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Antibacterial Potency of Fractions from *Psidium guajava* Leaves and Bark Crude Extracts against Bacterial Isolates Responsible for Foodborne Infection

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Article

Optimizing Antibacterial Activity of *Psidium guajava* Extracts Using Solvent Fractionation Method and Its Efficacy against Foodborne Pathogens

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Abstract: Guava (*Psidium guajava* L.) is a common fruit tree that grows in several tropical and subtropical parts of the world. The aim of this study was to employ the use of liquid-liquid fractionation to comparatively investigate the antibacterial potential of crude extracts of Guava leaves and bark against selected organisms obtained from food, the isolates includes; *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Bacillus cereus*, and *Staphylococcus aureus*. The phytochemical assay was carried out using standard methods. Analysis of the extracts showed the presence of tannin, phenol, flavonoid, and terpenoid in all the extracts, while steroid and saponin were absent in some. The agar well diffusion method was employed for the assessment of the antimicrobial properties of the extracts. The ethyl acetate and aqueous fractions from the stem bark acetone extract generally showed better antimicrobial activity compared with other extracts from leaves. The extract was active both against gram-positive bacteria (*Bacillus cereus* and *Streptococcus pneumonia*) and gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus*) at a varying zone of inhibition. The results of the study affirmed the potential of guava leaves and bark as novel antibacterial agents while supporting their application for treatment in traditional medicine. The study likewise suggests that the optimization of these extracts could maximize their efficiency as an antimicrobial agent and invariably for food safety.

Keywords: antibacterial activity; *Psidium guajava*; phytochemical screening; liquid-liquid fractionation

Introduction

Foodborne diseases resulting from bacterial contamination during food processing or consumption of spoiled food are a common cause of illness and death especially in developing countries [1,2]. Common foodborne pathogenic bacteria such as *Escherichia coli*, *Bacillus cereus*, *Listeria monocytogenes* *Salmonella* Typhi, and *Staphylococcus aureus* among others have been well-documented to produce toxins that result in a diverse range of disease conditions [3]. Incidences of community foodborne disease outbreaks are frequently reported in low and middle-income countries, with children being the most vulnerable [4]. Food preservation plays a critical role in the control of foodborne diseases. However, the conventional use of chemicals as preservatives has been faulted as possessing a harmful effect on human health [5–7]. Likewise, the treatment of infectious disease conditions with a commercial antibiotic has proven less effective due to the increasing incidence of antimicrobial resistance [8]. Conventional medications are also relatively expensive for treatment in

low and medium-income economies [9]. These combined concerns have prompted the intense research and development of alternatives for food preservation and treatment of foodborne diseases. Several emerging methods have been developed. Of these, the traditional plant-based approach to foodborne disease treatments, food preservation, and packaging has received great attention for safety, antimicrobial efficiency, and biodegradability [2,10,11]. In addition to its inexpensive cost and accessibility, it, therefore, remains plausible to consider ethnomedicinal plants as a potential source of antimicrobials for food preservation and treatment of foodborne diseases.

Guava (*Psidium guajava* L.) is a common fruit tree that grows in several tropical and subtropical parts of the world. It is widely valued because of its edible fruit and its leaves [12,13]. Guava leaves are used traditionally for the treatment of several ailments around the world. In West Africa, the leaves are utilised as a key recipe in herbal preparation for the treatment of malaria which is endemic to the region. It is reported to contain phytochemicals such as flavonoids, carotenoids, and polyphenols among others [14]. Quercetin has been isolated as one of the major compounds in the leaves [15]. The ethnobotanical use and bioactivity of Guava leaves include but are not limited to wound healing, antidiabetic, as cough sedative, anti-inflammatory etc. [16]. The twig of the Guava plant is popularly used as a chewing stick in Western Nigeria which is used in cleaning by brushing against the teeth and gum [17]. The use of this local chewing stick and extracts has been reported to reduce the presence of tartar/dental plaque formation on the tooth. Hence the investigation of the extracts for its antibacterial activities, especially it pertains to oral health [18]. As a result of the potentials of the different Guava parts, the leaves, and stem bark have been the focus of several antimicrobial studies. Due to its relative antibacterial activity, the Guava plant has the potential to serve as a plant-based alternative for food preservation and the treatment of disease conditions resulting from food poisoning. However, there is yet to be an exhaustive study comparing the bioactivity of the plant extract based on polarity. It has been documented that most parts of a plant can contain natural bioactive constituents, likewise, the polarity of extracts could significantly influence its activity. The constituent of some plant phytochemicals can complicate and buffer the activity of others. Therefore, the aim of this study was to employ the use of liquid-liquid fractionation to investigate the comparative antibacterial activity of the crude extracts of Guava leaves and bark against selected food isolates; *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Bacillus cereus* and *Staphylococcus aureus*.

Materials and Methods

Plant Material

Psidium guajava leaves and stem bark were harvested from Guava trees at the Orchard Garden, Landmark University, Omu-aran, Kwara State (8°07'30.9"N; 5°04'53.8"E). The plant samples were rinsed under running tap water. Part of the collected fresh leaves were stored at 4°C and the remaining plant sample air dried. The dried materials were pulverised with an electric blender and stored in a labelled air-tight container prior use.

Crude Extract Preparation

Approximately 300 g of pulverized dried leaves were extracted with 1500 mL of distilled water, acetone, and ethanol in Erlenmeyer flasks, the same was done for the fresh leaves. Similarly, 200g of dried stem bark was macerated in 1000 mL of distilled water and acetone. The setup was wrapped in aluminium foil to avoid evaporation and exposure to light. This was then placed on a Rocking Laboratory Shaker for 72 hours of agitation at 70 rpm. Subsequently, the mixture was decanted and filtered through a Whatman filter paper [19]. The filtrate was concentrated using a Rotary Evaporator, dried at 45°C and stored in the refrigerator at 4°C until use.

Fractionation of Crude Extracts

For each crude extract, 20 g was reconstituted in 100 mL of distilled water and manually agitated for 10 minutes. The mixture was transferred to a separating funnel, where 300 mL n-Hexane was

added, shaken vigorously and allowed to partition for 24 hours. The n-Hexane fraction was collected in sterile container and the procedure for partition was repeated to collect ethyl acetate fraction. The solvent from fractions were evaporated at 45°C in a water bath [20].

Qualitative Phytochemical Screening

Test for phytochemicals in crude *Psidium guajava* leaves and stem bark extract was analysed for saponins, tannins, phenols, flavonoids, terpenoids and steroids using standard protocols [21,22] as guide to the procedure adopted.

Organisms Used in Study

The test bacteria used in this experimental study were isolated from food samples. They were identified by morphological, cultural, and biochemical characteristics. The list of isolates include; two strains of Gram negative (*Escherichia coli*, and *Pseudomonas aeruginosa*) and three strains of Gram positive (*Staphylococcus aureus*, *Streptococcus pneumonia* and *Bacillus cereus*) bacteria. Fresh culture of each isolates was utilized in assessing antibacterial activity of the Guava leaf and stem bark crude extracts.

Antibacterial Activity of Extracts

A stock preparation of 100 mg/mL of each plant extract fraction was prepared in solution of 10% DMSO. The agar well diffusion technique was used in assessing antibacterial properties of each fraction. For each test organism, a fresh 18 to 24 hour culture which was plated on Nutrient Agar was utilised as inoculum at 0.5MarFarland (containing about 1.5×10^8 CFU/mL) in normal sterile saline water [23]. The inoculum was swapped on a freshly prepared solidified Muller Hinton Agar prepared in accordance with the manufacturer's instruction. Wells of 9 mm were made in the inoculated Muller Hinton Agar using a cork borer. The wells were filled with the prepared test fraction and ciprofloxacin as a positive control. The cultures were incubated at 37 °C for 24 hours. The diameter of zone of inhibition around the wells was recorded as level of antibacterial activity. Each assay was carried out in replicate.

Statistical Analysis

Result of zones of inhibition was analysed using IBM-SPSS Statistics version 26 (IBM Corp., USA) software and reported as mean \pm standard deviation ($p < 0.05$).

Results

The results of qualitative phytochemical screening analysis of Guava leaf and stem bark extracts are presented in Table 1. All the extracts were present for Tannin, Phenol, Flavonoid, and Terpenoids. Saponin was present in the aqueous leaf extract and the stem bark aqueous and acetone extract but was absent in the leaf ethanol and acetone extracts. The stem bark showed the absence of Steroids. However, steroids was present in the leaf extracts excluding acetone leaf extract.

Table 1. Qualitative analysis of Guava (*Psidium guajava*) leaf and stem bark extracts.

Phytochemicals	Leaf extract			Stem bark extract	
	Aq	EtOH	Ace	Aq	Ace
Saponin	+	-	-	+	+
Tannin	+	+	+	+	+
Phenol	+	+	+	+	+
Flavonoid	+	+	+	+	+
Terpenoid	+	+	+	+	+
Steroids	+	+	-	-	-

Key: (+) = Present; (-) = Absen.t.

The antibacterial activity of the fractions from *Psidium guajava* leaves and stem bark against *E. coli* (29.0 ± 1.0 mm to 14.0 ± 1.0 mm) is shown in Table 2 compared to the standard antibiotics Ciprofloxacin (53.0 ± 1.0 mm). Based on zone of inhibition, the widest inhibition diameter was reported for Ethyl Acetate fraction of Stem bark acetone extract at 29.0 ± 1.0 mm, followed by 23.5 ± 1.5 mm in n-Hexane fraction of Fresh leaf aqueous extract and 21.0 ± 1.0 mm in Ethyl Acetate fraction of Dried Leaf ethanol extract. The least activity was reported for Ethyl Acetate fraction Fresh leaf aqueous extract (14.0 ± 1.0 mm).

Table 2. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Escherichia coli*.

Crude Extract		Fractions (100 mg/mL)		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	Aq	16.5 ± 1.5	18.0 ± 1.0	19.5 ± 0.5
	EtOH	20.0 ± 2.0	21.0 ± 1.0	15.5 ± 0.5
	Ace	20.5 ± 1.5	19.5 ± 0.5	17.0 ± 1.0
Fresh Leaves	Aq	23.5 ± 1.5	14.0 ± 1.0	16.5 ± 1.5
Stem Bark	Aq	-	-	17.0 ± 1.0
	Ace	16.5 ± 1.5	29.0 ± 1.0	17.0 ± 1.0
Ciprofloxacin (Control)		53.0 ± 1.0	53.0 ± 1.0	53.0 ± 1.0

Test of fractionated crude extract from *Psidium guajava* leaves and stem bark against *P. aeruginosa* is shown in Table 3, with activity ranging from 34.0 ± 1.0 mm to 13.5 ± 1.5 mm. n-Hexane fraction of dried leaf acetone extract had the largest zone of inhibition (34.0 ± 1.0 mm). Stem bark acetone extract had activity at 27.5 ± 2.5 mm for its aqueous fraction and 26.1 ± 1.00 mm for ethyl acetate fraction. The water fraction of the dried leaf aqueous extract was least in zone of inhibition at 13.5 ± 1.5 mm.

Table 3. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Pseudomonas aeruginosa*.

Crude Extract		Fractions (100 mg/mL)		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	Aq	20.0 ± 0.0	15.5 ± 1.5	13.5 ± 1.5
	EtOH	19.0 ± 1.0	24.0 ± 1.0	17.0 ± 0.0
	Ace	34.0 ± 1.0	24.0 ± 1.0	16.5 ± 1.5
Fresh Leaves	Aq	23.5 ± 1.5	11.5 ± 0.5	14.0 ± 1.0
Stem Bark	Aq	-	-	19.0 ± 1.0
	Ace	15.0 ± 1.0	26.0 ± 1.0	27.5 ± 2.5
Ciprofloxacin (Control)		51.0 ± 1.5	51.0 ± 1.5	51.0 ± 1.5

Results of inhibitory activity of fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Streptococcus pneumonia* ranged from 27.5 ± 1.5 mm to 11.5 ± 0.5 mm, where the control Ciprofloxacin was 41.5 ± 1.5 mm. Ethyl Acetate fraction of Dried leaf ethanol extract and Stem bark acetone extract showed antibacterial activity against *Streptococcus pneumonia* at 27.5 ± 1.5 mm and 27.0 ± 1.0 mm respectively (Table 4). The ethyl acetate and aqueous fractions for dried leaf acetone extract both exhibited activity at 21.0 ± 1.0 mm against *Streptococcus pneumonia*. The least activity (11.5 ± 0.5 mm) was recorded for the n-Hexane fraction of Fresh leaf aqueous extract.

Table 4. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Streptococcus pneumonia*.

Crude Extract		Fractions (100 mg/mL)		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	Aq	12.0 ± 1.0	16.5 ± 1.5	21.0 ± 1.0
	EtOH	13.5 ± 0.5	27.5 ± 1.5	25.5 ± 3.5
	Ace	16.5 ± 0.5	26.0 ± 1.0	26.0 ± 1.0
Fresh Leaves	Aq	11.5 ± 0.5	21.0 ± 1.0	24.0 ± 0.0
Stem Bark	Aq	-	-	22.5 ± 0.5
	Ace	19.5 ± 0.5	27.0 ± 1.0	24.5 ± 0.5
Ciprofloxacin (Control)		41.5 ± 1.5	41.5 ± 1.5	41.5 ± 1.5

The antibacterial activity of the fraction of crude extracts from *Psidium guajava* against *Bacillus cereus* ranged from 29.0 ± 1.0 mm to 12.0 ± 1.0 mm, with the control (Ciprofloxacin) 59.0 ± 1.0 mm (Table 5). Ethyl acetate fraction from Stem bark acetone extract showed the highest inhibition against *Bacillus cereus* at 29.0 ± 1.0 mm. Stem bark aqueous extract was 24.5 ± 0.5 mm and fractions of dried leaf ethanol extract at 24.0 ± 1.0 mm. The lowest activity was in the aqueous fraction of fresh leave aqueous extract (12.0 ± 1.0 mm).

Table 5. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Bacillus cereus*.

Crude Extract		Fractions (100 mg/mL)		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	Aq	13.0 ± 1.0	23.0 ± 1.0	17.0 ± 1.0
	EtOH	23.0 ± 1.5	20.5 ± 0.5	24.0 ± 1.0
	Ace	16.0 ± 1.0	19.5 ± 0.5	23.0 ± 1.0
Fresh Leaves	Aq	19.5 ± 0.5	20.0 ± 2.0	12.0 ± 1.0
Stem Bark	Aq	-	-	24.5 ± 0.5
	Ace	16.5 ± 1.5	29.0 ± 1.0	23.5 ± 1.5
Ciprofloxacin (Control)		59.0 ± 1.0	59.0 ± 1.0	59.0 ± 1.0

The range of inhibition of fractions from *Psidium guajava* leaves and stem bark crude extracts was from 19.5 ± 0.5 mm to 30.0 ± 1.0 mm against *Staphylococcus aureus*. The control antibiotic was 43.5 ± 0.5 mm in zone of inhibition (Table 6). The widest zone of inhibition was observed in the aqueous fraction of Stem bark acetone extract (30.0 ± 1.0 mm). Aqueous fractions of dried leaf Ethanol and acetone extract show inhibition at 29.5 ± 0.5 mm and 26.5 ± 0.5 mm respectively. The lowest activity was reported for the aqueous fraction of dried leaf aqueous extract at 19.5 ± 0.5 mm.

Table 6. Antibacterial activity of Fractions from Guava (*Psidium guajava*) leaves and stem bark crude extracts and against *Staphylococcus aureus*.

Crude Extract		Fractions (100 mg/mL)		
		n-Hexane	Ethyl Acetate	Water
Dried Leaves	Aq	22.0 ± 1.0	21.5 ± 1.5	19.5 ± 0.5
	EtOH	20.5 ± 0.5	25.0 ± 1.0	29.5 ± 0.5
	Ace	22.0 ± 1.0	23.0 ± 0.0	26.5 ± 0.5
Fresh Leaves	Aq	24.5 ± 0.5	21.0 ± 2.0	23.0 ± 2.0
Stem Bark	Aq	-	-	20.5 ± 0.5
	Ace	22.0 ± 2.0	26.0 ± 1.0	30.0 ± 1.0
Ciprofloxacin (Control)		43.5 ± 0.5	43.5 ± 0.5	43.5 ± 0.5

Discussion

According to the WHO, Traditional Medicine Strategy (2014-2023), traditional medicine has continued to play a vital role in the treatment and management of diseases, especially in primary health care [24,25]. Therefore medicinal plants are continuously being sourced from the environment and prepared into herbal products. Different techniques are employed in this preparation, which includes but not limited to maceration, infusion, decoction, hot steam extraction among others [26]. For efficiency in plant extraction and yield; increasing the surface area of solvent and sample is important. The solvent type also has a complementary effect on the combination of active ingredients to be extracted [27].

This study reports on the presence of phytochemicals containing the active component of the plant extract. The choice of extraction solvent was based on availability, sustainable utilisation and report of prior antibacterial activity from other studies [28–30]. Water is polar and is commonly used in traditional settings for the extraction of plant bioactive components in the treatment of disease conditions. Ethanol and Acetone are solvents of mid-polarity. While ethanol is cheap and likewise commonly used similarly as water in folk medicine, studies have reported acetone to be capable of isolating antimicrobial compounds with high activity. Acetone as a solvent also has an advantage of easy removal from solution compared to water and ethanol [31,32]. Likewise, acetone has been reported to be nontoxic to bioassay systems and generally have high antimicrobial activities in several studies, [33]. Results from this study's qualitative phytochemical screening showed a wide range of compounds present in both *Psidium guajava* leaf and stem bark (Table 1). This included Tanin, Phenol, Flavonoid and Terpenoid, this is consistent to other reports [34]. However, absence of Saponin in Leaf ethanol and acetone extract shows effect of solvent polarity on the type of phytocompound extracted. Likewise, both extracts from the stem bark for aqueous and acetone was absent for Steroids. Liquid-liquid fraction is a technique for partitioning the range of compounds in a mixture based on polarity [35]. During the fractions of the crude extracts from *Psidium guajava* leaf and stem bark, choice of solvent was based on polarity. n-Hexane is a non polar solvent, regularly used for the defatting process of plant extracts. Ethyl acetate is a mid polar solvent and has the capacity to elute compounds in its mid polarity range. The liquid liquid fractionation process therefore resulted in varying amount of fractions except in the stem bark aqueous extract, where the n-Hexane and ethyl acetate fraction did not result in any yield.

Food contamination may pose a great threat to the human population, especially in cases resulting from infectious diseases and food poisoning [36]. In this study the antibacterial potential of different fractions of *Psidium guajava* leaf and stem bark extracts was assessed against five (5) isolates from food sources. The isolates included *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumonia*, *Bacillus cereus* and *Staphylococcus aureus*. The ethyl acetate and aqueous fractions from the stem bark of acetone extract generally showed better antimicrobial activity compared with other extracts from leaves. For *Bacillus cereus* and *Streptococcus pneumonia* which are both gram positive bacteria and *Escherichia coli* (gram positive), the ethyl acetate fraction of the stem bark of acetone extract showed the most potent activity. While the aqueous fraction of acetone extract was most inhibitory for *Staphylococcus aureus* (gram positive). This shows that phytocompounds in the stem bark has a validated potential mechanisms of antimicrobial activity against both gram positive and negative bacteria. In the leaf extracts, n-Hexane fraction from dried leaf acetone extract showed highest activity for *Pseudomonas aeruginosa* (gram negative). In this study, acetone extracts generally gave a higher anti-microbial activity. Nevertheless, all fractions of the extracts showed considerable bioactivity against the test bacteria isolates. The present study in addition has demonstrated the importance of the extratcted fractions in optimising bioactivity.

Conclusions

This study shows the potential of identifying novel antibacterial agent from *Psidium guajava* leaf and stem bark, while optimising its potential application for treatment in traditional medicine. In the light of latest trends in plant natural products research and development, green synthesis of nanoparticles from guava stem and leaves could aid increase antibacterial activity, for food security,

sustainable agriculture and in treatment regimen, [37–39]. Following the Sixty-second **World Health Assembly**-WHA resolution on Traditional Medicine (WHA62.13) for safety purposes; it is advocated that toxicological analysis be performed on the extracted fractions in dose dependent manner. Further work is suggested to isolate antibacterial compounds and characterize them from the fractions especially the stem bark and analyse same for their therapeutic and optimal development into food and pharmaceutical products.

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Conflicts of Interest: The authors declare that no conflict of interest exists in respect of this study.

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