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ANTIBACTERIAL POTENCY AND PHYTOCHEMICAL SCREENING OF *Psidium guajava* STEM BARK EXTRACTS AGAINST DENTAL PATHOGENS

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ABSTRACT

Investigation into the antibacterial potency and phytochemical properties of Psidium guajava stem bark extracts was carried out against dental pathogens using disc diffusion method. The bacteria isolates (Lactobacillus spp and Streptococcus mutans) were obtained from patients established to have dental infection. The extracts were obtained using methanol and warm – water (aqueous) as solvents for extraction. The result of the antibacterial activity shows that the methanolic extract of the Psidium guajava stem bark against the bacteria isolates were 13,16,17 and 19mm for Lactobacillus spp and 12,18,19 and 18mm against Streptococcus mutans at the various concentrations of 10mg/ml, 30mg/ml, 50mg/ml and 70mg/ml used respectively while 11,12,12 and 18mm and 10,12,16 and 18mm was recorded for the aqueous extract against Lactobacillus spp. and Streptococcus mutans at the concentrations of 10mg/ml, 30mg/ml, 50mg/ml and 70mg/ml of the concentrations used respectively. The MIC and MBC result indicates that the plant extract has both bacteriostatic and bacteriocidal abilities. The phytochemical compounds detected, could be responsible for the antibacterial potentials of the stem bark of Psidium guajava extracts. This suggests that Psidium guajava stem bark has anti-bacterial potency especially against the test bacteria.

Keywords: Investigation, Antibacterial, Potency, Extracts, Against, Dental, Pathogens, Disc, Diffusion, Method, phytochemical, MIC, MBC

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Introduction

Psidium guajava (also known as Guava in English) is a well-known tropical tree that's abundantly grown for fruit and the plant belongs to the family Myrtaceae. *Psidium guajava* plant and all of its parts are known to have an old history of medical value and the plant can grow on a big range of soils. The plant fruit (Guava) is ovoid or round berry in shape. Its exocarp and Mesocarp vary in colours ranging from green, pink to yellow (Gelvez – Torres, 2008). The mesocarp can either be thin or thick while the endocarp can have few or many seeds depending on the variety, the seeds are rounded, triangular and very hard. The fruit can weigh between 25 to 500g, the polar diameter of the fruit varies from 3 and 12cm and the equatorial from 3 to 5cm (Sanchez-urdaneta *et al*., 2007). The fruit is rich in vitamin C content which is higher than some citrus fruits. The moisture contents of the fruit make it highly perishable. The sugar content is increased during maturation and the skin softening affects the appearance and quality. The overripe fruits are a target for phytosanitary problem (Garcia *et al.*, 2010 and Insuasty, 2007). The plant has a very wide spreading network of branches, which are usually curved display of opposite leaves with small pestoles of between 3 to 16cm. the leaves are wide and clear green in colour and have clear and prominent veins (Bashir and Goukt, 2002). It is a common shade or shrub tree in most household's gardens in the tropics. The tree is easily identified by its distinctive thin, smooth, copper coloured bark that flakes off, with a greenish layer appearance beneath. The guava plant bark is thin and has green colour spots. It can be easily removed from its long straps. It has a huge content of antimicrobial and antibacterial compounds (Bashir and Goukh, 2002). The Ethanolic extracts of stem bark of the guava plant have a high antibioc activity with a large number of phytochemicals including essential oils, polysaccaharides, minerals, vitamins, enzymes and triterpenoids acid, alkaloids, steroids, glycosides, tannins, flavonoids and saponins (Dewanto *et al.*, 2002).

Local preparations made from the leaves and/or bark of the *Psidium guajava* plant have been reported to be useful for the treatment of diarrhea, dysentery, sore-throat, vomiting, stomach upsets and vertigo. They have also been found to be effective in regulating menstrual periods (Holetz *et al.*, 2012). The stem bark have also been used as a twig stick by many locals in the tropics, to remove debris and germs from the teeth and jaw.

Human oral cavity is one of the most dynamic habitats for a number of bacterial species where they undergo intense interspecies competition to form multispecies biofilm structure. Various species of the genus *Streptococcus, Lactobacillus, Lactococcus, Enterococcus, Staphylococcus, Corynebacterium, Veillonella and Bacteroids* are the prominent bacteria commonly found in the oral cavity. *Streptococcus* and *Enterococcus* are two important members of the oral cavity because they can shift their lifestyle from beneficial microflora on the surface of oral cavity and oropharynx to destructive pathogens when they gain access into the oral tissue and bloodstream. Some of the diseases caused by oral bacteria include dental caries, periodontitis, endocarditis, pharyngitis, pneumonia, meningitis etc (Garcia, 2010). Most of the oral *Streptococcus* are gram positive facultative anaerobes demonstrating highly efficient survival strategies such as the ability to adhere hard and soft tissues, cell-cell communication, biofilm formation and to cope up with the rapidly changing oral environment (Garcia, 2010).

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Dental diseases are infectious microbiological disease of the teeth that can result to localized dissolution and destruction of the calcified tissues. It is the second most common cause of tooth loss and is found universally, irrespective of age, sex, caste, creed or geographic location. It is considered to be a disease of civilized society, related to lifestyle factors, but heredity can also play a role. In the late stages, it causes severe pain, is expensive to treat and leads to loss of precious man – days (Garcia, 2010). Besides, poor oral health affects the functions of mastication and speech, and ultimately the overall well-being of an individual but the disease can be preventable.

Problem Statement

Despite the availability of different kinds of oral hygiene preparations e.g. tooth paste that is available to keep the oral cavity safe and healthy, this has not been helpful for the maintenance of oral hygiene and in keeping the oral resident flora versus that of transient microbes in check and this has led

to formation of plaque in the chew thereby resulting in to periodontal infection of the tooth, that is why periodontal disease is still a serious health challenge and most of these oral hygiene preparations and antibiotics used for prevention and treatment of Dental diseases are synthetic and could cause side effects such as; toxicity, allergy of the body and are also expensive when available. However, the practices of using twig-stick has been an ancient practice from time immemorial and has been very effective in maintaining oral hygiene and in the prevention of periodontal disease. Plant based products are safer, cheaper, available and renewable in nature. Therefore, the use of *Psidium guajava* stem bark as twig stick has been largely practiced in most rural communities in Africa including Nigeria and this research is sort with the view to investigate and determine the potency of *Psidium guajava* stem bark against bacterial isolates from patients established to have dental caries in Birnin Kebbi metropolis. Therefore, the aim of this research work is to determine the antibacterial potency and phytochemical properties of *Psidium guajava* stem bark extracts against bacteria isolates from patients with dental caries.

METHODOLOGY

Sample collection

The *Psidium guajava,* stem bark used in this study were collected within Birnin Kebbi metropolis, Kebbi state (Nigeria). These samples were well packaged and brought to the laboratory in polythene bags for identification and extraction process.

Sample preparation

The samples were processed by washing of the stem bark, chopped into smaller pieces and dried under shade for 14days. The dried stem barks were pounded into powder form with the aid of a pestle and mortar.

Extraction of Plant Material

About 200g of the powder sample was weighed and soaked in 500ml of methanol and was allowed to stay for three (3) days, this was then sieved and the extract was then placed on a water

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bath and concentrated at 40^0C for evaporation to take place. The extracts were then collected and stored for use. For the aqueous extracts, 200g of the pounded sample was poured into 500ml of warm water and was allowed to stay for three days. It was then sieved and concentrated on water bath for 4 days for evaporation to take place.

Media Preparation

The specific media (Nutrient Agar, Nutrient broth Agar, Blood Agar and Mueller Hinton Agar) were prepared and used in this research work, according to the manufacturer instructions as contained in the label of the container.

Isolation of Bacteria

Sterile swab sticks were moistened with normal saline, and were used to collect bacteria from patients established to have dental infection in Birnin Kebbi, Kebbi State. The specimens were transported aseptically to the Microbiology Laboratory of Waziri Umaru Federal Polytechnic, Birnin Kebbi for microbiological investigations. The swabs were removed from the tubes and inoculated into blood agar and incubated at 37^0C for 24hours. The colonies that developed from the blood agar were Gram stained to determine the morphology of the bacteria and were also subjected to some specific biochemical test in order to identify the bacterial to species level. The bacteria isolates were then preserved in slant.

Gram Staining and Microscopy

A clean glass slides were obtained and using the sterile technique a smear of each of the microorganisms from fresh culture was prepared, and heat – fixed. The smear was gently stained with crystal violet and left for 1-2 minutes. This was then rinsed rapidly with water, followed by treatment with gram's iodine solution and left for 1 minute which increased interactions between the bacteria cell and the dye, so that the dye was more tightly bound or the cell was more strongly stained. The iodine was then poured off, blotted and the smear was then decolorized by washing with 95% ethanol until no more stain ran from the side. The slide was then washed with running water and counter stained with safranin for 2minutes and was then washed with water and was allowed to dry and was then observed under a light microscope at x100 objectives. The morphology of the bacteria was then determined (Cappuccino and Sherman, 2010).

Biochemical Test

The bacteria were then subjected to the following biochemical test (catalase, indole, coagulase, oxidase, citrate, urease, glucose, lactose, sucrose, H_2S, methyl red and voges praukuer test) after determination of the bacteria morphology. This was done in order to identify the bacteria to species level (Zimmer, 2000).

Preparation of Disc

Whatman no. 1 filter paper is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven (Oyeleke and Manga, 2008).

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Formulations of concentrations of the extracts

Exactly 1g of the methanol extract was weighed and placed in 10ml of distilled water; this was done for 3g, 5g and 7g to obtain various concentrations of 10mg/ml, 50mg/ml and 70mg/ml respectively. The same procedure was repeated for the aqueous extracts.

Sensitivity Test

The prepared discs were soaked in the already prepared various concentrations of the extracts and then removed to allow them to dry. The dried discs were then placed into the already prepared Mueller Hinton agar contained in a petri dish, after striking the medium with the test bacteria. This was done in duplicate. This preparation was then incubated at 37^0C for 24hours. This was removed from the incubator after 24hours, the zone of inhibition was then measured, recorded and expressed in millimeters. Oral B tooth paste was used as a positive control. The experiment was conducted in duplicate and the average value was calculated.

Determination of Minimal inhibitory Concentration (MIC) and Minimal bactericidal Concentration (MBC) Test

The minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the crude extracts on the isolated bacteria were determined according to the method proposed by Sarnie *et al.* (2005) and Omori *et al.* (2012). Twelve sterile test tubes were used and 1ml of sterile nutrient broth was dispensed from test tube 12, a stock solution of each of the Stem.

Phytochemical Screening

The Phytochemical analysis of stem bark extracts were carried out by Standard qualitative methods (AOAC, 2000).

Test for Alkaloids

The test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloid.

Test for Flavonoids

On addition of cone HCl in methanol extract of the material, a red colour appeared which indicated the presence of flavonoids.

Test for Glycoside

The extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with $Ba(OH)_2$. The remaining extract contained the glycosides. The hydrolysis of the solution done with concentration of sulfuric acid and after the hydrolysis the presence of sugar was determined with the help of Fehhling's solution.

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Test for Steroids

The extract was mixed with 3ml of Chloroform and 2ml conc. of sulfuric acid was poured from the side of the test tube and the colour of the ring at the junction of two layers was noted. A red colour showed the presence of steroids.

Test for Tannins

Extract was added in 1% ferric chloride and the colour was observed. Bluish black colour appeared which disappeared on addition ofH_2SO_4; a yellow brown precipitate showed the presence of tannins.

Test for Saponins

Extracts were diluted with water to 20 ml and this was shaken in a graduated cylinder for 15min. Formation of 1 cm layer of foam indicates the presence of saponins.

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FINDINGS AND DISCUSSIONS

Table 1: Bacteria Isolated from Patients with Dental Infection

Gam Reaction & Biochemical test	Bacteria Isolates *Lactobacillus spp*	*Streptococcus mutans*
Gram reaction	**Gram +ve Rod**	**Gram +ve Rod**
Catalase	**-ve**	**-ve**
Coagulase	**-ve**	**-ve**
Indole	**-ve**	**-ve**
Oxidase	**-ve**	**-ve**
Urase	**-ve**	**-ve**
Citrase	**-ve**	**-ve**
Manitol	**-ve**	**-ve**
Methyl Red	**-ve**	**-ve**
Voges Proskauer	**-ve**	**-ve**
Sucrase	**-ve**	**-ve**
Glucose	**-ve**	**-ve**
Lactase	**-ve**	**-ve**
H2S	**-ve**	**-ve**
Gas	**-ve**	**-ve**
Spore	**-ve**	**-ve**

Key

-ve = positive

-ve = Negative

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Table 2: Antibacterial Activity of Guava Stem Bark Extract

Extracts	Isolates	Zone of inhibition (mm) at varying concentrations				
		10mg/ml	30mg/ml	50mg/ml	70mg/ml	0ral B Paste
Methanolic	L.S	13.00	16.00	17.00	19.00	22.00
	S.M	12.00	18.00	19.00	18.00	18.00
Aqueous	L.S	11.00	12.00	12.00	18.00	22.00
	S.M	10.00	12.00	16.00	18.00	18.00

Key
Mg/ml = milligram/ml, mm = millimeter, L.S =*Lactobacillus* spp, S.M =*Streptococcus mutans*

Table 3: The MIC and MBC result of guava stem bark extract against the test Isolates

Extract	Isolates	Mic-Value mg/ml	MBC-Value mg/ml
Methamolic	L.S	4.375	8.75
S.M		2.188	4.375
Aqueous	L.S	4.375	8.75
	S.M	8.75	17.5

Key:
MIC = Minimal Inhibitory Concentration
MBC = Minimal Bactericidal Concentration
L.S =*Lactobacillus* spp,

S.M =*Streptococcus mutans*

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Table 4: Result of phytochemical screening of methanoic and aqueous extract of guava stem bark

Phytoconstituents	Methanol extract	Aqueous extract
Alkaloids	+	++
Flavanoid	+	+
Tannins	+	+
Saponins	++	+
Cardiec Glycoside	++	++
Antraquinones	-	-
Terpenes	++	++

Key: +++ = Highlypresent;++ = moderately present; + = slightly present; - = Not detected

Discussion

Results in Tale 1 above shows that only two different species namely *Lactobacillus spp* and *Streptococcus mutans* were isolated from patients with dental infection, which indicated that these bacteria are among the predominant bacteria associated with dental infection. This result is in line with the study of Marcelin *et al.* (2003) who isolated similar bacteria from patients with dental infection.

The result of antibacterial activity of Methanolic and aqueous extract of guava stem bark against *Lactobacillus spp* and *Streptococcus mutans* shows higher zone of inhibition at varying concentrations of the extract but when compared with the oral B control, lower and equal zone of inhibition at different concentration were obtained. The result shows that methanolic extract of guava stem bark has 13.00mm, 6.00mm, 7.00 and 19.00mm zone of inhibition at 10mg/ml, 30mg/ml, 50mg/ml and 70mg/ml of the concentrations respectively against *lactobacillus spp.* While, on *Streptococcus mutans,* it also shows that aqueous extract of guava stem bark has 10.00mm, 12.00mm, 16.00mm and 18.00mm zone inhibition at 10mg/ml, 30mg/ml, 50mg/ml and 70mg/ml concentrations respectively. The result shows that higher zone of inhibition was achieved at higher concentration of both extracts. The oral B paste used as positive control was effective against the test bacteria even when compared with the effect of the extracts against the test bacteria. The result obtained in this study corroborated with the report of Maspalma, (2013) against *Lactobacillus spp.* who reported higher zone of inhibition in the control group. The study is also in line with the study done by Dubois and Wagner, (2000). The higher antibacterial activity observed in this study could be attributed to the various phytochemical components presence in the guava stem bark, which has been implicated by various authors as strong antimicrobial agents which are toxic to microorganisms (Dubois and Wagner, 2000).

The results of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were shown in table 3 above. It shows that the MIC (4.375 mg/ml) and MBC (8.75mg/ml) values were found to be the same for both extracts when tested against *Lactobacillus spp* but shows variation of values against *Streptococcus mutans.* The aqueous extract has MIC value of 8.75mg/ml and MBC value of 17.5mg/ml while the methanolic extract has MIC value of 2.188mg/ml and MBC value of 4.375mg/ml when tested against *Streptococcus mutans.* These results confirm that there's antibacterial activity of guava extracts against the

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bacteria isolated from the patients with oral infections. This study also corroborates with the report of Dubois and Wagner, (2000).

The result of Phytochemical screening of methanolic and aqueous extract of guava stem bark were shown on Table 4, the result shows the presence of important phytochemicals (Alkaloids, Flavanoid, Tannins, Saponins, Cardiec Glycoside and Terpenese detected in both the extract except Antraquinones which were not detected in both methanolic and aqueous extract of guava stem bark. The presence of these secondary metabolites in plants, produce some biological activity which are responsible for the antibacterial activity observed in this research work. These active constituents can be used to search for bioactive lead compounds that could be used in the partial synthesis of more useful drugs.

Conclusion

The present study determined the antibacterial activity of guava stem bark extracts against the bacteria isolates from patients with oral infections. The study revealed that two *Lactobacillus spp* and *Streptococcus mutans* were isolated from patients with dental infection. It also revealed that the antibacterial activity of Methanolic and aqueous extract of guava stem bark against *Lactobacillus spp.* and *Streptococcus mutants* shows higher zone of inhibition at varying concentrations of the extract. But when compared with oral B paste control, lower and equal zone of inhibition at different concentrations were obtained. The higher antibacterial activity observed in this study could be attributed to the various phytochemical components presence in the guava stem bark, which has been implicated by various authors as strong antimicrobial agents which are toxic to microorganisms.

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