and bear fruits quickly. The fruits are enjoyed by birds and monkeys, which disperse guava seeds and cause spontaneous dumps of guava saplings to grow throughout the. The leaves and bark of guava tree have a long history of medicinal uses (Ismail, 2012).

Uses and Properties

It was reported that *Psidium guajava* leaf extract has a wide spectrum of biological activities such as anticough, antibacterial, haemostasis, antidiarrhoeal and narcotic properties, and antioxidant properties (Biswal, 2013).

According to El-Ahmady *et al*. (2013), the leaf extract is used to treat diarrhoea, abdominal pain, convulsions, epilepsy, cholera, insomnia and has hypnotic effect. Some studies reported that the leaf extract and its derivative identified as quercetin has effect on the intracellular calcium levels in gastrointestinal smooth muscle, in cardiac muscle cell and in neuromuscular junction.

Assessment of two medicinal plants, *Psidium guajava* L. and *Achillea millefolium* L., in in vitro and in vivo assays: Study on the cytotoxicity and mutagenicity of the plants provide info on its safety for use as therapeutic agents (Biswal, 2013).

Antihypertensives and Antidiarrheal: In the study, *P. guajava* leaf extracts was more active than D. *mespiliformis* in their antagonistic effects on caffeine-induced calcium release from the sarcoplasmic reticulum of rat skeletal muscle. Results might explain their use as antihypertensive and antidiarrheal agents in traditional medicine through an inhibition of intracellular calcium release.

Hypoglycemic/Hypotensive: The leaf of *Psidium guajava* is used extensively in African folk medicine. The study shows that the aqueous leaf extract of *P. guajava* possesses hypoglycemic and hypotensive properties and provides pharmacological credence to the folkloric use of the plant for type-2 diabetes and hypertension in some rural African communities.

Anti-Ulcer: Study showed rats pretreated with P guajava extract from fresh tender leaves showed antiulcer activity in aspirin-induced gastric ulcer model with a significant reduction of ulcer index, pepsin activity, free and total acidity, volume and mucus content of gastric juice. antioxidants and reduced oxidative stress and also increase the level of HDL cholesterol significantly.

Antibacterial: Study evaluated the antibacterial activities of aqueous and ethanol-water extracts from leaves, roots and stem bark of *P. guajava*. The AE of leaves roots and stems were active against gram-positive bacteria Staphylococcus aureus and B. subtilis and virtually ineffective against E. coli and P. aeruginosa. The EW showed higher activity than the AE.

Leaves Extracts / Differences in Hypoglycemic Potential: In a mice model, study showed the water soluble, edible alcohol, and edible alcohol-soluble extracts of wild *Psidium guajava* leaves may have different hypoglycemic potential.

Hepatoprotective / Leaves: Study in male and female rats showed the aqueous extract of *P. guajava* leaves may be hepatoprotective (not hepatotoxic), with hematopoietic potentials.

Anticancer Activity / Review: Review of a limited number of studies revealed guava extracts may have anti-cancer activity. One study tested guava fruit extract against a proliferation of cancer cell lines. One study in mice used a combination of bark, leaf, and root extract to inhibit growth of B16 melanoma cells.

Corrosion Inhibition / Mild Steel: Study evaluated the corrosion inhibition behavior of an extract of guava leaves towards mild steel in HCl media. Results showed the extract has good inhibition efficiency (IE) and acts as a mixed-type inhibitor. As extract concentration increases, IE also increases.

Hepatoprotective / Leaves: Study evaluated the hepatoprotective activity of *P. guajava* in CCl4-, paracetamol-and thioacetamide-induced liver injury. Results showed significant reduction of liver enzymes and bilirubin. Higher doses prevented increases in liver weight.

Antihyperglycemic/Unripe Fruit Peel: Study evaluated the glycemic potential of an aqueous extract of unripe fruit peel in STZ-induced diabetic rats. Results showed normal, mild, and severely diabetic rat models had hypoglycemic and antidiabetic effect.

Analgesic/Antipyretic/Dried Leaves: Study of an ethanol extract produced significant reduction of pyrexia in yeast induced hyperpyrexia and hot plate latency assay. Analgesic activities were observed in early and late phase of formalin induced paw licking tests in rats.

Anti-epileptic / Leaves: Study evaluated the anti-epileptic activity of a leaves extract of *P. guajava* in seizure induced by maximal electroshock and pantaloon territorialize. Results showed the leaves extract at higher and medium doses produced highly significant and sustained increases in onset of convulsions and decrease in rate of convulsion. Activity may be due to presence of flavonoids and saponins.

Antimicrobial Properties

More than twenty identified compounds from *Psidium guajava* leaf have been reported (. The major components are: β-selinene, β-caryophyllene, caryophyllene oxide, squalene, selin-11-en-4α-ol, guaijavarin, isoquercetin, hyperin, quercitrin and quercetin-3-Ogentobioside, morin-3-O-α-L-lyxopyranoside and morin-3-O-α-Larabopyranoside, β-sitosterol, uvaol, oleanolic acid and ursolic acid (Goncalves *et al*., 2008).

Our recent phytochemical screening of *Psidium guajava* leaf showed tannins in aqueous extract, anthocyans, alkaloids, flavonoids, tannins and steroids/terpenoids in ethanolic extract. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. Guava fruit is higher in vitamin C than citrus fruits (80 mg of vitamin C in 100g of fruit) and contains appreciable amounts of Vitamin A as well. Guava fruits are also a good source of pectin (Ibe *et al.,* 2015).

The leaves of guava are rich in flavonoids, particularly quercetin. It has demonstrated antibacterial and anti-diarrheal effects and is able to relax the intestinal smooth muscle and inhibit bowel contractions. Guava has antioxidant properties attributed to polyphenols found in its leaves. Leucocyanidin, luectic acid, ellagic acid and amritoside have been isolated from the stem bark. Five constituents, including one new pentacyclic triterpenoid:guajanoic acid and four known compounds-beta-sitosterol, uvaol, oleanolic acid and ursolic acid, have been recently isolated from the leaves of *P. guajava* (Balakrishnan *et al*., 2011).

Flavonoids

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities (Birdi *et al*., 2011).

Flavonoids have been shown to have a wide range of biological and pharmacological activities in *in vitro* studies. Examples include anti-allergic, anti-inflammatory, antioxidant, anti-microbial (antibacterial, antifungal, and antiviral), anticancer, and anti-diarrheal activities. Flavonoids have also been shown to inhibit topoisomerase enzymes and to induce DNA mutations in the mixed-lineage leukemia (*MLL*) gene in *in vitro* studies. However, in most of the above cases no follow up *in vivo* or clinical research has been performed, leaving it impossible to say if these activities have any beneficial or detrimental effect on human health. Biological and pharmacological activities which have been investigated in greater depth are described below (Balakrishnan *et al*, 2011).

In Vivo Flavonoid-rich grape-seed extract has been shown to have antioxidant activity in *in vivo* studies with rats, protecting their gastrointestinal mucosa against the reactive oxygen species generated by acute and chronic stress. In the absence of any additional *in vivo* data, it is impossible to say if these findings are generalizable to all flavonoids. Also, without any clinical studies, it is impossible to say if the antioxidant activity of grape-seed flavonoids offers any protection against oxidative stress in the human gastrointestinal tract (Periyasami and Mahalingam, 2010).

Research at the Linus Pauling Institute and the European Food Safety Authority shows that flavonoids are poorly absorbed in the human body (less than 5%), with most of what is absorbed being quickly metabolized and excreted. These findings suggest that flavonoids have negligible systemic antioxidant activity, and that the increase in antioxidant capacity of blood seen after consumption of flavonoid-rich foods is not caused directly by flavonoids, but due to increased production of uric acid resulting from excretion of flavonoids from the body (Pandey and Shweta, 2011).

Inflammation has been implicated as a possible origin of numerous local and systemic diseases, such as cancer, cardiovascular disorders, diabetes mellitus and celiac disease. Preliminary studies indicate that flavonoids may affect anti-inflammatory mechanisms via their ability to inhibit reactive oxygen or nitrogen compounds. Flavonoids have also been proposed to inhibit the pro inflammatory activity of enzymes involved in free radical production, such as cyclooxygenase, lipoxygenase or inducible nitric oxide synthase, and to modify intracellular signaling pathways in immune cells (Offor, 2015).

Procyanidins, a class of flavonoids, have been shown in preliminary research to have anti-inflammatory mechanisms including modulation of the arachidonic acid pathway, inhibition of gene transcription, protein expression and activity of inflammatory enzymes, as well as secretion of anti-inflammatory mediators (Offor, 2015).

Antibacterial effects of Flavonoids have been shown to have

(a) direct antibacterial activity,

(b) synergistic activity with antibiotics, and

(c) the ability to suppress bacterial virulence factors in numerous *in vitro* and a limited number of *in vivo* studies. Noteworthy among the *in vivo* studies is the finding that oral quercetin protects guinea pigs against the Group 1 carcinogen *Helicobacter pylori*. Researchers from the European Prospective Investigation into Cancer and Nutrition have speculated this may be one reason why dietary flavonoid intake is associated with reduced gastric carcinoma risk in European women. Additional *in vivo* and clinical research is needed to determine if flavonoids could be used as pharmaceutical drugs for the treatment of bacterial infection, or whether dietary flavonoid intake offers any protection against infection (Pandey and Schweta, 2011).

Tannins

Tannins are polyphenolic compounds that are very complex. Because of the phenol group, the tannins can react with formaldehyde (condensation polymerization) to form thermosetting products that can be used as an adhesive. Antibacterial effectiveness of tannin contained in the leaves of plants such as guava is influenced by the concentration of tannins. The higher levels of tannin antibacterial activity will increase. Because of its importance in the treatment of guava leaf, the quality, safety and benefits should be improved through research and development (Pandey and Schweta, 2011).

Guava Leaf Extract as a Potent Cure for Bacterial Infections

Gonclaves *et al.* (2008) showed that the antimicrobial effect of essential oils and methanol, hexane, ethyl acetate extracts from guava leaves. The extracts were tested against diarrhea-causing bacteria: *Staphylococcus aureus, Salmonella spp. and Escherichia coli*. Strains that were screened included isolates from seabob shrimp, *Xiphopenaeus kroyeri* (Heller) and laboratory-type strains. Of the bacteria tested, *Staphylococcus aureus* strains were most inhibited by the extracts. The methanol extract showed greatest bacterial inhibition. No statistically significant differences were observed between the tested extract concentrations and their effect. The essential oil extract showed inhibitory activity against *S. aureus* and *Salmonella spp* (Gonclaves *et al.*, 2008)

The antibacterial testing of Guava (*Psidium guajava*) leaves extract was carried out by Agar well diffusion method. Amongst the tested extracts the result suggested that methanolic extracts of leaves showed significant antibacterial activity compared with standard drug (liprofloxacin). The guava leaves exhibited a distinct resistance in some strains of bacteria involved in present study at the concentration of 200ug/ml. In comparison to liprofloxacin the methanolic extract offered significant protection against *Staphylococcus aureus* & *Escherichia coli*. The antibacterial potential exhibited by guava leaves extract may be contributed to the presence of the Flavanoids as found in the preliminary phytochemical investigation (Ismail *et al*, 2012).

Richard *et al*. (2013), investigated the effects of *Psidium guajava* on organisms responsible for skin disorders, specifically the fungi *Microsporum gypseum* and *Trichophyton mentagrophytes* and bacteria *Staphylococcus aureus*, and *Staphylococcus epidermidis*.

Henry *et al*. (2020) in a more recent study on the *In vitro* antibacterial activity of *Psidium guajava* (guava) leaves extract on carbapenem-resistant *Klebsiella pneumoniae* causing multi drug resistant systemic infections used agar well diffusion and well microplate dilution of the ethanolic extract of the guava leaves was used to determine the effectiveness of *Psidium guajava* on carbapenem-resistant *K. pneumoniae*. The antimicrobial compounds responsible for the antibacterial activity were screened using standard methods. The active zones of inhibition were observed in *P. guajava* leaves extract concentrations of 50, 100 and 200 mg/ml. The minimum inhibition concentration and minimum bactericidal concentration of ethanolic extract of guava leaves was 6.25 mg/ml indicating significant antibacterial activity against the carbapenem resistant *K. pneumoniae*. The antibacterial activity of the leaves extract may be attributed to the presence of flavonoids and other antimicrobial phytochemicals in the guava leaves extract.

In another study, Abdullah *et al*. (2019) carried out studies on the antibacterial activity of *Psidium guajava* leaf and stem bark extracts against clinical isolates of *S. aureus* and *S. typhi* in Kano, Nigeria. The phytochemical screening of the plant materials for various phytochemical constituents was conducted using laboratory methods. The sensitivity of each extracts against the isolates was determined using the agar well diffusion and broth dilution method was employed for the determination of Minimum inhibitory concentration of the extracts. The result of preliminary phytochemical screening of the extracts showed the presence of Alkaloid, saponin, phenol, flavonoids, Glycoside, Anthraquinones, terpenoid and tannin. The antibacterial activity of the extracts showed that the extracts were active against the isolates with *Staphylococcus aureus* being the most susceptible isolate with average zone of inhibition of 16.05mm and *S. typhi* the least susceptible with average zone of inhibition of 15.21 mm. Statistical analysis of the result showed methanol extract is more effective than aqueous extract while the stem bark of the plant showed higher efficacy than the leaf. However, there is no statistical differences on the effectiveness of the extract at $p<0.05$. Finding of the study justify the antibacterial efficacy of leaf and stem bark of *Psidium guajava*.

Das and Goswami (2019) carried out studies on the antifungal and antibacterial property of Guava leaf extract and the role of phytochemicals in its efficacy. The antifungal and antibacterial property of guava leaves were estimated using Bacillus subtilis (Gram positive bacterial strain), Escherichia coli (Gram negative bacterial strain), Saccharomyces cerevisiae (Yeast, fungal strain) and Aspergillus niger (Mould, fungal strain) strains. The growth of gram-positive bacterial and fungal strains were inhibited strongly, whereas gram negative bacterial strain displayed less sensitivity against the antimicrobial (antifungal and antibacterial) property of guava leaf extract. Zone inhibition assay also confirmed the result. Phytochemical analysis (qualitative and quantitative) revealed that guava leaf extract was rich in wide range of poly phenols. It was found that guava leaves are rich in phenols, flavonoids and tannins whereas components like alkaloids, flavonoids, saponins and triterpenes are present in comparatively lesser amounts. As polyphenols have strong antimicrobial property, it can be concluded that rich source of phenols, flavonoids and tannins are the probable cause of antimicrobial property of guava leaves.

Biswas *et al*. (2013) in a study to determine the antimicrobial activities of leaf extracts of Guava (*Psidium guajava*) on two gram-negative and gram-positive bacteria reported similar reports with those of Das and Goswami (2019). The guava leaves were extracted in four different solvents of increasing polarities (hexane, methanol, ethanol, and water). The efficacy of these extracts was tested against those bacteria through a well-diffusion method employing 50 μL leaf-extract solution per well. According to the findings of the antibacterial assay, the methanol and ethanol extracts of the guava leaves showed inhibitory activity against gram-positive bacteria, whereas the gram-negative bacteria were resistant to all the solvent extracts. The methanol extract had an antibacterial activity with mean zones of inhibition of 8.27 and 12.3 mm, and the ethanol extract had a mean zone of inhibition of 6.11 and 11.0mm against *B. cereus* and *S. aureus*, respectively.

In Jos, Plateau State, Egga *et al*. (2014) reported on the preliminary phytochemical, antimicrobial and proximate analysis of tender leaves of *Psidium guajava L*. The phytochemical screening revealed the presence of all metabolites and compounds tested for such as flavonoids, tannins, reducing sugar, terpenes, saponins, anthraquinones and alkaloids. The proximate analysis gave a moisture content of 3.67%, ash value of 3.295%, acid insoluble ash of 0.25%, alcohol soluble extractive value of 21.34%and water extractive value of 3.54 %. The antimicrobial screening of methanol extract showed activity against the tested organisms. The antimicrobial screening of hexane and ethyl acetate also showed activity against the tested organisms. The result indicated that the leaves had a potential antimicrobial activity and was concentration dependent. The chromatographic analysis of the extracts showed presence of variety of compounds. This therefore, supports the traditional medical use of *Psidium guajava*.

MATERIALS AND METHODS

Study Location

This study was carried out at Charis Rhema Laoratories, High level, Makurdi, Benue State. The city is located in central Nigeria along the Benue River. It is located on latitude 7.73˚N and longitude 8.53˚E and situated 104 meters above sea level (The world Gazetteer, 2013). The annual rainfall ranges from 150mm to 180mm, and the mean temperature ranges from 27^{0}C to 38^{0}C, Along the banks of the Benue River which is a major tributary to the Niger river. It is an agricultural catchment area and has a variety of potentials in human capital and material resource.

Sample collection

Fresh and healthy leaves of *Psidium guajava* were obtained at locations within Makurdi, Benue State and stratified at the Plant Science and Biotechnology herbarium in Benue State University. The leaves were washed in distilled water and allowed to dry at room temperature for 7 days. The leaves were then pulverized into coarse powder using blending machine.

Isolation and Identification of Bacterial Species

Bacterial species were isolated from urine samples of urinary tract infected (UTI) patients referred to the Microbiology Laboratory Unit of Federal Medical Centre, Makurdi. The urine samples were cultured by streaking on MacConkey Agar (MA) and Mannitol Salt Agar (MSA), and incubated at 37 °C for 24 hours. Identification of the bacterial isolates was based on the cultural and morphological and biochemical characteristics such as, Indole, Methyl Red, Citrate utilization, Catalase, Coagulase, Nitrate reduction, Urease and Sugar fermentation tests following standard microbiological procedures as described by Chesbrough (2017).

Extraction of *Psidium guajava* Leaves

Extracts were prepared following the method described by S'anchez *et al.* (2010). Briefly, 100 g of dried pulverized guava leaves were soaked in 500 mL of water, methanol and ethanol for 24 hours at room temperature, under occasional shaking. Extraction was repeated three times, and the extracts obtained were filtered using Whatman filter paper number 1. After that, the extracts were concentrated to dryness under reduced pressure using a rotary evaporator at 45 °C.

Phytochemical Analysis

Test for Alkaloids

1mL of the extract was added into 3 drops of draggendorff"s reagent and was observed for the presence of orange precipitate which gives preliminary evidence for the prescence of alkaloids (Chesbrough, 2017).

Test for Quinones

Extracts suspended in ethanol (1 mL) were treated with 1mL of concentrated sulfuric acid. Formation of red colour showed the presence of quinones (Chesbrough, 2017).

Test for Glycosides

One ml of glacial acetic acid, 3 drops 5% W/V ferric chloride and concentrated sulphuric acid were added to test tubes containing 2 ml of extracts and observed. The disappearance of reddish brown colour at the junction of two layers and bluish green in upper layer indicates the presence of glycosides (Chesbrough, 2017).

Test for Tannins

Extracts were treated with 1mL of 5% ferric chloride. The presence of tannin was indicated by the formation of bluish black or greenish black precipitate (Chesbrough, 2017).

Test for Flavonoids

Few fragments of magnesium metal ribbon (3-4 pieces) was added to 1 mL of the extracts, followed by drop wise addition of concentrated hydrochloric acid. Formation of pink or red colour indicated the presence of flavonoids (Chesbrough, 2017).

Test for Saponins

The 2 mL of distilled water was added to extracts suspended in ethanol and was shaken vigorously. The formation of profuse foam layer indicated the presence of saponins (Chesbrough, 2017).

Test for Terpenoids

One mL of acetic anhydride and 5 drops of concentrated sulfuric acid (H_2SO_4) was added to the extracts. A colour change from violet to blue confirms the presence of steroids and formation of blue-green ring indicates the presence of terpenoids (Chesbrough, 2017).

Determination of Antibacterial Activity of *Psidium guajava* leaves

Antibacterial activity of crude extracts (aqueous, methanol and ethanol) of *P. guajava* leaves was carried out using cup-plate agar diffusion bioassay as follows; 100 μL of fresh culture (Standardized to 0.5 McFarland) was spread uniformly on a sterile Mueller-Hinton agar (MHA) plates and allowed to air dry. After that, wells of 6 mm in diameter were made in the MHA plates using a sterilized cup-borer and the base was seeded with molten MHA and approximately 100 μL for each concentration (50 mg/L, 25 mg/L, 12.5 mg/L, 6.25 mg/L, 3.125 mg/L, 1.56 mg/L and 0.78 mg/L) of the extract was dispensed into the wells and the plates were allowed to stand for 1 hr at room temperature for pre-diffusion and then incubated at 37 °C for 24 hours and the diameter zone of inhibition against the test strain is measured and recorded. Ciprofloxacin (5μg) was used as control (Biswas *et al*., 2013)

Data analysis

The data gotten during this laboratory study were subjected to analysis of variance (ANOVA), and the Fisher's Least Significant Different (FLSD) was used to ascertain the significance between the different treatment means at 5% level of probability (Adeniran *et al*., 2012).

RESULTS

Data from the experiment was computed, analysed and presented in the tables below. Table 1 presents the phytochemical screening of *Psidium guajava* extracts. Phenols were present at all concentrations (100, 80 and 60 mg). Alkaloids were not present while flavonoids and saponins were all effective at all concentrations. Tannins were potent at two concentrations (80 and 60mg), while it was not effective at 100mg.

Table 2 shows the effect of the N-hexane and methanolic extracts of *Psidium guajava* extracts on some selected microorganisms. The effect of these

Table 1: Phytochemical Screening of *Psidium guajava*

Phytochemical	100	80	60
Phenol	+	+	+
Alkaloids	-	-	-
Flavonoids	+	+	+
Tanin	-	+	+
Saponin	+	+	+

Table 2: Effects of N-hexane and Methanolic Extracts of *Psidium guajava* on Some Selected Microorganisms

Extract	Zones of Inhibition (mm)			
	Klebsiella sp	E. coli	S. aureus	Salmonella typhi
0.0% N-hexane/100% Methanol	19.00±2.00	-	17.67±0.58	
20% N-hexane/80% Methanol	18.33±0.58	-	16.00±1.00	-
60% N-hexane/40% Methanol	21.67±2.08	-	16.67±1.53	-
FLSD (P<0.05)	NS		NS	

Values are mean ± Standard Deviation

NS = No significant difference

FLSD = Fisher's Least Significant Difference

extracts on *Klebsiella sp.* were slightly higher at 60% N-hexane/40% methanol concentration than 0.0% N-hexane/100% methanol concentration but with no significant difference between them (P=0.213). The effect of 0.0% N-hexane/100% methanol was also observed to be slightly higher than the effect at 20% N-hexane/80% methanol. However, there was also no significant difference between them (P=0.116).

An evaluation of the effect of the extracts on *S. aureus* also showed a slightly higher concentration at 0.0% N-hexane/100% methanol concentration(solvent) (17.67±0.58mm) than at 60% N-hexane/40% Methanol concentration (16.67±1.53mm) with no significant difference between them (P=0.544). No significant difference was also observed when compared with the effect at 20% N-hexane/80% Methanol concentration (P=0.234). It was further observed that the extracts showed no activity against *E. coli* and *Salmonella typhi* respectively.

Table 3 shows the minimum inhibitory concentration and the Maximum Bacterial Concentration of the plant extracts for the respective organisms. It was observed that the MIC for *Klebsiella sp*. was the same for *S. aureus* (P=1.000) while the MBC for *S. aureus* was slightly higher (50.00±19.80) than that of *Klebsiella sp* (25.00±0.00). There was however, no significant difference between them (P>0.05).

Table 3: Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration of the Plant Extracts

Test Organisms	MIC	MBC
Klebsiella sp	25.00±1.41	25.00±0.00
Salmonella sp	-	-
E. coli	-	-
S. aureus	25.00±0.00	50.00±19.80

Values are Mean ± SD in 2 replications

- NS = Not significant
- FLSD = Fisher's Least Significant Differenc

DISCUSSION

This study was carried out to determine the phytochemical and antibacterial activity of *Psidium guajava* (Guava) extracts on bacterial isolates of *Klebsiella sp., E. coli, S. aureus, Salmonella typhi*. The results of the phytochemical screening in this study suggest that the antibacterial efficacy of *Psidium guajava* extracts could be due to the activities of phenols, flavonoids, tannins and saponins which were observed to possess potent characteristics that present them as the active ingredients in the extracts of guava. The antibacterial activity in extracts of Guava (Psidium guajava) can be attributed to various reasons and characteristics, including:

1. Presence of flavonoids: Guava extracts contain flavonoids like quercetin, kaempferol, and rutin, which have been shown to exhibit antibacterial properties.
2. Phenolic compounds: Guava extracts contain phenolic compounds like gallic acid, ellagic acid, and ferulic acid, which have antimicrobial properties.
3. Tannins: Guava extracts contain tannins, which can bind to bacterial cell walls, inhibiting their growth.
4. Essential oils: Guava extracts contain essential oils like limonene, beta-

caryophyllene, and alpha-pinene, which have antimicrobial properties.

5. Presence of triterpenoids: Guava extracts contain triterpenoids like ursolic acid and oleanolic acid, which have antimicrobial properties.

The findings from this study closely align with the work of Henry *et al*. (2020) who reported that flavonoids and other phytochemicals played an important role in the inhibition of bacterial growth.

The results from this study also correspond to the works of Ismail *et al*. (2012) who stated that the antibacterial potential exhibited by guava leaves extract may be as result of the presence of Flavonoids found in the preliminary phytochemical investigation in their research. The result also conforms to the work of Abdullah *et al*. (2019) who reported the presence of Alkaloids, saponin, phenol, flavonoids, glycosides, anthraquinones, terpenoid and tannin in their study.

The zones of inhibition reported in this study closely align with the work of Abdullah *et al*. (2019) who reported an average zone of inhibition of 16.05mmmm which is particularly close to the findings in our study for *Staphylococcus aureus*. The findings in our study however contrasts with theirs in that there was no inhibition while their study reported inhibition zones of up to 15.21mm. The study also aligns with the works of Biswan *et al*. (2013) who reported mean inhibition rates of 12.3mm and 11.0mm in methanol and ethanol extracts against isolates of *Staphylococcus aureus.*

The findings from this study observed antimicrobial activity against *Klebsiella sp.* and *S. aureus*. The strong relationship between concentration and the activity reported in this study agrees with the findings of Egga *et al*. (2014) who reported a concentration-dependent antimicrobial activity of *Psidium guajava* extracts against the tested organisms. This agrees with the finding of Das and Goswani (2019) who stated that the growth of gram-positive organisms was inhibited strongly by the extracts. The findings from this study however disagrees with the former in that the growth of Gram-negative bacteria also showed strong sensitivity in this study unlike the results from their finding which suggested a lower sensitivity.

CONCLUSION

The findings in this study suggest that the phytochemical properties of Guava play a very vital role in the inhibition of bacterial growth. The study also discovered a significant relationship between extract concentration and the zones of inhibition at 60% N-hexane/40% methanol for *Klebsiella sp*., and 0.0% N-hexane/100% methanol for *S. aureus.* Flavonoids, tannins, saponins and phenols are the active compounds in guava that helped in the inhibition of growth on the sampled isolates.

RECOMMENDATIONS

From the findings in this study, it is recommended that:

i. Extracts of guava be used as potent medicines for inhibiting the growth of *Klebsiella* and *Staphylococcus aureus*.
ii. The concentration of extracts be taken into consideration while preparing guava for better potency.
iii. More research be carried out on the potency of guava extracts on other gram positive and negative bacteria.

REFERENCES

1. Abdullah, D., Zarlis, M., Pardede, A. M. H., Anum, A., Suryani, R., Hidayati, P. I., and Setiyadi, D. (2019, November). Expert System Diagnosing Disease of Honey Guava Using Bayes Method. In *Journal of Physics: Conference Series*, 1361(1):012-054).
2. Adeniran, I., El Harchi, A., Hancox, J. C. and Zhang, H. (2012). Proarrhythmia in KCNJ2-linked short QT syndrome: insights from modelling. *Cardiovascular research*, *94*(1):66-76.
3. Adikwu, P., Oyiwona, E. G., Johnson, A., Awua, Y., Hassan, A. O., Adenugba, T. O. and Ebiega, E. T. (2022). Antibacterial Activity of *Psidium guajava* Leaf and Stem Bark Extracts on Selected Bacteria in Ugbokolo, Benue State, Nigeria. *Advances in Microbiology*, *12*(10):569-578.
4. Balakrishnan, N., Balasubramaniam, A., and Begum, S. (2011). Antibacterial and free radical scavenging activities of stem bark of *Psidium guajava* Linn. *International Journal of Drug Development and Research*, *3*(4):255-260.
5. Birdi, T. J., Daswani, P. G., Brijesh, S. and Tetali, P. (2011). In vitro antigiardial and antirotaviral activity of *Psidium guajava* L. leaves. *Indian Journal of Pharmacology*, *43*(5):616.

6. Biswas, B., Rogers, K., McLaughlin, F., Daniels, D. and Yadav, A. (2013). Antimicrobial activities of leaf extracts of guava (*Psidium guajava* L.) on two gram-negative and gram-positive bacteria. *International Journal of Microbiology*, 13-27.
7. Chesbrough, H. (2017). The future of open innovation: The future of open innovation is more extensive, more collaborative, and more engaged with a wider variety of participants. *Research-Technology Management*, *60*(1), 35-38.
8. Das, M., and Goswami, S. (2019). Antifungal and antibacterial property of guava (*Psidium guajava*) leaf extract: Role of phytochemicals. *International Journal for Health Sciences and Research*, *9*(2):39-45.
9. Egga, E. S., Adeyanju, O. and Agyeno, O. E. (2014). Preliminary phytochemical, antimicrobial and proximate analysis of tender leaves of *psidium guajava* l in jos, plateau state, nigeria.20-30.
10. El-Ahmady, S. H., Ashour, M. L., and Wink, M. (2013). Chemical composition and anti-inflammatory activity of the essential oils of *Psidium guajava* fruits and leaves. *Journal of Essential Oil Research*, *25*(6), 475-481.
11. Firn, R. (2010). Nature's Chemicals. Oxford University Press, Oxford. Pp 74-75
12. Gonçalves, F. A., Andrade Neto, M., Bezerra, J. N., Macrae, A., Sousa, O. V. D., Fonteles-Filho, A. A. and Vieira, R. H. (2008). Antibacterial activity of GUAVA, *Psidium guajava* Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from Seabob shrimp, Xiphopenaeus kroyeri (Heller). *Revista do Instituto de Medicina Tropical de São Paulo*, *50*, 11-15.
13. Henry, K. H., Reuben, E. A., Bright, K. A., David, B., Blessing, N., and Mark, A. (2020). In vitro antibacterial activity of *Psidium guajava* (Guava) leaves extract on carbapenem-resistant *Klebsiella pneumoniae* causing multi-drug resistant systemic infections. *Journal of Medicinal Plants Research*, *14*(9), 475-479.
14. Ibe, C., Maduagwu, N. L., Oladele, R. and Alamir, O. (2015). Antibacterial activity of Psidium guajava Linn. stem bark extracts on multi drug resistant (MDR) community acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). *Education*, 20-28.
15. Ichoron, N., Tyoer, S., James, E. J., and Igoli, J. O. (2019). A survey of medicinal plants used as traditional medicine in Ukum and Ogbadibo Local Government Areas of Benue state, Nigeria. *Plants Environment*, *1*, 5-11.
16. Ismail, M., Minhas, P. S., Khanum, F., Sahana, V. M. and Sowmya, C. (2012). Antibacterial activity of leaves extract of Guava (Psidium Guajava). *International Journal of Research in Pharmaceutical and Biomedical Sciences*, *3*(1), 1-2.
17. Kumar, M., Tomar, M., Amarowicz, R., Saurabh, V., Nair, M. S., Maheshwari, C., and Satankar, V. (2021). Guava (*Psidium guajava* L.) leaves: nutritional composition, phytochemical profile, and health-promoting bioactivities. *Foods*, *10*(4):752.
18. Muhammad, A. A. and Ibrahim, R. B. (2019). Review of Medicinal Plants with Antianaemic Activity Found in Nigeria. *Scholars International Journal of Biochemistry*. 2(8): 225-22
19. Offor, C. E., Ugwu, P. C., Okechukwu, P. M. and Igwenyi, I. O. (2015). Proximate and phytochemical analyses of *Terminalia catappa* leaves. *European Journal of Applied Sciences*, *7*(1), 09-11.
20. Pandey, A. and Shweta. (2011). Antibacterial properties of *Psidium guajava* leaves, fruits and stems against various pathogens. *International Journal of Pharmaceutical Research and Development (IJPRD)*, *3*(11):15-24.
21. Periyasamy, A., and Mahalingam, K. (2010). Phytochemical screening and antimicrobial activity from five Indian medicinal plants against human pathogens. *Middle East Journal of Scientific Research*, *5*(6):477-482.
22. Preethi, R., Devanathan, V. V. and Loganathan, M. (2010). Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. *Advances in biological Research*, *4*(2):122-125.
23. Richard, F. T., Joshua, A. T. and Philips, A. J. (2013). Effect of aqueous extract of leaf and bark of guava (*Psidium guajava*) on fungi *Microsporum gypseum* and *Trichophyton mentagrophytes*, and bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Advancement in Medicinal Plant Research*, *1*(2), 45-48.
24. Sukanya, D. A., Thanet, P. and Chatchai, W. (2017). Alpha-glucosidase inhibitory activity and phytochemical investigation of *Borassus flabellifer* Linn. *African Journal of Pharmacy and Pharmacology*, *11*(3), 45-52.
25. "The World Gazetteer". Archived from the original on 9 February 2013. Retrieved 2 Febuary 2022.
26. Zahidah, W. N. Noriham, A. and Zainon, M. N. (2013). Antioxidant and antimicrobial activities of pink guava leaves and seeds. *Journal of Tropical Agriculture and Food Science*, *41*(1), 53-62.

APPENDIX

Appendix 1: Demonstration of Guava Leaves