



***In vitro* Antibacterial Activity of *Psidium guajava* Leaves Extracts against Clinical Isolates of *Salmonella* specie**

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Abstract

Dried leaves of *Psidium guajava* obtained from Bayero University, Kano old campus were extracted using water, ethanol and chloroform solvents via percolation method. The leaves extracts were subjected to phytochemical screening to detect presence of secondary metabolites. The extracts were further tested for antibacterial activity against clinical isolates of *Salmonella* Typhi, *Salmonella* ParatyphiA and *Salmonella* Paratyphi B using agar well diffusion method. The results of phytochemical screening indicated the presence of tannins, flavonoids, steroids, terpenoids and phenols while those of the antibacterial activity indicated that highest zone of inhibition was observed in water extracts against *S. Paratyphi* B, (12mm), followed by water extracts against *S. Paratyphi* A (9.6mm) with the least being water extracts against *S. typhi* (8mm). *S. Paratyphi* B was resistant to both ethanolic and chloroform extract while *S. Typhi* was found to be resistant to chloroform extracts only. *S. Paratyphi*A was however, found to be the most sensitive bacterium to all the extract regardless of extraction solvent.

Keywords: Antibacterial activity, *Psidium guajava*, extracts, agar-well diffusion, clinical isolates, typhoid fever.

INTRODUCTION

The leaves and bark of *Psidium guajava* tree have a long history of medicinal uses that are still employed today (Nwinyi *et al.*, 2008). The leaves and bark of the guava plant have been used to treat diarrhea, other gastrointestinal disorders, toothaches, colds, and swelling, in Africa (Rabe and Vanstaden, 1997).

The genus *Psidium* belongs to the family Myrtaceae, which is considered to have originated in tropical South America. Guava crops are grown in tropical and subtropical areas of the world like Asia, Egypt, Hawaii, Florida, Palestine, and others. The genus *Psidium* comprises approximately 150 species of small trees and shrubs in which only 20 species produce edible fruits and the rest are wild with inferior quality of fruits (Scopus *et al.*, 2011). The most commonly cultivated species of *Psidium* is *P. guajava* L. which is the common guava. Other species are utilized for regulation of vigor, fruit quality improvement and resistance to pest and disease (Scopus *et al.*, 2011).

Guava is a phytotherapeutic plant used in folk medicine that is believed to have active components that help to treat and manage

various diseases. The many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions (Abdelrahim *et al.*, 2002, Scopus *et al.*, 1999 and Scopus *et al.*, 1992). This plant has also been used for the controlling of life-changing conditions such as diabetes, hypertension, and obesity (Abdelrahim *et al.*, 2002) and (Sunagawa *et al.*, 2004).

The aim of this work was to determine phytochemical composition and antibacterial activity of *P. guajava* extracts against clinical isolates of *Salmonella* Typhi, *Salmonella* Paratyphi A and *Salmonella* Paratyphi B.

MATERIALS AND METHODS

A. Preparation of Sample

Fully matured leaves, of *Psidium guajava* were collected from Bayero University Kano, Old Campus, their identity was confirmed by a botanist, in the Department of Plant Biology of the institution and voucher specimens were deposited in the departmental herbarium.

The Sample was given a voucher number BUKHAN 0336. The plant leaves were washed thoroughly with running tap water, shade dried, homogenized to fine powder and stored in an air tight bottle (Aliyu, 2006).

B. Extraction Procedure

Fifty grams (50g) each of the dried powdered plant leaves was weighed into 3 different glass containers and sequentially extracted with 250ml for Ethanol and chloroform and 500ml for aqueous by percolation method for one week. The extracts were filtered using Whatman No. 1 filter paper. Each of the resulting filtrate was then concentrated by complete evaporation of solvent at room temperature except for aqueous extract which was evaporated in a water bath at 45°C. The filtrate was carefully labeled, weighed and transferred into sterile air tight glass containers after which it was stored in the refrigerator for further use (Fatope *et al.*, 1993).

C. Phytochemical Analysis

Both water, ethanol and chloroform extracts of the leaves were subjected to phytochemical screening in order to test for the presence or absence of secondary metabolites using the method described by (Sofowara, 1993).

D. Bioassay

i. Test Organisms

Salmonella Typhi, *Salmonella* Paratyphi A, and *Salmonella* Paratyphi B, were collected from Department of Microbiology, Aminu Kano Teaching Hospital, Kano, Nigeria.

ii. Confirmation of Test Bacteria

The test bacteria were subjected to various biochemical tests for identification of catalase, oxidase, indole, motility, citrate utilization, urease production, hydrogen sulfide production as well as acid and gas production. And observation of cultural growth characteristics such as ability to grow readily on simple media over a range of pH 6-8, temperature 15-41°C with optimum temperature of 37°C. Large colonies, circular and smooth on MacConkey and Deoxycholate citrate media and colourless colony colour due to absence of lactose fermentation (Cheesbrough, 2006).

iii. Standardization of Inoculum

Using sterile inoculation wire loop, a loopful of the test isolate was picked from an overnight

culture of the test organism and transferred into a tube of saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard (Cheesbrough, 2006).

iv. Antimicrobial Susceptibility Test

The agar well diffusion method was used for the antimicrobial susceptibility test. Mueller Hilton agar was prepared according to manufacturer's specification and a standardized inoculum was swabbed on the agar. Four wells of 6mm each were made in each plate using a sterile cork borer of 6mm. The wells were filled with 0.1ml of different diluted concentrations of the extract with the aid of sterile pipettes. Standard antibiotic was used as positive control. While sterile distilled water was used as negative control. The plates were allowed to stand for 15 minutes on a table to allow pre diffusion of the extracts followed by incubation at 37°C for 24 hours. Diameters of zones of inhibition were measured using transparent plastic ruler (Dahiru *et al.*, 2013).

v. Determination of Minimum Inhibitory Concentration of the Extracts (MIC)

Plants extracts that showed activity in the agar well diffusion method were used for the determination of Minimum Inhibitory Concentration (MIC). The extracts were prepared by serial doubling dilution using Dimethyl Sulfoxide (DMSO). Concentrations of 200µg/ml, 100µg/ml, 50µg/ml, 25µg/ml, 12.5µg/ml and 6.25µg/ml. A stock solution of the extract and that of amoxicillin were serially diluted in test tubes containing double strength Nutrient broth. Equal volume of the extract in a nutrient broth (i.e. 2ml each) was dispensed into sterilized test tube.

Specifically 0.1 ml of the standardized inoculum was added to each of the test tubes above. Tube containing broth and extracts without inocula serve as a positive control while tubes containing broth and inocula without extract served as negative control. The tubes were incubated at 37°C for 24 hours (Fatope *et al.*, 1993).

MIC was recorded as lowest concentration of the extract inhibiting the visible growth of the bacteria. This was carried out by comparing the tubes with the control tubes against a source of light with white background and some contrasting black lines.

vi. Determination of Minimum Bactericidal Concentration (MBC)

MBC was determined by sub-culturing samples from the MIC tubes that showed no turbidity on Mueller Hilton agar plates separately and then incubated at 37°C for 24 hours. After the incubation the plates were observed for presence or absence of growth. The least concentration of the extract at which no bacterial growth was observed was considered as the MBC (NCCLS, 2008).

RESULTS

Table 1 showed the physical properties; colour, odour, texture of the chloroform, ethanol and water leaves extracts as well as their percentage yield. The solvents extraction potential was ranked this way in accordance with the findings; ethanol > water > chloroform. Ethanol was therefore the best solvent for extraction in comparison to the other solvents used.

The result of the phytochemical screening of water, ethanol and chloroform extracts of *P. guajava* leaves is shown in Table 2. The results showed the presence of these secondary metabolites namely saponins, tannins, flavonoids and terpenoids in both extracts,

phenols were found in water while steroids were found in ethanolic extracts. Lastly alkaloids and glycosides were not found in any of the extracts.

The result of antibacterial activity of the *P. guajava* extracts against the test bacteria is shown in Table 3. Highest zone of inhibition was observed by water extracts against *S. Paratyphi B*, (12mm), followed by chloroform extracts against *S. Paratyphi A* (10mm) and the least being water and ethanolic extracts against *S. Typhi* (8mm) each. *S. Paratyphi B* was resistant to both ethanolic and chloroform extract while *S. Typhi* was found to be resistant to chloroform extract only. *S. Paratyphi A* appeared to be the most sensitive to all the extracts regardless of solvent.

The result of the MIC of *P. guajava* leaves extracts is shown in Table 4. The results showed that aqueous and ethanolic extracts had MIC ranges of (6.25µ/ml-50µ/ml), (25µ/ml-50µ/ml) respectively while chloroform maintained MIC of 25µ/ml for all the tested bacteria. While MBC values range from (12.5µg/ml -50µg/ml, 25µg/ml-100µg/ml and 50µg/ml - 200µg/ml) for aqueous, ethanolic and chloroform extracts respectively.

Table 1: Physical Properties of *P. guajava* Extracts

Solvent	Color	Odor	Texture	Sample (g)	Amount Recovered(g)	% Yield
Chloroform	Dark green	Minty	Gummy	50	0.56	1.12
Ethanol	Deep green	Minty	Gummy	50	3.03	6.06
Water	Reddish brown	Chocolate	Sticky	50	2.5	5.00

Table 2: Phytochemical constituents of *P. guajava* Extracts

EXTRACT	Alkaloid	Saponin	Tannin	Flavonoid	Steroid	Glycoside	Terpenoid	Phenol
	S	S	S	S	S	S	S	S
Aqueous	-	+	+	+	-	-	+	+
Ethanolic	-	+	+	+	+	-	+	-
Chloroform	-	+	+	+	-	-	+	-

Key: + = present, - = absent

Table 3: Antibacterial Activity of *P. guajava* Extracts Using Agar well Diffusion Method

Test Organisms	AE (µg/ml)				EE (µg/ml)				CE (µg/ml)				Amx (µg/ml)			
	50	100	200	400	50	100	200	400	50	100	200	400	50	100	200	400
<i>S. Typhi</i>	0.0	0.0	7.0	8.0	0	0	7	8	0	0	0	0	12	12	15	18
<i>S. Paratyphi A</i>	8	8.3	9	9.6	0	0	0	8.3	7.8	8.5	9.5	10	9	11	11	14
<i>S. Paratyphi B</i>	9.5	10	12	12	0	0	0	0	0	0	0	0	8	9	14	16

Key: AE = Aqueous Extract, EE = Ethanolic Extract, CE = Chloroform Extract, Amx = Amoxicillin

Table 4: MIC and MBC of *P. guajava* Extracts

S/N	Test Organisms	Aqueous Extract		Ethanollic Extract		Chloroform Extract		Amoxicillin	
		MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml	MIC µg/ml	MBC µg/ml
1	<i>S. typhi</i>	50	100	25	100	25	50	12.5	25
2	<i>S. Paratyphi A</i>	50	100	50	100	25	50	6.25	25
3	<i>S. Paratyphi B</i>	6.25	50	25	50	25	50	6.25	12.5

Key: MIC= Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration

DISCUSSION

Result of the physical properties of *P. guajava* leaves extracts is presented in Table 1. From the result the extracts appeared dark green, deep green to reddish brown for chloroform, ethanol and water extracts respectively with gummy and sticky textures. Highest percentage yield of *P. guajava* extracts was observed in ethanolic extracts (6.06w/w), then water extracts (5.00w/w) and the least being chloroform extracts (1.12w/w).

The results of the phytochemical screening of water, ethanol and chloroform extracts of *P. guajava* is shown in Table 2. From the results the major secondary compounds found are saponins, tannins, flavonoids and terpenoids. These phytochemicals have been reported for antimicrobial activity (Singh and Bhat, 2003). These are the suggestive components to which the antimicrobial activity observed could be attributed. Begum and Siddiqui, (2002) reported isolation of two triterpenoids; guavanoic acid and guava coumaric acid from the leaves of guava. Arima and Danno (2002) also isolated and identified four flavonoids from leaf extracts of *P. guajava* which were found to inhibit the growth of *Salmonella enteritidis* and *Bacillus cereus*.

Pritesh and Zara (2015) reported that alkaloids has pain killing and poisonous effect but sometimes help in important cure. Flavonoids have been referred to as nature's biological response modifiers, because it's inherent ability to modify the body's reaction to allergies. It possesses various pharmacological

roles including anti-microbial activities (Duraipandiyan *et al.*, 2006). Tannins were also reported for in vitro antibacterial activity (Lü *et al.*, 2004).

The result of the antibacterial activity of the *P. guajava* extracts against the test bacteria is shown in Table 3. Highest zone of inhibition was observed by water extracts against *S. Paratyphi B*, (12mm) and the least activity wise being water and ethanolic extracts against *S. Typhi* (8mm). *S. Paratyphi B* was resistant to both ethanolic and chloroform extract while *S. Typhi* was found to be resistant to chloroform extract only.

This finding corroborates with a work on methanolic extracts of guava reported by Lin *et al.* (2002) where *P. guajava* showed significant inhibitory activity against the growth of 2 isolates of *Salmonella spp.* And oppose the finding of Nascimento *et al.* (2000) in which the *P. guajava* extracts was able to have inhibitory effects against *Staphylococcus* and *Bacillus* and no effect on the *Escherichia* and *Salmonella* which are all gram negative bacteria.

CONCLUSION

The study indicated that leaves extracts of *P. guajava* had antibacterial activities against all the test bacteria and as such provided scientific support for the traditional use of the plant in treating typhoid fever. However, further research should be carried out to study the implication of taking the leaves.

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