



# Journal of Applied Sciences

ISSN 1812-5654

**science**  
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## Preliminary Phytochemical and Antibacterial Evaluation of Crude Aqueous Extract of *Psidium guajava* Leaf

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**Abstract:** The leaves of *Psidium guajava* is used in folk medicine as an antidiarrhoic in Nigeria and many other countries of the world. It is also employed for the treatment of vertigo and regulation of menstrual periods. World health organization encourages and indicated that studies on medicinal plants should include both identification of chemical constituents and determination of the biological activities of such plants. In this preliminary study, phytochemical and antibacterial properties of crude aqueous extract of *Psidium guajava* leaf were evaluated. The extract was subjected to qualitative chemical screening for identification of various classes of active chemical constituents while disc diffusion method was used to determine the antibacterial properties of the extract against some gram positive and gram negative bacteria. The extract showed the presence of tannins, saponins, carbohydrates, flavonoids, steroids and cardiac glycosides. The extract inhibited the growth of *Salmonella typhi* and *Klebsiella pneumoniae*, but has no effect on the growth of *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus fecalis* organisms. The study revealed some antibacterial properties of the extract that justify the use of the plant in folk medicine. However, further studies need to be carried out to identify the potentials of the plant to be considered as a natural source of antibacterial agent.

**Key words:** Phytochemistry, antibacterial properties, crude aqueous extract, *Psidium guajava*

### INTRODUCTION

*Psidium guajava* L. of the family *Myrtaceae*, is found in many parts of the world where the fruit is eaten and the plant is reputed to possess several medicinal properties. The leaves and/or stem bark is used in folk medicine as an antidiarrhoic in Nigeria and other West African countries, Latin America and Southeast Asia (Hawkes, 1983). It is also employed for the treatment of vertigo and regulation of menstrual periods.

In Africa, particularly in Nigeria, herbal medicine has become a part of the people's culture with about 70% depending mainly on traditional medicine (Akinniyi and Tella, 1991; Osuinde and Isibor, 1998; Ajoku *et al.*, 2001; Atawodi, 2001; Banso and Olutimayin, 2001) due to high cost of conventional primary health service. Many herbs including the decoction from the leaves of *P. guajava* have been used in folk medicine for the control of bacterial diseases. World health organization (Anonymous, 1977) indicated that studies on medicinal plants should include both identification of chemical constituents and determination of the biological activities of such plants.

The aim of the present study was to identify the chemical constituents and evaluate the *in vitro* antibacterial properties of the aqueous extract of the leaves since information on these is lacking. The information obtained may provide validation for its reported medicinal uses.

### MATERIALS AND METHODS

**Sample collection and identification:** Fresh samples of the leaves without stalk were collected from the University of Maiduguri campus, Maiduguri, Nigeria. The plant was identified and authenticated by Dr. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Nigeria. A voucher specimen (Chemistry 242 B) was deposited in the Department of Chemistry, University of Maiduguri, Maiduguri.

**Preparation of extract:** The fresh leaves of *P. guajava* collected were air-dried in the laboratory, ground into fine powder and stored in a glass container at 4°C. Eight hundred grams of the powdered sample was exhaustively extracted with distilled water using a reflux method. The

crude aqueous extract obtained was concentrated *in vacuo*, brown in colour and yielded 33.75% (w/w). It was properly labeled and stored in the refrigerator at 4°C until used (Trease and Evans, 1989). All work was carried out in accordance with the general guidelines for methodologies on research and evaluation of traditional medicine (WHO, 2000).

**Phytochemical analysis:** The crude aqueous extract of *P. guajava* was subjected to qualitative chemical screening for identification of the various classes of active chemical constituents such as carbohydrates, tannins, phlobatannins, saponins, cardiac glycosides, steroids/triterpenoids, flavonoids, anthraquinones and alkaloids. The phytochemical analysis was done according to standard methods (Trease and Evans, 1989, 1997).

**Microbial cultures:** Laboratory isolates of the pure culture of Gram positive (*Staphylococcus aureus* and *Streptococcus fecalis*) and Gram negative (*Salmonella typhi*, *Klebsiella pneumoniae* and *Escherichia coli*) organisms were obtained from the Department of Veterinary Medicine Laboratory, University of Maiduguri, Nigeria. The isolates were separately cultured on a nutrient agar plate for 24 h. Twenty milliliters of the medium was poured in to sterile medium sized Petri-dish and allowed to solidify. A colony of each test organism was sub cultured on 10 mL nutrient broth and incubated at 37°C for 8 h. One milliliter of the broth culture was then used to flood the agar plates.

**Concentration of extract:** Stock solution of the crude aqueous extract was prepared by dissolving known weight of the extract in known volume of distilled water. The following concentrations were prepared, 100, 200 and 400 mg mL<sup>-1</sup> of the crude extract. Standard antibacterial agent (oxytetracycline, Cipla Ltd., Mumbai, India.) at a concentration of 10 mg mL<sup>-1</sup> was also used on all the bacteria to compare the zones of inhibition with that of the extract.

**Antimicrobial sensitivity testing:** Disc diffusion method as described by National Committee of Clinical Laboratory Standards (1993) was used to determine the antibacterial activity of the crude extract. Sterilized filter papers (whatman No. 1; 6 mm in diameter) soaked in different beakers containing the dissolved extracts of different concentrations. They were dried at 50°C and placed on plates with the different organisms. The plates were

incubated at 37°C for 48 h. After incubation, the inoculated plates were observed for zones of inhibition in milliliter diameter using a transparent ruler.

The sensitivity or susceptibility of the test bacteria to standard drug was tested using inoculated agar plate and oxytetracycline at a concentration of 10 mg mL<sup>-1</sup>. The zones of inhibition were measured and compared with those of the plant extract.

## RESULTS

The result indicates the presence of tannins, saponins, carbohydrate, flavanoids, steroids and cardiac glycosides (Table 1). The antibacterial efficacy of the crude aqueous extract against some bacterial organisms is presented in Table 2. The crude aqueous extract inhibited the growth of *Salmonella typhi* and *Klebsiella pneumoniae*, but has no effect on the growth of *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus fecalis* organisms.

Table 1: Phytochemistry of the crude aqueous extract of *Psidium guajava* leaf

Phytochemical constituent	Phytochemical test	Inference
Tannin	Ferric chloride	+++
	Lead acetate	+++
	Formaldehyde	++
Saponin	Frothing	+++
Carbohydrate	Molish's test	+++
	Free reducing sugar	+++
	Combined reducing sugar	+++
	Barfoed's test	-
Flavonoid	NaOH	++
	Ferric chloride	+++
	Lead acetate	++
	Shinoda's test	++
Alkaloid	Dragendorff's test	-
	Mayer	-
	Wagner	-
Phlobatanin	HCl	-
Steroid	Lieberman's test	+
	Salkowski's test	+
	Keller-Kiliani	+++
Cardiac glycoside	General test	++
Anthraquinone	Free anthraquinone	-
	Combined anthraquinone	-

Key: + = Low concentration; ++ = Moderate concentration; +++ = High concentration - = Absent

Table 2: Antibacterial properties of crude aqueous extract of *Psidium guajava* leaf

Concentration of extract	Zone of inhibition (mm)				
	<i>Staph. aureus</i>	<i>Strep. fecalis</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Klebsiella pneumoniae</i>
100 (mg mL <sup>-1</sup> )	R	R	R	7.0	9.0
200 (mg mL <sup>-1</sup> )	R	R	R	8.0	10.0
400 (mg mL <sup>-1</sup> )	R	R	R	9.0	11.0
Oxytetracycline					
10 (mg mL <sup>-1</sup> )	20	20	22	20.0	10.0

Key: R = Resistant

## DISCUSSION

The phytochemical screening of *P. guajava* leaf extract showed classes of useful chemical compounds such as tannins, saponins, flavonoids, steroids and cardiac glycosides. Cho *et al.* (2003) reported that medicinal plants may contain many kinds of chemical components and that their biological activities are not usually attributable to a single moiety. Flavonoids have attracted a great deal of attention in relation to their potential for beneficial effects on health. Over the past few years, several experimental studies have demonstrated biological and pharmacological properties of many flavonoids, especially their antimicrobial activity (Narayana *et al.*, 2001), anti-inflammatory (Middleton *et al.*, 2000), antioxidant (Packer *et al.*, 1999; Robak and Marcinkiewicz, 1995) and anti-tumour (Castillo *et al.*, 1989; Inoue and Jackson, 1999) effects, which are associated with free radical-scavenging actions.

Saponins generally lower the surface tension and possess emulsifying activities. They tend to alter the permeability of the cell wall and hence exert a general toxicity on all organized tissues. They are known to possess some antibacterial activity. According to Birk and Petri (1980) saponin combines with cell membrane sterole to produce changes in cell morphology leading to lysis.

Tannins are diverse organic substances with various compositions that have pronounced physiological astringent properties that hasten the healing of wounds and inflamed mucus membranes (Tyler *et al.*, 1988). Tannins also decrease bacterial cell proliferation by blocking key enzymes of microbial metabolism. Glycosides apart from exerting pronounced physiological effects are also known to have antiseptic properties (Robbinson, 1967; Frantisek, 1991).

The present study indicates that the aqueous extract has antibacterial activity against some bacterial (*Salmonella typhi* and *Klebsiella pneumoniae*) organisms. The inhibition of some bacterial growth *in vitro* by the crude extract could be due to the presence of some active principles in the extract. These active principles may have acted alone or in combination to inhibit the growth of the bacterial organisms. The activity of the *P. guajava* water extract against *Salmonella typhi* organism is very interesting since it is well known that this organism is difficult to treat in clinical settings in the developing countries including Nigeria. The study revealed some antibacterial properties of the extract that justify the use of the plant in folk medicine. However, further studies need to be carried out to identify the potentials of the plant to be considered as a natural source of antibacterial agent.

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