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Antibacterial Properties of Ethanolic and Aqueous Leaf Extract of Guava (Psidium guajava) Against Selected Foodborne Pathogens

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Abstract

Psidium guajava, commonly known as guava, is best known for its antimicrobial potential. Rice and kunun aya are starchy, potentially dairy-based products with high moisture content and pH levels, and they also provide a nutrient-rich environment that can support the growth of microorganisms. Therefore, this study was carried out to evaluate the antibacterial activities of Guava leaf extracts against isolated foodborne pathogens in Kaduna State, Nigeria. In this study, Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, and Bacillus spp were isolated and characterized from rice and kunun aya using standard microbiological tests. Thereafter, aqueous and ethanol extractions of the dried guava leaves were carried out using the maceration method. Phytochemical screening of the extract was performed using standard techniques, while antibacterial activity against the isolated bacterium was assessed using the Agar well diffusion method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using a standard microbiological technique. Phytochemistry of the ethanol extract showed the presence of tannins, saponins, alkaloids, phenols, steroids, terpenoids, cardiac glycosides, and flavonoids, while the aqueous extract showed the presence of tannins, alkaloids, phenols, steroids, terpenoids, cardiac glycosides, and flavonoids. The antibacterial potential of ethanol and aqueous extracts was effectively demonstrated against isolated microorganisms. Staphylococcusus species were the most susceptible, exhibiting a notable mean zone of inhibition of 25.00±0.82mm when treated with the ethanol extract. Escherichia coli also responded significantly, showing a mean zone of inhibition of 23.75±0.50 mm with the same extract. The aqueous extract proved effective as well; Staphylococcus spp. displayed an inhibition zone of 23.00±0.60 mm, while Escherichia coli presented a slightly lower zone of 21.50±0.58mm.In contrast, Bacillus spp showed complete resistance, with no inhibition observed for either extract. The MIC shows 25mg/ml for Staphylococcus spp. and 50mg/ml for E. coli for the ethanol and aqueous extract, and the MBC shows 50mg/ml for Staphylococcus spp and 100mg/ml for E. coli for the aqueous extract and 25mg/ml and 50mg/ml for ethanol extracts. These findings underscore the potential of these extracts as antibacterial agents and provide a foundation for further research into their applications in combating bacterial infections.

Keywords: Antimicrobial, Extracts, Resistance, Phytochemistry

INTRODUCTION

Psidium guajava Linn is commonly known as guava. It is a tropical shrub and food plant belonging to the Myrtaceae family (Ravi and Divyashree, 2014). It grows up to 10 meters and is widely distributed in many countries. *Psidium guajava Linn*. is an economically important food plant with diverse medicinal properties. It has a short trunk, patchy, smooth, and peeling bark. The leaves are fleshy, dark green, and have prominent veins. It has white flowers, and the fruit contains pulp and small hard seeds (Morais-Braga *et al.*, 2016).

In ethnomedicine, the various parts of *P. guajava* – the stem, bark, fruits, leaves, and roots are used in the treatment of diseases such as diarrhea, rheumatism, and diabetes (Gutiérrez *et al.*, 2008), digestive problems, laryngitis, ulcers, malaria, cough, and bacterial infections (Ravi and Divyashree, 2014), wound healing, and pain relief (Metwally *et al.*, 2010). Many natives consume decoctions, infusions, and/or boiled preparations of *P. guajava*, either orally or topically, depending on the type of illness. For instance, *P. guajava* leaves can be applied to wounds, whereas aqueous leaf extract can be orally consumed to lower the blood

glucose level in diabetic patients (Gutierrez *et al.*, 2008). In several studies, guava showed significant antibacterial activity against common food-borne diarrhea-causing bacteria, including *Salmonella* spp., *E. coli*, *Shigella* spp., and *Staphylococcus* spp. (Gutierrez *et al.*, 2008).

Psidium guajava consists of important chemical constituents such as flavonoids, tannins, phenols, alkaloids, triterpenes, saponins, carotenoids, lectins, vitamins, carbohydrates, fatty acids, and glycosides (Gutiérrez *et al.*, 2008). The leaf of the guava, locally known as 'bayabas, ' is a plant of the family Myrtaceae. Research studies have shown that almost all of its parts have medicinal values. The phytochemicals of the plant show a potential for antibacterial activity. The leaves contain a plethora of beneficial phenolic compounds, including guaijaverin, quercetin, kaempferol, apigenin, catechin, chlorogenic acid, hyperin, gallic acid, epicatechin, myricetin, caffeic acid, and epigallocatechin gallate (Ojewole, 2005).

Chen *et al.* (2015) posited that guava leaf aqueous extract reduces blood glucose levels and accelerates plasma insulin levels. The leaf and bark extracts of *P. guajava* effectively enhanced glucose uptake in muscle cells and inhibited α-amylase activity. Guava is a widely cultivated tropical fruit found in numerous tropical and subtropical areas. The common guava (*Psidium guajava*), also known as lemon guava or apple guava, is a small tree belonging to the myrtle family (Myrtaceae) and is indigenous to Mexico, Central America, the Caribbean, and northern South America. The term guava also refers to other species within the genus Psidium, including the strawberry guava (*Psidium cattleyanum*) and the pineapple guava (*Feijoa sellowiana*). In 2009, global production of guava reached 55 million tons, with India accounting for 45% of this total. From a botanical perspective, guavas are classified as berries. (Morton, 1987).

Foodborne illnesses stemming from insidious pathogens like *E. coli*, *Salmonella*, *Staphylococcus*, *Shigella*, and *Pseudomonas* continue to cast a long shadow over public health, with millions of cases reported annually around the globe. The reckless and widespread misuse of standard antibiotics has sparked the emergence of antibiotic-resistant strains, complicating treatment efforts and endangering lives. In this context, it is vital to seek innovative and alternative strategies to effectively combat these relentless foodborne adversaries. Among nature's potential solutions, guava leaf extract stands out, showcasing remarkable antibacterial properties. However, its full potency against foodborne pathogens and its promise as a natural antimicrobial agent have yet to be thoroughly explored. Now is the critical moment to investigate this extraordinary plant and unlock its potential to safeguard our health and well-being (Banu and Sujatha, 2012).

Guava leaves are known for their vibrant green color and are valued in traditional medicine for various health benefits. They are effective in treating kidney inflammation, managing gastroenteritis, and addressing diarrhea in children caused by infections. Their therapeutic effects come from high dietary fiber, which improves digestion and regulates bowel movements. Additionally, guava leaves have strong antimicrobial properties that help fight harmful pathogens, thanks to their rich content of vitamin C and other phytochemicals (Farhana *et al.*, 2017).

In many cultures, guava leaves are also used for their antiseptic qualities to treat hypertension, pain, fever, and respiratory disorders, supporting overall health (Deguchi and Miyazaki, 2010). This research aimed to determine the antibacterial properties of ethanolic and aqueous leaf extract of guava (*Psidium guajava*) against selected foodborne pathogens such as *Staphylococcus aureus, Staphylococcus epidermis, Escherichia coli, and Bacillus* spp.

MATERIALS AND METHODS

Plant Collection and Leaf Extraction

Fresh leaves of *Psidium guajava* (guava) were collected from Nupawa Road, Mahuta, Kaduna, and authenticated at Kaduna State University, Department of Biological Sciences (Voucher No. KASU/BSH/318). The leaves were thoroughly washed, cut into smaller pieces, and air-dried at room temperature for 14 days before being ground into coarse powder and stored in airtight containers. Extraction was carried out following the procedure of Bengum (2014), where 50 g of the powdered guava leaf was soaked separately in 500 ml of ethanol and water for 72 hours. The mixtures were then filtered using sterilized Whatman filter paper, and the filtrates were evaporated in a water bath to obtain the crude extracts.

Phytochemical Screening

Phytochemical screening of the extracts was conducted using the method of Aliyu et al. (2021) to identify key bioactive compounds,

including alkaloids, saponins, flavonoids, cardiac glycosides, terpenoids, steroids, phenols, and tannins. The aqueous and ethanol extracts were appropriately diluted before analysis, and standard qualitative tests were performed, with positive results indicated by characteristic color changes or frothing. These included green coloration for alkaloids, persistent froth for saponins, blue or green coloration for phenols, green precipitate or blue-black coloration for tannins, pink or reddish coloration for flavonoids, a brown ring for cardiac glycosides, blue-green coloration for steroids, and a reddish-brown interface for terpenoids, indicate the presence or absence of these phytochemicals in the extracts.

Collection of Samples and Isolation of Microorganisms

Cooked rice and *kunun aya* samples were aseptically collected from multiple locations within Kaduna metropolis using sterile containers. Microbial isolation was performed following the protocol described by Cheesbrough (2017). Briefly, samples were subjected to six-fold serial dilution in sterile peptone water. One gram of rice or 1 mL of homogenized *kunun aya* was added to 9 mL of diluent to obtain a 10^{-1} dilution, followed by successive dilutions up to 10^{-6}. Aliquots (0.1 mL) from the 10^{-4} dilution were inoculated onto Nutrient Agar, Mannitol Salt Agar, and MacConkey Agar plates using the spread plate technique and evenly distributed with a sterile glass rod. Plates were incubated at 37 °C for 24 h. Following incubation, colonies were examined, and morphologically distinct isolates were subcultured onto fresh media to obtain pure cultures. Purified isolates were maintained on agar slants and subjected to microscopic examination and biochemical characterization using standard microbiological methods.

Gram Staining and Biochemical Characterization

Gram staining and biochemical characterization of bacterial isolates were performed following standard microbiological procedures (Cheesbrough, 2017). For Gram staining, thin smears were prepared on clean, grease-free slides, air-dried, heat-fixed, and sequentially stained with crystal violet, Lugol's iodine, decolorized with 95% ethanol, and counterstained with safranin. Slides were examined under oil immersion (×100 objective) to determine Gram reaction and cellular morphology. Biochemical identification of isolates was carried out using conventional assays. Catalase activity was determined by the production of bubbles upon exposure to 3% hydrogen peroxide. Coagulase activity was confirmed by clot formation in serum. Oxidase activity was assessed by the development of a dark purple coloration on oxidase reagent-impregnated filter paper. Voges-Proskauer test was performed using glucose phosphate peptone broth, with a pink-red coloration indicating a positive reaction after the addition of reagents. Endospore staining was conducted using malachite green and safranin, with endospores appearing green under microscopy. Methyl red test indicated acid production by a red color change, while indole production was confirmed by the formation of a red ring following the addition of Kovac's reagent.

Antibacterial Activity

Agar Well Diffusion Assay

The agar well diffusion method was performed according to Anibijuwan and Udeze (2009) with slight modifications. A standardized inoculum (0.5 McFarland) was prepared and uniformly inoculated onto Mueller-Hinton agar plates. Wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Each well was filled with 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL concentrations of plant extract. Distilled water served as the negative control, while ciprofloxacin-impregnated discs were used as the positive control. Plates were allowed to stand for 1 h at room temperature to facilitate pre-diffusion, then incubated at 37 °C for 24 h. After incubation, zones of inhibition were measured in millimeters using a ruler, and antibacterial activity was determined based on the diameter of clear zones around each well.

Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined using a broth microdilution method according to Ali et al. (2017) and Aliyu et al. (2021), with modifications based on Sanches et al. (2005). Test organisms were standardized to 0.5 McFarland turbidity prior to inoculation. Serial extract concentrations (100, 50, 25, and 12.5 µg/mL) were prepared in nutrient broth (5 mL per tube) and inoculated with standardized bacterial suspensions. Tubes were incubated at 37 °C for 24 h. MIC was defined as the lowest concentration exhibiting no visible growth, as indicated by absence or marked reduction in turbidity.

Minimum Bactericidal Concentration (MBC)

The MBC was determined by subculturing 0.1 mL aliquots from tubes showing no visible growth onto Mueller–Hinton agar plates. Plates were incubated at 37 °C for 24 h, and bacterial growth was assessed. The MBC was defined as the lowest extract concentration resulting in no observable growth (≥99% reduction in viable cells) on subculture.

RESULTS

The phytochemical constituents of aqueous and ethanol extracts of *Psidium guajava* leaves are presented in Table 1. Both extracts contained alkaloids, saponins, phenols, tannins, flavonoids, glycosides, steroids, and terpenoids. The cultural and biochemical properties of bacterial isolates obtained from cooked rice and *kunun aya* are summarized in Table 2. *Staphylococcus aureus* appeared as Gram-positive cocci with golden-yellow colonies and was positive for catalase, coagulase, and Voges-Proskauer tests. *Escherichia coli* was a Gram-negative rod producing large, smooth, mucoid colonies and was positive for catalase, indole, methyl red, and motility tests. *Bacillus* spp. were Gram-positive rods with rough, opaque colonies and showed catalase positivity, endospore formation, methyl red, Voges-Proskauer reactions, and motility. *Staphylococcus epidermidis* was Gram-positive cocci with creamy white

Table 1: Phytochemical Constituents of Ethanol and Aqueous Leaf of *P. guajava* Extract

Phytochemical Components	Ethanol Leaf Extract	Aqueous Leaf Extract
Alkaloids	+	+
Saponins	+	–
Phenols	+	+
Flavonoids	+	+
Tannins	+	+
Glycosides	+	+
Steroids	+	+
Terpenoids	+	+

Key: + = Detected, - = Not detected

Table 2: Cultural and Biochemical Characteristics of the Isolates from Rice and Kunun aya

Gram Reaction	Cell/Colony Morphology	Cat	Coa	Oxi	Endo	Ind	Meth	Mot	Vog	Probable Organism
Negative	Rod; pinkish, smooth, large, mucoid colonies	+	–	–	–	+	+	+	–	*Escherichia coli*
Positive	Rod; creamy, rough, large, opaque colonies	+	–	–	+	–	+	+	+	*Bacillus* spp.
Positive	Cocci; golden yellow, circular, smooth, mucoid	+	+	–	–	–	–	–	+	*Staphylococcus aureus*
Positive	Cocci; creamy white, circular, smooth, moist	+	–	–	–	–	–	–	+	*Staphylococcus epidermidis*

Key: + = Positive results, - = Negative results, Cat = Catalase, Coa = Coagulase, Oxi = Oxidase, Endo = Endospore, Ind = Indole, Meth = Methyl red, Mot = Motility, Vog = Voges-proskauer

The antibacterial activity of aqueous and ethanol extracts of *P. guajava* leaves (100–12.5 mg/mL) against the test isolates is presented in Table 3. Activity was concentration-dependent, with the highest inhibition recorded at 100 mg/mL. The ethanol extract showed greater zones of inhibition than the aqueous extract against all organisms. The highest inhibition was observed against *S. aureus* (24.00 ± 0.82 mm), followed by *S. epidermidis* (25.00 ± 0.82 mm) and *E. coli* (23.75 ± 0.50 mm). Overall, Gram-positive bacteria exhibited higher susceptibility compared to *E. coli*.

MIC and MBC values are presented in Table 4. For *Staphylococcus* spp., the MIC was 25 mg/mL for both aqueous and ethanol extracts, while for *E. coli* it was 50 mg/mL. MBC values indicated

bactericidal activity at 50 mg/mL (aqueous) and 25 mg/mL (ethanol) for *Staphylococcus* spp., and 100 mg/mL (aqueous) and 50 mg/mL (ethanol) for *E. coli*. Ethanol extracts demonstrated higher bactericidal potency than aqueous extracts.

Table 3: Antibacterial Activity of Guava Leaf Extract of Ethanol and Aqueous on *Staphylococcus* spp. and *E. coli* Isolates

Isolates	*S. aureus*	*S. epidermidis*	*E. coli*
Control (Ciprofloxacin)	26.00 ± 0.00	30.00 ± 0.70	24.00 ± 2.06
ALE 100	22.00 ± 0.82	23.00 ± 0.60	21.50 ± 0.58
ALE 50	20.00 ± 0.82	21.00 ± 0.60	17.75 ± 0.96
ALE 25	16.50 ± 5.80	16.50 ± 5.80	10.75 ± 1.71
ALE 12.5	9.25 ± 0.50	9.50 ± 0.82	7.25 ± 1.50
ELE 100	24.00 ± 0.82	25.00 ± 0.82	23.75 ± 0.50
ELE 50	22.25 ± 1.26	24.00 ± 0.82	20.00 ± 0.82
ELE 25	17.50 ± 1.29	17.50 ± 1.29	11.75 ± 0.96
ELE 12.5	11.00 ± 0.82	11.00 ± 0.82	9.25 ± 2.50
x^2	0.109	0	0.646
p-value	0.99	1	0.59

**Statistically significant association exists at p≤0.05
Key: ALE = Aqueous Leaf Extract, ELE = Ethanol Leaf Extract, mm = Millimeter, Values in mm = Mean, SD= Standard Deviation
Interpretation (CLSI, 2020): ≥15-20mm = Susceptible; 8-14mm = Intermediate; ≤7mm = Resistant.

Table 4: The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Ethanol and Aqueous Leaf of *P. guajava* Extract Against the Bacterial Isolates.

Bacterial Isolates	Solvent	MIC (mg/ml)	MBC (mg/ml)
Staphylococcus spp.	Aqueous	25	50
	Ethanol	25	25
Escherichia coli	Aqueous	50	100
	Ethanol	50	50

DISCUSSION

The phytochemical test results shows that, the ethanol extract has a positive result for alkaloids, saponins, phenols, flavonoids, tannins, glycosides, steroids and terpenoids tests, while the aqueous extract has a positive result for alkaloids, phenols, flavonoids, tannins, glycosides, steroids, terpenoids and a negative result for saponins, which can be possible due to the different concentrations and solvent used to dilute the leaf, this is similar to that obtained by other researchers, Anand *et al*. (2016) and Rao *et al*. (2017).

Staphylococcus spp. *E. coli* and *Bacillus* spp. were isolated from rice and kunun aya. Rice and kunun aya are starchy and potentially dairy-based products with high moisture content, pH level, and also provide a nutrient-rich environment that can support the growth of microorganisms like *Staphylococcus* spp, *Bacillus* spp, and *E. coli*. These factors contributed to the isolation of *Staphylococcus* spp. *E. coli*, and *Bacillus* spp. Contamination sources such as water used in processing the food, ingredients used, environmental contamination (like soil, air), human handling, and cross-contamination (utensils, skin, etc.) can increase the rate of contamination by these organisms. This can be prevented by taking precautions and proper personal hygiene when preparing the food, as reported by Ali *et al*. (2017).

The results for the ethanolic extract show that the 100, 50, and 25mg/ml concentrations of *P. guajava* leaf extracts showed inhibitory effects on *Staphylococcus* spp., and 100 and 50mg/ml showed Inhibitory effects on *E. coli*. In this research, *Staphylococcus* spp. were more inhibited by the plant extract compared to *E. coli*; this might be due to the difference in their cell wall composition.

The extract wasn't effective on *Bacillus* spp., which may be a result of the ability of *Bacillus spp*. spore-forming, which can make them resistant, and their cell wall structure, which helps to protect them against antimicrobial compounds found in guava leaf. It can also be due to insufficient concentration of active compounds in the guava leaf, which is too low to effectively inhibit *Bacillus* spp. as reported by Farhana *et al*. (2017).

The results for the ethanolic extract of the plant show that the ethanol extract was more effective than the aqueous leaf extract against

the *Staphylococcus* spp. having the highest mean zone of inhibition of 25.00±0.82mm, and *E. coli* having the highest zone of inhibition of 23.75±0.50mm, as shown on the ethanol extract. This is possible because ethanol has a broader solubility range, higher extraction efficiency, has antimicrobial properties, and is less prone to spoilage and degradation. Ethanol also reduces toxicity and has a lower risk of contamination. Ethanol is easier to concentrate and is more versatile, so ethanol is more recommended for plant extraction as reported by Azmir *et al.* (2013), and Dakappa *et al.* (2013).

The aqueous extract inhibited less compared to the ethanol extract, with the *Staphylococcus* spp. having the highest mean zone of inhibition of 23.00±0.60mm, and *E. coli* having the highest mean zone of inhibition of 21.50±0.58mm. Chi-square analysis showed no statistically significant difference in the antibacterial activity between the aqueous and ethanolic guava leaf extracts against the isolates. This means there was no statistically significant association between the extract type and antibacterial activity against the isolates. Both aqueous and ethanol guava leaf extracts possess significant antibacterial activity against the isolates.

To effectively combat bacterial growth, rigorous Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) tests were conducted to identify the precise extract concentrations required for inhibition or eradication. The findings of this research were compelling: the ethanol extract demonstrated a MIC of just 25 mg/ml against *Staphylococcus* spp. and 50 mg/ml for *E. coli*. Even more promising, the aqueous extract achieved a MIC of 50 mg/ml for *E. coli* while also reaching 25 mg/ml for *Staphylococcus* spp. These results highlight the potential of these extracts as powerful agents in combating bacterial infections.

The Minimum Bactericidal Concentration (MBC) values for the ethanol extract are 25mg/ml for *Staphylococcus* spp. and 50mg/ml for *E. coli*, while the MBC results for the aqueous extract show 50mg/ml for *Staphylococcus spp*. and 100mg/ml for *E. coli*.

CONCLUSION

Alkaloids, saponins, phenol, flavonoids, tannin, cardiac glycosides, steroids, acetic, and terpenoids were the phytochemicals detected in the extracts. At concentrations of 100 mg/ml and 50 mg/ml, the research findings demonstrated that both aqueous and ethanol extracts of *P. guajava* leaf exhibited strong antibacterial activity against *Staphylococcus aureus*, *Staphylococcus epidermis,* and *E. coli*, with the ethanol extracts exhibiting a greater inhibition than the aqueous extract. However, the extracts did not show any antibacterial activity against *Bacillus* spp. meaning that the leaf of *Psidium guajava* did not inhibit or kill the species of *Bacillus*. The results of the extract work on Gram-positive (*Staphylococcus* spp.) bacteria more compared to the Gram-negative (*E. coli*) bacteria, signifying that *Psidium guajava* leaf extract has a significant antibacterial activity against *Staphylococcus* spp. and *E. coli*. Based on the result obtained, *Psidium guajava* leaf can be used as a drug in the treatment of infections caused by *S. aureus, S. epidermis,* and *E. coli,* such as diarrhea, gastroenteritis, food poisoning, vomiting, and abdominal cramps from consuming contaminated food, etc.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTION

Author A designed the study and carried out all the laboratory investigations. B and C- reviewed the protocols and procedures, and made the final corrections with Author A.

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