Preliminary Phytochemical and Antibacterial Activity of Ethanolic and Aqueous Stem Bark Extracts of *Psidium guajava*

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ABSTRACT

To establish the scientific basis for the use of *Psidium guajava* in traditional medicinal practices in Northern Nigeria, the ethanolic and aqueous stem bark extracts of *Psidium guajava* were screened for the presence of some phytochemicals and antibacterial activity against *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella* spp. The phytochemicals were analyzed using the standard methods of phytochemical analysis, while the antibacterial activities were analyzed using agar well diffusion method. The results indicated presence of tannins, saponins, flavonoids, alkaloids, steroids and cardiac glycosides in the two extracts. The results revealed that the two extracts possess wider antimicrobial activities against the tested organisms at a concentration of 100 mg mL$^{-1}$ which compares favourably with the standard antibiotic streptomycin as positive control. However, ethanolic extract demonstrated a better activity than the aqueous extract with the most susceptible organism being *S. Faecalis* and the least activity was reported for *Salmonella* spp. Both the ethanol and aqueous extracts of *P. guajava* may provide a target for drug discovery.

Key words: Medicinal plant, antibacterial activity, *P. guajava*, phytochemicals

INTRODUCTION

Infectious diseases are the world’s leading cause of premature deaths, killing almost 50000 people every day. In addition, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Mulligan et al., 1993; Davies, 1994; Huebner et al., 1998; Akinsulire et al., 2007). The present scenario of emergence of multiple drug resistance to human pathogenic organisms has necessitated a search for new antimicrobial substances from other sources including plants. Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties (Iyengar, 1986; Chopra et al., 1992). The substances that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs.

The present study was aimed at establishing the scientific basis for the use of this plant in traditional medicinal practices in our locality. Both the ethanolic and aqueous stem bark extracts of the plant were screened for the presence of phytochemicals and antimicrobial activities against *Staphylococcus aureus*, *Streptococcus faecalis*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella* spp.
Psidium guajava, is a shrub tree known as "gufọ̀" to Yoruba and "goba" to Hausa communities in Nigeria. P. guajava characteristically has very thin skins; the leaves are typically evergreen, opposite, short petioled, oval or oblong (Akinpelu and Onakoya, 2006). The plant is used in folk medicine to treat fevers, diarrhoea and as tonic in psychiatry. Clinical studies using phytodrugs made from leaves of P. guajava were found to be effective in the treatment of gastrointestinal disturbances. The bark extract of P. guajava is also used in the treatment of diarrhoea, stomach ache and diabetes (Akinpelu and Onakoya, 2006).

MATERIALS AND METHODS

Plant sample: Fresh P. guajava stem bark was separately collected from Bodinga, Sokoto State, Nigeria in June, 2012 and was identified and authenticated by a Botanist at the Biological Sciences Department, Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. Voucher samples were prepared and deposited in the Herbarium of the Botany Department of Kebbi State University of Science and Technology, Aliero for reference. The bark was later air-dried, powdered and stored in an air-tight container for further use.

Preparation of plant extracts: The ethanolic extract was prepared by soaking a sample (200 g) of powdered plant material in 90% ethanol (200 mL) for 48 h. Aqueous extract was prepared by soaking a sample (200 g) of powdered plant material in warm sterile water (200 mL) for 48 h. At the end of the extraction, each extract was filtered using Whatman filter paper. The filtrate was concentrated in vacuum at 30°C and stored at 4°C until further use.

Text organisms: The bacterial species used in this study were provided by the Department of Microbiology, Faculty of Science, Kebbi State University of Science and Technology, Aliero. The bacteria were grown and maintained on Trypticase Soy agars (TSA) at 37°C overnight.

Phytochemical screening: The two extracts were screened for the presence of major phytochemicals using standard qualitative methods as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1998). The plant extracts were screened for the presence of tannins, saponins, flavonoids, alkaloids, steroids, terpenoids, cardiac glycosides and anthraquinones.

Antibacterial activity assay: Agar well diffusion method was employed to assay for the antibacterial activity (Russell and Furr, 1977). The test organisms (bacterial isolates) were first grown in already prepared nutrient agar for 15 h before use. The inoculum suspensions were standardized and then the antibacterial activities of the two plant extracts tested at a concentration of 100 mg mL⁻¹ each in the medium. The plates were later incubated at 37°C for 24 h after which zone of inhibition (diameter) formed was determined as an indication of antibacterial activity. These effects were compared with that of the standard antibiotic streptomycin at a concentration of 1 mg mL⁻¹.

RESULTS

The result for the phytochemicals analysis of both the ethanolic and aqueous stem bark extracts is shown in Table 1. While Table 2 represents the results for the antibacterial activity of the two extracts against the test organisms.
Table 1: Phytochemical constituents present in the ethanol and aqueous stem bark extracts of P. guajava

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Ethanol extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

Note: +: Present in small concentration, ++: Present in a moderately high concentration, +++: Present in a very high concentration, -: Not detected.

Table 2: Zone of inhibition (mm) of the solvent extracts (100 mg mL⁻¹) of the stem bark of P. guajava against tested bacteria

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zones of inhibition (mm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol (100 mg mL⁻¹)</td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>18±0.68</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>23±2.21</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>12±0.85</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>22±0.96</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>28±0.56</td>
</tr>
</tbody>
</table>

Note: Values are presented as Means±SD of three replicates

DISCUSSION

The result for the phytochemical analysis is presented on Table 1. The result revealed the presence of different phytochemicals that slightly varied with the solvent used for the extraction. The results show the presence of tannins, saponins, flavonoids, alkaloids, steroids and cardiac glycosides in both the two extracts of Psidium guajava. Anthraquinones were not detected in the ethanolic extract, while terpenoids and steroids were not detected in the aqueous extracts of Psidium guajava. This might be as a result of different extraction abilities of varied solvents (Njoku et al., 2010).

The antibacterial activity is presented on Table 2. In this study the results showed that the two extracts of the bark of P. guajava possess antimicrobial activities against the tested organisms at a concentration of 100 mg mL⁻¹ (Table 2). The two extracts compared favourably with the standard antibiotic streptomycin. Ethanolic extract demonstrated a better activity than the aqueous extract with the most susceptible organism being S. faecalis and the least activity was reported for Salmonella spp. Both the extracts demonstrated a broad spectrum of activity. The present study compares favourably with that of the antibacterial activity of the methanolic extract of P. guajava on Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa (Abdelrahim et al., 2002).

The presence of alkaloids, saponins, terpenoids and tannins in the stem bark extracts of P. guajava has medicinal implications. These phytochemicals are known to be biologically active. The presence of tannins was found to play a role in antifungal, antibacterial, astringent and antibiotic activities (Akiyama et al., 2001; Lu et al., 2004). Tannins were also found to form irreversible complexes with proline-rich proteins (Hagerman and Butler, 1981) resulting in the inhibition of the cell protein synthesis.
In addition to antimicrobial activity exhibited by tannins, they also react and form complex with proteins to provide the typical tanning effect. This is important medicinally for the treatment of inflamed or ulcerated tissues (Mota et al., 1985). Tannins-containing herbs as their main component are astringent in nature and are used in the treatment of intestinal disorders such as diarrhoea and dysentery, thus exhibiting antimicrobial activity. One of the largest groups of chemical produced by plants is the alkaloids and their amazing effect on humans has led to the development of powerful pain killer medications. Terpenoids also act as antibiotics to protect plants from pathogenic microorganisms (Aliyu et al., 2008).

CONCLUSION

The results of the present work revealed the presence of most of the phytochemicals with various biological activities. This might be responsible for the observed antibacterial activity against test organisms. The results also indicate that both the ethanolic and aqueous extracts demonstrated wider activity against the test organism which compares well with the standard drug streptomycin.

Further research is needed in the areas of isolation and characterisation of the active components responsible for the observed antibacterial activities.

REFERENCES