

Preliminary Phytochemical, Antimicrobial and Proximate Analysis of Tender Leaves of *Psidium guajava* L in Jos, Plateau State, Nigeria

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Abstract

Phytochemical screening, proximate, chromatographic analyses and the antimicrobial activities of tender leaves of *Psidium guajava* of the family *Myrtaceae*, were carried out. 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*.

Keywords: Antimicrobial, Phytochemical, Proximate analysis, *Psidium guajava*, Chromatographic analysis, Jos.



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1. Introduction

Guavas are plants in the myrtle family (*Myrtaceae*). Common names are guava (English), gioba (Hausa), goifa (Yoruba), gova (Igbo), guayaba (Spanish), goyave (French), goeajaaba (Dutch), [1]. A native of tropical America, it is now planted as a fruit tree in West Africa. The seeds are distributed by man, and animals, (mainly monkey and birds) and are cultivated throughout the tropics. A small tree of about 6- 8m high, bark is grayish brown, hard or very rough and resistant to termites. The fruits are up to 4 inches long, fleshy, globosely, ovoid or pear-shaped; generally yellowish or white when ripe. They contain a mass of small seeds embedded in the endocarp, though some are seedless or nearly so. It is said to be higher in vitamin C than citrus – 80mg of vitamin C in 100g of fruit and also contains an appreciable amount of vitamin A [1]. The seeds are numerous, flattened and kidney-shaped. The plant is angiospermic and dicotyledonous. Leaves are up to 4.5 inches long, opposite, elliptic/ellipsoidal and the veins are prominent on the lower surface. The tender leaves are a lighter shade of green than the matured leaves and are curly and unfurling. The leaves of *Psidium guajava* are aromatic when crushed. The flowers are white, singly borne on short stalks in leaf axis, sepals 4, and petals 5. Leaves are scented and contain 6% of fixed oil, 0.365% of a cineol-based essence, 3.15% resin, 8.5% tannins and other metabolites. [1]. The main components of the essential oil are pinene, β -pinene, limonene, β -bisobolene, caryophyllene oxide, β -copanene, quercetin and curcumene [2]. The essential oil from the leaves has also been shown to contain nerolidol, β -sitosterol, ursolic and guayavolic acids have also been identified [3].

Leaves are particularly rich in flavonoids and distinctly rich in quercetin [4]. Much of the leaves therapeutic activity is attributed to the flavonoids, they demonstrate great antibacterial activity [5]. Quercetin (fig 1) is thought to contribute to the antidiarrhoeal effect of *Psidium guajava* leaves. It is able to relax intestinal smooth muscle and inhibit bowel contraction [6].

The leaf methanolic extract also showed in vitro antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus mirabilis* and *Shigella dysenteria* [3]. The effectiveness of the leaf extract against *Staphylococcus aureus* and a strong antimicrobial action have been reported on Gram-positive and Gram-negative organism [7]. The flavone derivatives isolated were reported to inhibit the growth of *Staphylococcus aureus* in a dilution of 1:1000 [5]. The leaves of guava tree in decoction are recommended for gastroenteritis, chronic diarrhea etc. the young leaves and shoots are used for dysentery and diarrhea [8]. It has been reported that the

quercetin present can inhibit the intestinal movement and reduce capillary permeability in the abdominal cavity and this may explain the antidiarrheal mechanism of *Psidium guajava* extract [8]. In addition, other flavonoids and triterpenes in guava leaves e.g Quaijavarin in (fig 2) show antispasmodic activity [9]. Guava leaves also have antioxidant properties which are attributed to the polyphenols in the leaves.

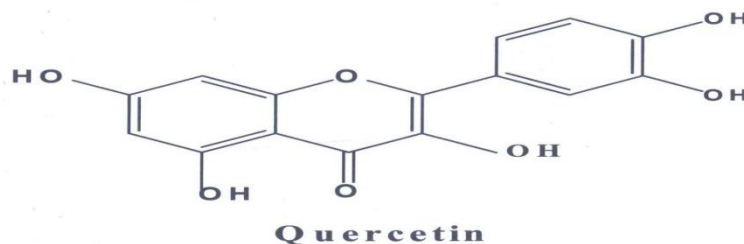


Figure 1.

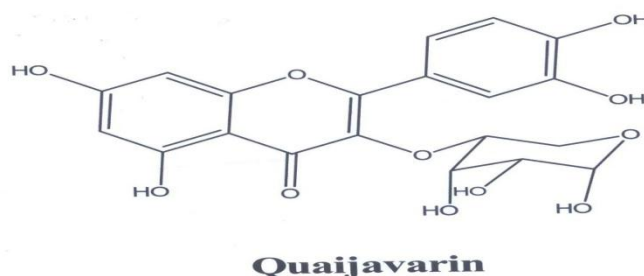


FIGURE 2

Tender leaves are chewed to bring relief in tooth ache (analgesic) in Ghana [10]. They are chewed for stomach ache and piles [10]. When boiled with lemon grass (*Cymbopogon citrates*), an adjuvant, the decoction is used to cure cough. This decoction is also taken in Senegal for tracheobronchitis [10].

The active principles of many drug found in plants are secondary metabolites [4]. Therefore, basic phytochemical investigation of its extracts for major phyto-constituents is also vital. In the present study the extracts of *Psidium guajava* were screened for phytochemical constituents, antimicrobial activity and proximate analysis.

2. Materials and Methods

Plants used for this study were collected from Jos metropolis, Plateau State, Nigeria. The identity of plant materials was confirmed and authenticated at the Herbarium of the Department of Plant Science Technology, University of Jos.

2.1. Preparation of Plant Extracts

The plant material was dried at room temperature and then powdered using a grinder. The powdered sample (100g) was subjected to soxhlet extraction using 300ml of each of the solvents (methanol, hexane and ethyl acetate). The resulting extracts were concentrated on a hot water bath and kept for further investigation.

2.2. Phytochemical Screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods [11]. The extracts were screened for the presence of glycosides, alkaloids, tannins, flavonoids, saponins, anthraquinones and terpenes.

2.3. Test Organisms

Standard strains of *S. aureus*, *S. pyogenes*, *Bacillus subtilis*, *Candida albicans* and *Pseudomonas aeruginosa* were obtained from the Department of Medical Microbiology, Jos University Teaching Hospital, Jos, Nigeria.

2.4. Antimicrobial Screening

MIC of the ethanolic and aqueous extract of *Psidium guajave* which showed the highest antibacterial activity in the disc diffusion assay were determined based on broth dilution technique with a standard method [12]. Inoculation of microorganisms were prepared from 12 hours broth cultures. Stock solutions of extracts (4000µg/ml) were diluted with nutrients broth cultures. Stock solutions of extracts (4000µg/ml) were diluted with nutrient broth in serial tenfold dilutions using nutrient broth to make dilution ranging from 250µg/ml to 4000µg/ml and inoculated with 0.2ml of the test microorganisms. The inoculated tubes were then incubated at 37⁰c for 24hours and were inspected for non-turbidity. The lowest concentration of the extract which prevented visible growth was noted and recorded as the minimum inhibitory concentration (MIC).

2.5. Proximate Analysis

Analysis of tender leaves of *Psidium guajava* for moisture content, total ash, acid insoluble ash, alcohol extractive value and water extractive value using a standard method by Trease and Evans [13] was carried out.

Table-1. Phytochemical screening of methanol, hexane and ethyl acetate extracts of tender leaves of *Psidium guajava*

	Methanol extract	Hexane extract	Ethyl acetate extract
Flavonoid	++	+	-
Tannins	+++	++	-
Reducing sugar	+	+	+
Terpenes	++	+	-
Saponins	+	++	+
Anthraquinones	+++	++	+
Alkaloid	+	++	-

+++ = High concentration; ++ = moderate concentration; + = low conc.; - = absent

Table-2. Proximate analysis of tender leaves of *Psidium guajava*

Moisture content	3.67%
Total ash	3.29%
Acid insoluble ash	0.25%
Alcohol extractive value	21.34%
Water extractive value	3.54%

Table-3. RF values for 70% methanol, hexane and ethyl acetate extracts

Spots	70% Methanol	Hexane	Ethyl acetate	70% methanol	Hexane	Ethyl acetate
1	0.8167	0.51	0.35	0.7142	0.58	0.4761
2	0.95	0.816	0.533	0.8571	0.827	0.6507
3	-	0.933	0.8167	0.9523	0.931	0.7619
4	-	-	0.883	-	-	0.8730
5	-	-	0.95	-	-	0.9047
6	-	-	-	-	-	0.9523
7	-	-	-	-	-	-

Table-4. Minimum Inhibitory Concentration (MIC) of the tender leaves of *Psidium guajava* (methanol extract) Against tested microorganisms

Test organism	Concentration µg/ml				
	250µg/ml	500µg/ml	1000µg/ml	2000µg/ml	4000µg/ml
<i>Pseudomonas aeruginosa</i>	-	-	0+	+	+
<i>Bacillus subtilis</i>	-	0+	+	+	+
<i>Candida albicans</i>	-	-	0+	+	+
<i>Streptococcus pyogenes</i>	-	0+	+	+	+
<i>Staphylococcus aureus</i>	-	-	0+	+	+

O+ = minimum inhibitory concentration; + = inhibition; - = No inhibition (turbidity)

Table-5. Minimum Inhibitory Concentration (MIC) of the tender leaves of *Psidium guajava* (hexane extract) against tested microorganisms

Test organism	Concentration µg/ml				
	250µg/ml	500µg/ml	1000µg/ml	2000µg/ml	4000µg/ml
<i>Pseudomonas aeruginosa</i>	-	-	0+	+	+
<i>Bacillus subtilis</i>	-	0+	+	+	+
<i>Candida albicans</i>	-	-	0+	+	+
<i>Streptococcus pyogenes</i>	-	0+	+	+	+
<i>Staphylococcus aureus</i>	-	-	0+	+	+

O+ = minimum inhibitory concentration; + = inhibition; - = No inhibition (turbidity)

Table-6. Minimum Inhibitory Concentration (MIC) of the tender leaves of *Psidium guajava* (ethyl acetate extract) against tested microorganisms

Test organism	Concentration µg/ml				
	250µg/ml	500µg/ml	1000µg/ml	2000µg/ml	4000µg/ml
<i>Pseudomonas aeruginosa</i>	-	0+	+	+	+
<i>Bacillus subtilis</i>	-	-	0+	+	+
<i>Candida albicans</i>	-	-	0+	+	+
<i>Streptococcus pyogenes</i>	-	-	0+	+	+
<i>Staphylococcus aureus</i>	-	-	0+	+	+

O+ = minimum inhibitory concentration; + = inhibition; - = No inhibition (turbidity)

3. Result

The results of the phytochemical screening, proximate analysis, antimicrobial tests for the methanol, hexane and ethyl acetate are presented in Tables 1-6.

4. Discussion

Phytochemical screening (Table 1) revealed the presence of flavonoid, tannins, reducing sugar, terpenes, saponins, anthraquinones and alkaloid. The chemical constituents present in the extracts have some therapeutic values. Tannins are plant metabolites well known for their antimicrobial properties [14]. Flavonoids have both antifungal and antibacterial activities. They possess anti-inflammatory activity [3, 15]. Flavonoid, terpenes and alkaloid are known to have antimicrobial and bactericidal properties against several [11].

In the antimicrobial studies, the majority of the organisms were more sensitive to methanolic extract. According to Trease and Evans [13], the anti-bacterial activity and inhibitory effect of plant extracts may be due to the presence of secondary metabolites.

In Table 4, the methanolic extract of tender leaves of *P. guajava* was active against the entire microorganisms. It has MIC value of 500µg/ml against *Bacillus subtilis* and *Streptococcus pyogenes*, 100 µg/ml against *Pseudomonas aeruginosa*, *Candida albicans*, and *Staphylococcus aureus*.

These findings are consistent with Etuk, et al. [16] who reported that the bark extract of *P. guajava* had antidiarrheal activity *in vitro*. Previous reports have demonstrated the antidiarrheal activity of tannins [17], Flavonoids [18] and saponins [9]. In Table 2, the low moisture content (3.67%) implies that the plant can be stored for a long time and not affected by microbial attack. The ash content (3.29%) buttresses the claim that it is highly organic and medicinal. In Table 3, the components of the extract separated on the plates showed that variety of compounds are present in the extracts.

5. Conclusion

The result of this experiment showed that tender leaves of *P. guajava* may have some valuable anti-microbial activities. This property tends to support the traditional medicinal stage in the treatment of bacterial infections. The result justified the use of the plant in the treatment of diseases of microbial origin in herbal medicine.

6. Contribution/ Originality of This Study

This study contributes to the existing literature and the scientific justification of the medicinal potential of this plant. The findings in this research validate its use among the low income people such as farmers, villagers and native communities. It is also likely to establish a scientific bases and proof for its potential use in folk medicine for the treatment of infectious diseases produced by common pathogenic microorganisms.

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