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Full Length Research Article

ANTIBACTERIAL ACTIVITY OF GUAVA (*PSIDIUM GUAJAVA L.)* EXTRACTS ON *STAPHYLOCOCCUS* AUREUS ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS ATTENDING A TERTIARY-CARE HOSPITAL

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ABSTRACT
The uses of herbal treatment are one of the possible ways to treat diseases caused by multi drug resistant bacteria. In this study, the phytochemical and antimicrobial effect of *Psidium guajava* (*l.*) leaf and stem extracts were investigated using well diffusion method against *Staphylococcus aureus* isolates recovered from urine sample of patients with urinary tract infection (UTI) attending Murtala Muhammad Specialist Hospital, Kano. The results revealed that the plant contained some bioactive compounds which includes; Alkaloids, Flavonoids, Anthraquinones, Amino acid, Saponins, Tannins, Reducing sugar, Glycoside and Phenolic compound. The antimicrobial activity of the plant showed that the plant leaf and stem extracts (Ethanolic and Aqueous) had an antibacterial activity against the test isolates with varying mean zones of inhibitions ranging from 10mm to 24mm. However, the organic solvent extract showed more effect compared to the aqueous extract. The present study therefore, suggested that the plant (*Psidium guajava L.*) can be used as an alternative to chemotherapeutic agents.

Keywords: Antimicrobial Activities, Inhibition, Phytochemical, *Psidium guajava*, Urinary Tract Infection.

INTRODUCTION
The uses of herbal treatment are one of the possible ways to treat diseases caused by multi drug resistant bacteria. Though many pharmaceuticals industries have produced a number of antibiotics from several years, but in many cases it was observed that the cultures were showing resistance against the medicines (Cohen, 1992). *Psidium guajava* is a small evergreen shrub native to tropical America that has neutralized in South East Asia and Africa. It grows up to 35 feet tall and widely grown for its fruits in tropics. It is a member of the *Myrtaceae* family, with about 133 genera and more than 3800 species. The leaves and bark of *Psidium guajava* tree have a long history of medical uses that are still employed today (Nwanyi *et al*., 2008). There are over 20 compounds present in leaves, stems, bark and roots of *P. guajava* (Lozoya, 1994). The leaves of guava contain an essential oil rich in cineol, tannins triterpenes, flavanoids, resin, eugenol, malic acid, fat, cellulose, chlorophyll, mineral salts, and a number of other fixed substances (Ncube *et al*., 2008). The leaves were used in USA as an antibiotic in the form of poultice or decoction for wounds, ulcers and toothache. Guava fruits also contain vitamin C vitamin, iron calcium and phosphorus (Lozoya, 1994).
The pharmacological actions and the medicinal uses of methanolic extracts of guava leaves in folk medicine include the treatment of various types of gastrointestinal disturbances such as vomiting, diarrhoea, inhibition of the peristaltic reflex, gastroenteritis, spasmolytic activity, dysentery, abdominal distention, flatulence and gastric pain (Ross 2003). *P. guajava* have been known to have antimicrobial (Chah *et al*., 2006), anti-inflammatory (Ojowole, 2006), antimalarial (Tona *et al*., 1999), and antiglycemic (Ojowole, 2005) activities. It has been used to treat wounds (Chah *et al*., 2006), acne (Qadan *et al*., 2005), cough (Jairaj *et al*., 1999) and dental diseases (Razak *et al*., 2006), diabetes and hypertension (Begum *et al*., 2004). Leaves, root, and bark extracts are used for treatment of diarrhea and cholera (Ahmed and Beg, 2001). *Guajava* leaf extract contains guajava polyphenol that has an anti-oxidation action and flower and leaf of the plant have been reported to have antibiotic activity (Andrew, 2001).
The leaves of *Psidium guajava* have been shown to exhibit both gram-positive and gram-negative bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella enteridis*, *Bacillus cereus*, *Proteus* species, *Shigella* species and *Escherichia coli* (Perez *et al*., 2008).
In the present study, the extracts (Both aqueous and ethanolic) from leaves and stem of *Psidium guajava* were screened for Antibacterial activity against *Staphylococcus aureus* recovered from patients with Urinary tract infection as well as to determine the phytochemicals present in sample which are responsible for antibacterial activity.

MATERIALS AND METHODS

Plant materials
The plant material used in this research is the of the leaves and stem back of *Psidium guajava* (Plate 1) which were collected from botanical garden of Government Secondary School Gundutse, Kura Local Government Area of Kano State at about 08:30 a.m. Identification and authentification of the plant materials was done by trained plant taxonomist at Herbarium unit in the department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria with the following voucher number 3253. After authentification, a voucher plant specimen was deposited in the herbarium of the University for future reference. The sample was washed with water to remove dust and rinsed with distilled water. Sample was air dried for two-weeks and pulverized into powder form using sterile mortar and pestle in the laboratory as described by

Mukhtar and Tukur, (1999). The powdered sample was bagged in a black polythene bag and store in air tight container for further work.

Preparation of plant extract
The ethanol and aqueous extract of the plant samples were carried out according to Bengum (2014). Twenty five grams (25g) of the powdered leaves and stem back were weight out and dissolved in 250ml of both solvent in a sterile beaker and allowed to stand for seven days. The mixture was filtered using Whatman No.2 filter paper and the extracts were evaporated to dryness using rotary evaporator and water bath. The solid residues obtained were reconstituted in DMSO and water at stock concentration, stored in the refrigerator at 4^0C until used.

Antimicrobial assay of extracts
The agar well method was used to determine the antibacterial activity of the plant extracts. 0.1ml of the standardized organism (0.5 McFarland standards) were introduced separately and thoroughly mixed with Mueller Hilton Agar in a sterile Petri dish and allowed to set then labeled. A sterile cork borer 6mm was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 200mg/ml, 150 mg/ml, 100mg/ml and 50 mg/ml while the 5th well contained the solution used for the study to serve as control. Tetracycline (Chi Pharmaceutical Limited, Lagos Nigeria) 125mg/ml, was used as control in this research. These were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37^0C for 24 hours. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters along straight line i.e. 180^0 to each other and the mean of the readings were then calculated (Anibijuwan and Udeze, 2009).

Phytochemical screening
This was done on different extract to ascertain the presence of bioactive component present in the leaves and stem back of *Psidium guajava.* The presence of Alkaloid, Saponin, Glycoside, Tannins, Flavonoids, Steroid, Terpenoids, Anthraquinones, Protein and Amino acid were determined using procedure described by Sofowora (1993).

RESULTS
The antibacterial activity of aqueous and ethanolic leaf extract *Psidium guajava* were indicated in Table 1. The result showed that the mean diameter of zone of inhibition of extract on the test isolate and the highest zone of inhibition recorded was 23.3mm from 200mg/ml ethanolic leaf extract while the lowest zone of inhibition was 10.6mm from 50mg/ml aqueous extract.
The antibacterial activity of aqueous and ethanolic stem back extracts of *Psidium guajava* were indicated in Table 2 showing the mean diameter of zone of inhibition of extract on the test isolate and the highest zone of inhibition recorded was 21.3mm from 200mg/ml ethanolic leaf extract while the lowest zone of inhibition was 08.6mm from 50mg/ml aqueous extract.
Table 3 showed that the phytochemicals were present in both leaf and stem of *Psidium guajava.* Both leaf and stem contain all the tested phytochemicals except Steroid

Table 1: Mean diameter zones (with standard error) of inhibition of aqueous and ethanolic leaf extract *Psidium guajava*

CONCENTRATION (mg/ml)		PETRI DISH/ZONE OF INHIBITION (mm) 1	2	3	MEAN
	50	11.0	10.0	11.0	10.6±0.27
ALE	100	12.0	11.0	12.0	11.6±0.27
	150	12.0	12.0	13.0	12.3±0.27
	200	16.0	17.0	16.0	16.3±0.27
CONTROL		18.0	19.0	19.0	18.6±0.27
	50	12.0	11.0	11.0	11.3±0.27
ELE	100	18.0	19.0	18.0	18.3±0.00
	150	20.0	21.0	20.0	20.3±0.27
	200	23.0	24.0	23.0	23.3±0.27

ALE = Aqueous Leaf Extract. ELE = Ethanolic Leaf Extract.

Table 2: Mean diameter zones (with standard error) of inhibition of aqueous and ethanolic stem back extract of *Psidium guajava*

CONCENTRATION (mg/ml)		PETRI DISH/ZONE OF INHIBITION (mm) 1	2	3	MEAN
	50	13.0	13.0	14.0	13.3±0.27
ALE	100	15.0	16.0	14.0	15.0±0.00
	150	20.0	21.0	22.0	20.0±0.00
	200	23.0	24.0	23.0	23.3±0.27
CONTROL		18.0	17.0	18.0	17.6±0.27
	50	15.0	14.0	15.0	14.6±0.27
ELE	100	17.0	17.0	18.0	17.3±0.27
	150	21.0	21.0	22.0	1.3±0.27
	200	24.0	3.0	24.0	23.6±0.27

ALE = Aqueous Leaf Extract. ELE = Ethanolic Leaf Extract

Table 3: Phytochemical constituents of leaves and stem back of *Psidium guajava*

S/N	PHYTOCHEMICAL	LEAF EXTRACT	STEM EXTRACT
1.	Alkaloids	+	+
2.	Saponin	+	+
3.	Phenol	+	+
4.	Flavonoid	+	+
5.	Protein and Amino acid	+	+
6.	Tannin	+	+
7.	Reducing Sugar	+	+
8.	Anthraquinone	+	+
9.	Steroid	_	_
10.	Terpenoid	+	+

+ = Presence of phytochemical, - = Absence of phytochemical.

Plate 1: *Psidium guajava* tree

DISCUSSION

The present study investigated the antimicrobial activity of *Psidium guajava* leaf and stem extracts; the results showed that both aqueous and ethanolic extracts of guava leaf and stem inhibited the growth of the *Staphylococcus aureus* tested. These results support the findings of Viera *et al*. (2001), Egharevba *et al*. (2010) and Biwas *et al*. (2013) which also reported the antibacterial effect of guava leaves extracts and found that they inhibited the growth of *S. aureus*. However, the ethanolic extract showed stronger inhibition than the aqueous extract against the organisms. This result is in conformity with that of Pandey, (2012) who reported that the antibacterial activity ethanolic extract of *Psidium guajava* leaf and stem showed stronger anti-bacterial activity than aqueous extract. The present result is contrast with the findings of (Elekwa *et al*., 2008; Emmanuel, 2010 and Biwas *et al*., 2013) who reported higher antimicrobial activity of aqueous extract of *Psidium guajava* than that of ethanolic extract.

The results of the present study on the antibacterial activity of stem and leaf of *Psidium guajava* on the tested isolates revealed that stem extract possess higher antimicrobial activity than corresponding leaf extract. This hold true with the results of Elekwa *et al*. (2008) in which the antibacterial study on the effect of *Psidium guajava* showed that the stem extract is more effective than leaf extract. The observed inhibition of *Staphylococcus aureus* in this study suggests that guava possesses compounds containing antimicrobacterial properties that can effectively suppress the growth of *Staphylococcus aureus* when extracted using ethanol as a solvent. It has been observed that gram-positive bacteria such as *Staphylococcus aureus* have a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts of plants (Burt, 2004; Qadan *et al*., 2005; Rameshkumar *et al*., 2007 and Stefanello *et al*., 2008). Belemtouri *et al*. (2006) reported that the strong antibactericidal activity exhibited by the leaf extracts of *Psidium guajava* was possibly due to the protein degradading activity of the extracts. Ceceres *et al*. (1993) describe the antibiotic activity of the aqueous extract of dried leaves and bark of *P. guajava* to guajaverin and psidiolic acid

The result of the preliminary phytochemical analysis of leaf and stem back extract, (ethanol and water) of *P. guajava* revealed the presence of the following chemical constituents; Alkaloid, saponin, phenol, flavonoids, protein and amino acid, anthraquinones, terpenoid and tannin. Earlier work have revealed the presence of alkaloids, flavonoids, glycosides, poly-phenols, reducing compounds, saponins and tannins in the aqueous extract of *Psidium guajava* leaf (Uboh *et al.,* 2010). This result is in line with the work of Ugoh and Nneji (2013) and Offo (2015) who also reported similar finding on the phytochemical of guava leaf extract, which contain alkaloid, saponin, flavonoids, phenol, steroid, tannin, protein and glycoside. Pandey and Shweta, (2012) reported the phytochemicals mainly present in *Psidium guajava* were reducing sugar, tannin, saponin, phlobatannin, terpenoid, alkaloid and phenols. The finding is also similar to that of Joseph and Priya, (2012) where the preliminary phytochemical analysis of leaf, stem back and root back extracts of *Psidium guajava* showed the presence of carbohydrate, glycoside, saponin, Anthraquinones, flavonoids, tannins and alkaloids. It has been documented that different solvents have diverse solubility capacities for different phytochemical constituents (Marjorie, 1999).

Tannins found in the phytochemical analysis may be responsible for the antibacterial effects. Akiyama *et al*. (2001), in their study of the antibacterial action of tannins against *S. aureus*, attributed the antimicrobial mechanisms to their (I) astringent property (II) toxicity, and (III) complexation of metal ions. It is reported that these phytochemicals are known to exhibit medical and physiological activities. For example, tannins are polyphenolic compounds that bind to proline rich protein that interferes with protein synthesis (Sanches *et al*., 2005) and have shown to antibacterial activity (Min *et al*., 2008). Flavanoids are hydroxylated polyphenolic compounds known to be produced by plants in response to microbial infections by various microorganisms *in vitro* (Cowan, 1999). Their ability has been attributed to their ability to form complexes with extracellular and soluble proteins as well as bacterial cell walls (Trease and Evans, 1989). Terpenoids although mainly used for their aromatic qualities have also be found to potential agents against inhibiting bacteria (Tsuchiya *et al*., 1996). Saponins which are glycosides have been found to have inhibitory effects on gram- positive organisms such as *S. aureus*. It has also been reported that *Psidium guajava* stem bark can be used to treat malaria because it presents antiplasmodial activities possibly due to the presence of Anthraquinones, Flavanoids, Seccoirridoids and Terpenoids (Nundkumar and Ojewole, 2002). Therefore, the results of this study justifies that the phytochemical solvents of stem and leaf extracts of *P. guajava* possess antibacterial properties that could inhabit microorganisms as well as believed to contribute in a way as humans continue to source for total cure for infectious diseases especially with the growing trends of antimicrobial resistance.

Conclusion

The medicinal uses of these plants *Psidium guajava* are supported by the presence of phytochemical constituents present in them and the antimicrobial activities they exhibit. The results obtained from this study showed that the plant contains bioactive chemical compounds and also possesses antibacterial activities against *Staphylococcus aureus*. The ethenolic extracts of *Psidium guajava* had higher antimicrobial activity against *Staphylococcus aureus* than aqueous extracts. Plant-based antimicrobials have enormous therapeutic and preferential potential; they can serve the desired purpose with lesser side effects that are often associated with synthetic antimicrobials used presently. Based on these findings, the application of the decoction of leaf and stem of the plant in ethno medicine is justified and leaves and stem of *Psidium guajava* possesses the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections caused by *S. aureus*. Hence, the need to exploit the potentials of these plants especially in areas of traditional medicine and pharmaceutical industries arises.

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