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Full Length Research Paper

Preliminary phytochemical and antimicrobial investigations of the stem bark and leaves of *Psidum guajava L.*

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Extracts (ethanol, methanol and aqueous) of the leaves and stem barks of *Psidum guajava L* were studied for their phytochemical constituents. Results of the phytochemical screening revealed the presence of alkaloids (all the extracts), saponins (ethanol and methanol), cardenolides with steroided rings, and cardenolides with deoxy sugar (all the extracts). Thin layer chromatographic separation of ethanol and methanol extracts gave three spots each with Rf values ranging from 0.60 – 0.70. Only the aqueous extract inhibited *Bacillus subtilis* and *Fusarium spp*. The presence of these constituents tends to support the uses of this plant medicinally.

Key words: Alkaloid, cardenolides, extracts, saponin.

INTRODUCTION

Guava (*Psidum guajava L.*) belongs to the Family Myrtaceae (Willis, 1985). It is a low growing tree of 6 to 25 ft (1.8 to 7.5 m) high, but in some varieties may reach 40 ft (12 m) under favourable conditions. *P. guajava* is an American fruit which is well know throughout the tropics, and in some countries it has become naturalized on waste lands.

Guava tree has long history of medicinal uses that are still employed till date. The infusion decoction made from the leaves and/or bark of the tree has been used for treatment of diarrhea, malaria and dysentery (Burkill, 1997). It has been used in the treatment of sore throats, vomiting and menstrual complications (Wagner and Parmsonerth, 1994). Tender leaves are chewed for bleeding gum and bad breath. It is said to prevent hang-over if chewed before drinking (Edeoga, Federal University, of Agriculture, Umudike Nigeria, Personal Communication). Indians also use it as a douche for vaginal discharge, and to tighten vaginal walls after childbirth (Sofowora, 1984). A decoction of the bark or leaves is used topically to treat wound and skin sores. These therapeutic uses of guava are expected to be due to the presence of some components. In the present study, the phytochemical constituents of guava extracts and their antimicrobial activities are investigated.

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MATERIALS AND METHODS

Sample collection and preparation

Stem-bark and leaves samples of guava were collected from identified guava *(P. guajava L.)* plants, and processed in the Plant Science and Biotechnology Laboratory of Abia State University, Uturu, Nigeria. Authentication was done by a Taxonomist in that Department. The stem-bark and leaves were air-dried for a period of seven days at room temperature. The air dried stem-bark and leaves were ground to 0.50 mm mesh size.

Sample extraction

Powdered guava leaves and stem-barks were weighed 170 g each into three containers labeled A, B and C for leaves and D, E and F for stem-bark. About was kept in 800 ml each of ethanol, methanol and distilled water were added to samples A, B and C respectively for leaves and samples D, E and F respectively for stem-bark. The mixture was left for 72 h with occasional shaking. The samples were taken in triplicate. The extracts (200 – 300 ml) were obtained using a sieve cloth and concentrated to 50 – 75 ml using rotary evaporator.

Phytochemical screening

Basic phytochemical screening comprising chemical tests to detect the presence of alkaloids, tannins, saponins, anthraquinones and

Table 1. Detection of phytochemical constituents of extracts of leaf (A) and stem-bark (B) extract of *P. guajava L*.

Tested Material	Yield (%w/w/)	Steroidal Nucleus	Deoxy Sugar (Cardiac glycosides)	Cardenolides	Tannins	Alkaloids	Saponins	Anthraquinone
Leaf extract (A)								
Ethanol	2.96	+	+	+	+	+	-	-
Methanol	4.20	+	+	-	+	+	+	-
Aqueous	8.02	-	+	-	+	+	-	-
Stem-bark extract (B)								
Ethanol	2.72	+	+	+	+	+	+	-
Methanol	3.84	+	+	-	+	+	+	-
Aqueous	7.65	-	+	-	+	+	-	-

Key note: +, Present; –, Absent.

cardiac glycosides were carried out according to the methods of Sofowora (1984).

Thin layer chromatography

The TL separation was carried out using precoated KCl silica gel @ 60^0A TLC plate size 20 x 20 cm, and n-hexane: ethylacetate (15:85) Solvent system. Spots were identified by UV lamp (245 nm) and the Rf values calculated.

Antimicrobial studies

The micro-organisms used include *Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Candida albican, Fusarium spp,* and *Geotrichum candidum*. The agar diffusion method was used for the antimicrobial activities (Okeke et al., 2001). Ampicillin, Tetracycline, Eventamycin, antibiotics for bacteria and Fulcin for fungi were used as controls.

Minimum inhibitory concentration (MIC) of the extracts that inhibited the test organisms was determined using the serial dilution method (Okeke et al., 2001).

RESULT AND DISCUSSION

The phytochemical screening revealed the presence of alkaloids, saponins, steroidal rings and deoxy sugars characteristic of cardenolides (Table 1). Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents (Chabner and Horwitz, 1990; Noble, 1990). Alkaloids also interfere with cell division, hence the presence of alkaloids in the plant makes it a possible remedy in the treatment of cancer.

Saponins are glycosides of both triterpenes and sterols having hypertensive and cardiac depressant properties (Trease and Evans, 1985), hence the presence of these metabolites in *P. guajava L* tend to support its medical uses.

Cardiac glycosides have been found to be effective in congestive heart failure, regardless of the cardiac rhythm and that the beneficial effect is brought about by its direct action to increase the force of myocardial contraction. It also acts directly on the smooth muscles of the vascular system. They exert a number of effects on neural tissues and this indirectly influence the mechanism and electrical activities of the heart, and modify vascular resistance and capacitance (Branndwald et al., 1961). The fore-going would suggest the use of *P. guajava L* as a cardiac tonic.

The three spots indicated from the TLC results (Table 2), for each of these ethanolic and methanolic extracts confirmed the presence of the three major constituents (saponins, alkaloids and cardiac glycosides).

The antimicrobial activities of the extracts of the stem-bark and leaves of the *P. guajava L*. are represented in Table 3.

The antimicrobial studies showed that aqueous extract of *P. guajava L* had inhibitory effects on *Fusarium spp* and *B. subtilis* with zones of inhibition of 20 and 16 mm respectively, while the ethanolic extracts exhibited no inhibitory effects on these microorganisms. This shows that the plant could be used to treat skin infections and other infections caused by these micro-organisms (Burkill, 1997).

The MIC of the aqueous extract of *P. guajava* against *Fusarium spp* and *B. subtitis* found to be 5 and 3 mg/ml respectively, indicating that the aqueous extract was highly potent against these microorganisms. Of the control drugs, Amplicine was not effective against any of the test organisms while Gentamycin and Tetracycline showed inhibitory effects against the organisms. Gentamycin was however the most potent of the two with zones of inhibition range of 18 to 22 mm, the highest being against *B. subtilis* (22 mm). Tetracycline exhibited inhibitory effects only against *E. coli* (16 mm) and *P. aeruginosa* (18 mm). The zones of inhibition of effective extracts were close to those of the control drugs and falls with the Kirby Bain's Standard for antimicrobial studies. Moreover, the MIC results, 3 and 4 µg/ml for *B. subtilis* and 5 and 4µg/ml for *Fusarium species* were appreciably low which account for the potent of these extracts.

The medicinal uses of this plant are supported by the

Table 2. Characteristics of TLC spots of crude extracts of the leaf (A) and stem-bark (B) extract of *P. guajava L*.

Tested material	No of spots	Colour	Rf.
Leaf extract (A)			
Ethanol	3	Pink	0.64
		Light Pink	0.36
		Faint yellow	0.60
Methanol	3	Pink	0.62
		Faint orange	0.50
		Light orange	0.70
Aqueous	-	-	0.00
Stem-bark extract (B)			
Ethanol	3	Pink	0.64
		Light Pink	0.36
		Faint yellow	0.60
Methanol	3	Pink yellow	0.62
		Faint orange	0.50
		Light orange	0.70
Aqueous	*	-	0.00

Key note: –, No separation.

Table 3. Antimicrobial activity of leaf (A) and stem-bark (B) extract of *P. guajava L.* Antimicrobial activity was measured as inhibition zone (mm) using 200 µg extract/ml.

Microorganism	Extracts (200µg/ml) inhibition zone (mm)							
Bacteria	**Methanol**	**Ethanol**	**Aqueous**	**Ampicillin**	**Gentamycin**	**Tetracycline**	**Fulcin**	**MC aqueous extract (µg/ml)**
Leaf extract (A)								
B. subtilis	-	-	6	-	12	-	ND	3
E. coli	-	-	-	-	12	6	ND	-
P. aeruginosa	-	-	-	-	10	8	ND	-
Fungi								
C. albicans	-	-	-	ND	ND	ND	6	-
Fusarium spp	-	-	10	ND	ND	ND	-	5
G. candidan	-	-	-	ND	ND	ND	4	-
Stem-bark extract (B)								
Bacteria								
B. subtilis	-	-	4	-	10	-	ND	2
E. coli	-	-	-	-	10	6	ND	-
P. aeruginosa	-	-	-	-	8	7	ND	-
Fungi								
C. albicans	-	-	-	ND	ND	ND	7	-
Fusarium spp	-	-	12	ND	ND	ND	-	4
G. candidan	-	-	-	ND	ND	ND	4	-

Key note: ND – Not detectable.

presence of the above mentioned constituents and the antimicrobial activities. Hence, the need to exploit the potentials of this plant especially in areas of traditional medicine and pharmaceutical industries arises.

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