Determination of Phytochemicals and Anti-Bacterial Properties Evaluation of the Leaves Extracts of *Psidium guajava* (L) Myrtaceae

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**ABSTRACT**

The current study sought to assess the phytochemicals and antimicrobial activities of *Psidium guajava* Linnaeus leaves extracts. The contents of healthy free fresh leaves of *Psidium guajava* Linnaeus were analyzed for antibacterial efficacy using dichloromethane (DCM), palm wine, and n-hexane solvents. The presence of several chemical components was revealed by phytochemical analysis of the extracts. The Agar disc diffusion method was used to assess antibacterial activity against five clinically significant organisms. The extract's zone of inhibition against each organism was measured. The presence of phytochemicals such as tannins, terpenoids, flavonoids, alkaloids, and phenol were discovered. Saponin was not found in all of the solvents tested. The antibacterial results showed that the n-hexane fraction had 44 mm as zone inhibition against *Bacillus subtilis* at 25 mg/ml, followed by 42 mm in *Escherichia coli* at the same concentration, which was significantly higher than the standard amoxicillin at 25 ug. The highest zone of inhibition for DCM fraction was at 25 mg/ml (35 mm) and 10 mg/ml (25 mm) against *Pseudomonas aeruginosa* and *E. coli*, respectively, with similar zones of inhibition to the standard. Finally, resistance was observed in *P. aeruginosa*, but significant inhibition was observed against *B. subtilis* (30 mm), *E. coli* (15 mm), and *S. aureus* (15 mm) at 15%. As a result, *Psidium guajava* leaf extracts contain phytochemicals as well as significant antibacterial properties, particularly against *B. subtilis* and *E. coli*, and can be used to obtain useful lead compounds for the eventual synthesis of medicinally significant antioxidant and antibacterial agents.

**Keywords:** Antibacterial, Disc diffusion, maceration, phytochemicals, *Psidium guajava*.

I. INTRODUCTION

Ethnomedicine is the study of traditional medical practice, which is an important part of indigenous peoples' culture and health interpretation in many parts of the world [1]. Concerns are growing around the world about the rising cost of synthetic drugs, along with their toxicological profile and uncertain efficacy [2]. These anomalies compel researchers to investigate alternative drug sources made from medicinal plants which have few or no known side effects when used to treat microbial infections. In medical science, folk medicine, and as chemical entities or frameworks for synthetic drugs, medicinal herbs are the most abundant bio-resource of drugs [3]. The identification of medically significant metabolites in widespread and prevalent plants would reduce the excessive usage of well-known, rare medicinal plants [4].

Aspirin, atropine, digoxin, ephedrine, morphine, quinine, reserpine, vincristine, and vinblastine are all plant-derived drugs, as are many plants' steroidal sapogenins that act as semi-synthetic intermediates to the steroidal drugs [5].

*Psidium guajava* tree leaves and bark have a long history of traditional medical applications that are still used presently [6]. The leaves, stems, bark, and roots of *P. guajava* contain over 20 substances [7].

Guava leaves contain cineol, tannins, triterpenes, flavonoids, eugenol, malic acid, chlorophyll, mineral salts, and a variety of other fixed substances. [8]. Carotenoids and polyphenols such as galloacetin and leucocyanidin are also found in guava leaves [9]. Among the phytochemicals found in guava are polysaccharides, vitamins, essential oils, mineral deposits, enzymes, proteins, sesquiterpenoids, triterpenoids [10], [11], alkaloids, glycosides, steroids, flavonoids, tannins, and saponins [12]-[14]. Tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins,
vitrahs, fiber, and fatty acids are present in high percentages. Antibacterial activity has been demonstrated for flavonoids. Quercetin is thought to help guava’s anti-diarrhoea effect by relaxing the intestinal smooth muscle and preventing bowel contractions. Anti-spasmodic activity is also demonstrated by other flavonoids and triterpenes found in guava leaves [9]. Guava’s antioxidant properties are due to the polyphenols found in the leaves [12]-[14]. The leaves were used as an antibiotic in the form of a poultice or decoction in the United States for wounds, ulcers, and toothache. Guava fruits contain a lot of Vitamin C, iron, calcium, and phosphorus [7]. P. guajava has been shown to have antimicrobial, anti-inflammatory, antimalarial, and anti-hyperglycemic properties [15]. It has been used to treat wounds, acne, cough, diabetes, and hypertension [16]. Staphylococcus aureus, Bacillus cereus, Proteus species, Pseudomonas aeruginosa, Salmonella enteritidis, Streptococcus mutans, Escherichia coli, and Shigella species have all been shown to be inhibited by Psidium guajava leaves [12].

Plant phytochemical constituents and antimicrobial activities vary due to biochemical reactions within species, geographical location, extraction methods, and solvent used for extraction, among other factors. The fact is that phytochemical constituents differ due to geographical location [18].

Many studies on the antibacterial properties of guava essential oil have been conducted. They have various mechanisms of action in inhibiting microorganisms’ actions. These oils and extracts have been shown to permeate the lipid bilayer of the cell membrane, attempting to make it more permeable and enabling vital microorganisms’ cell content to leak [8]. Furthermore, because of the presence of flavonols, guava leaf tea aided in the control of influenza virus growth, including oseltamivir-resistant strains, by preventing viral entry into host cells [17], [20]. The current study aimed to determine the presence of various phytochemicals as well as the antimicrobial activities of P. guajava extracts on Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Bacillus subtilis.

II. Procedure

A. Plant Collection

Fresh white and pink Psidium guajava leaves were collected from Ogbiri Community, Sagbama Local Government Area, Bayelsa State, Nigeria. It was identified and authenticated at Niger Delta University’s Department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, Wilberforce Island, Bayelsa State, Nigeria, and processed according to Abubakar and Haque's instructions [21]. The plant was washed with running water and allowed to dry at room temperature. To produce small-size materials, the dry specimen was cut into smaller particles and air-dried at ambient temperature for 3 days before pulverizing. 200 g of the powdered plant was obtained from the leaves, from which 50 g was used for the different solvents’ extraction processes, respectively.

B. Plant Extraction

N-hexane, DCM, palm wine, and aqueous (water), were used for the extraction following the procedure by Abubakar and Haque [21]. About 50 g of the powdered leaves were weighed and dissolved in each solvent separately, allowed to stand for four days in a clean jar. After filtering with sterile cotton wool, the extracts were evaporated to dryness using a rotary vacuum evaporator and a water bath. The solid residues were reconstituted in ethanol and stored at 4 °C in the refrigerator till further use.

C. Phytochemical Analysis

Using qualitative standard methods, each extract was accessed for the presence of secondary metabolites such as alkaloids, anthraquinonine glycosides, cardiac glycosides, flavonoids, saponins, phenol, tannins, carbohydrates, phlobatannins, and terpenoids [1].

Alkaloids: For 5 minutes, the sample was boiled in 2 ml of hydrochloric acid in a water bath. The mixture was allowed to cool before being filtered. Mayer’s reagent was added to a 1ml portion of the filtrate. The presence of alkaloids is indicated by a creamy white precipitate.

Tannins: In a test tube, the sample was boiled in 20 ml of distilled water and then filtered. A few drops of 0.1% ferric acid were added, and the presence of tannins was indicated by a brownish-green coloration.

Saponin: The sample was boiled in 20 ml of water that had been distilled in a water bath and then filtered. 10 ml of the filtrate was shaken vigorously with 5 ml of distilled water to generate froth, which indicates the presence of saponin.

Flavonoids: 5 ml of 10% dilute ammonia solution was added to a portion of the plant extract's aqueous filtrate, followed by the addition of concentrated H2SO4. The presence of flavonoids is indicated by the extract's yellow coloration.

 Phenol: Phenol causes red litmus paper to turn blue.

Terpenoids: The Salkowski test was used to detect terpenoids. Extract (5 ml) was combined with chloroform (2 ml) and concentrated sulphuric acid to form a layer (3 ml). The presence of terpenoids was indicated by the formation of a reddish-brown coloration of the interface.

![Chemical constituents of Psidium guajava L.](image-url)
D. Antibacterial Screening

As test organisms, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, and Bacillus subtilis were used. The organisms were obtained from Niger Delta University's Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmacy. These organisms were streaked on agar plates and incubated at 37 °C overnight before being stored at 4 °C.

To standardize it, a small colony of the overnight culture was transferred into a bottle containing 2 ml of normal saline to make it turbid and compared to the McFarland standard of 0.5. The extracts' antibacterial activity was tested using overnight cultures of the tested bacteria. The test organisms were seeded into an already solidified sensitivity test medium on Petri dishes using a sterile swab stick (spread plate method), and three holes were made using a cork borer (6 mm).

A 50 mg/ml stock solution was used to prepare different concentrations of DCM and n-hexane extracts: 10 mg/ml, 20 mg/ml, and 25 mg/ml. Different percentage (% ) concentrations of palm wine extract 5% v/v, 10% v/v, and 15% v/v were also prepared. After 24 hours of incubation at 37 °C, the zones of inhibition were measured in millimeters using a transparent meter rule. Positive control was an antibiotic sensitivity disc (amoxicillin 25 μg), and the test organisms were also used as controls. Following incubation, observations were made and results were recorded.

III. RESULTS

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>DCM</th>
<th>n-Hexane</th>
<th>Palm Wine</th>
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<tbody>
<tr>
<td>Saponin</td>
<td>++</td>
<td>++ +</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
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<td>Alkaloids</td>
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<td>Flavonoids</td>
<td>++</td>
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<tr>
<td>Terpenoids</td>
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<td>+</td>
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<tr>
<td>Phenol</td>
<td>+</td>
<td>+ +</td>
<td>+</td>
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</tbody>
</table>

Keys: + Slightly Present, ++ Present in Large Quantity, - Not Present

IV. DISCUSSION

Using various solvents, the researchers discovered the presence of some phytochemicals as well as the antimicrobial potential of Psidium guajava. Previous research has found phlobatannins, saponin, flavonoids, steroids, terpenoids, polyphenols, and glycosides in Psidium guajava, but not triterpenoids, alkaloids, or anthraquinone [14]. Phytochemicals are non-nutritive chemical compounds produced by plants to protect themselves, but new research has discovered that they can also prevent humans from certain diseases [23]. Triterpenoids like guaionic acid and guavacoumaric acid were obtained and determined from the leaves of Psidium guajava, which had hitherto been shown to inhibit the growth of Salmonella enteritidis and Bacillus cereus [8]. The extracts of the leaves also contained alkaloids, flavonoids, tannins, phenol, and terpenoids, according to this study. The presence of tannin, terpenoids (h-hexane, DCM, and palm wine extracts), flavonoids (h-hexane and DCM fractions), alkaloids (n-hexane and palm wine extracts), and phenol was discovered in the plant's preliminary qualitative phytochemical analysis (DCM and palm wine extracts). Saponin was not found in all of the solvents tested (Table I).

Sacchetti and colleagues tested ethanol and water extracts of P. guajava leaves, stem, bark, as well as root against gram-positive and gram-negative bacteria, and aqueous extract was discovered to be more active against Staphylococcus aureus than just aqueous extract [23]. Guava leaf aqueous and organic extracts have been shown to inhibit antimicrobial-resistant clinical isolates of Staphylococcus aureus strains [8].

Previous research on the antimicrobial activities of guava leaves on various bacterial strains found that the leaves’ synergistic action with antibiotics increased their antimicrobial property. Protein synthesis, cell wall synthesis, and folic acid target drugs all showed this effect. The latter, on the other hand, did not discover a synergistic effect with gentamicin, potentially because the maceration time was shorter than the duration used and the solvent was different [17]. Thus, in the antibacterial assay, n-hexane and DCM were found to be better solvents for guava leaf antimicrobial constituent extraction. Escherichia coli showed the greatest susceptibility to the extract, with a zone of inhibition of 24 mm at 10 mg, 22 mm at 20 mg, and 42 mm at 25 mg concentration, indicating a high level of susceptibility. Staphylococcus aureus showed the most resistance to the extract at 10 mg, with a zone of inhibition of 10 mm measured at 20 mg, and a zone of inhibition of 14 mm measured at 2 mg, indicating resistance in the n-hexane fraction. Staphylococcus aureus showed very little susceptibility at 10 mg and 20 mg concentrations, but susceptibility at 25 mg concentrations. Bacillus subtilis was found to be very susceptible to DCM extract at a concentration of 25 mg.

At 25 mg concentration, Escherichia coli was also very susceptible. Pseudomonas aeruginosa demonstrated the greatest susceptibility at all percentage concentrations, whereas Bacillus and Escherichia coli demonstrated no susceptibility and thus resistance to the palm wine extract (Table II).

According to the DCM results, Escherichia coli, a gram-negative organism, was the most susceptible, while Staphylococcus was the most resistant. Susceptibility to Pseudomonas aeruginosa was observed with Palm wine extract at all concentrations, indicating that this extract in particular is very potent against this organism and can thus be used in the treatment of diseases caused by this organism such as UTI, Respiratory system infections, Bone and joint infections, and so on [24].

Susceptibility was observed at high concentrations but was very low at low concentrations, indicating that a low dose, this extract may produce little or no effect; thus, when using this solvent for guava leaf extraction, a high dose should be administered to give effective results.

Based on the results and the potency of the extracts against the organisms, Staphylococcus proved to be the most resistant. As a result, the antimicrobial properties of guava leaves may be ineffective against Staphylococcus aureus infections. In the same way, Escherichia coli and Pseudomonas aeruginosa were extremely sensitive to this leaf extract. Both of these organisms are gram-negative, which suggests that the antimicrobial activity of this leaf may
be stronger against gram-negative organisms than gram-positive organisms. As a result, these leaves can be used to treat infections and diseases caused by these organisms, such as urinary tract infections. When compared to the standard Amoxicillin (25 μg), it demonstrated more potent and consistent activity against all organisms, with zones of inhibition ranging from 41 mm, 20 mm, 37 mm, and 24 mm for S. aureus, P. aeruginosa, B. subtilis, and E. coli, respectively. This demonstrates that, despite guava leaves' observed activities against these organisms, especially gram-negative organisms, amoxicillin is a far more potent antibiotic. This could be because amoxicillin is used to treat infections caused by both gram-positive and gram-negative organisms.

V. CONCLUSION

This study sheds light on the antimicrobial potentials and other pharmacological activities of guava. According to the present research results, P. guajava leaves could represent a potential candidate in the quest for naturally occurring antibiotics against B. subtilis and S. aureus infections and/or diseases. This study has helped to provide some rational evidence for the use of guava leaves against certain diseases due to the presence of some useful phytochemicals. It can also be used to treat P. aeruginosa and E. coli infections, especially if the patient has developed a strong resistance to the potent synthetic drugs used to treat these infections.

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CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

REFERENCES