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Comparative studies of the anti-microbial effect of *Parkia biglobosa* (Dawadawa) and *Psidium guajava* (Guava) stem extracts on microbes commonly associated with oral infections

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Abstract
Plants have been used for the treatment of various diseases thousands of years ago and are still in use by about 80% of the world's population due to their effectiveness, availability and little or no side effects. *Psidium guajava* (guava) and *Parkia biglobosa* (dawadawa) are used locally in Ghana for treatment of dental problems. It has also been observed that the local people who use the stems of these plant as chewing sticks are protected from tooth decay and other dental problems that affect some people who use commercial tooth pastes. This study therefore sought to investigate the susceptibility of *Candida albicans*, *Streptococcus mutants* and *Escherichia coli* associated with oral diseases to the aqueous and 70% ethanol guava and dawadawa (stem/twine) extracts. The agar well diffusion and broth dilution assays were used to determine the antimicrobial activity and the MICs respectively. Phytochemical screening was also performed to determine the secondary metabolites present. All the extracts at 200 - 12.5 mg/ml were able to inhibit *Streptococcus mutants* and *Escherichia coli* with mean zones of inhibitions ranging from 18.67 ± 0.58 to 9.33 ± 0.58 and 28.0 ± 1.73 to 10.0 ± 1.73 respectively. *Candida albicans* was not susceptible to any of the extracts. The MICs of the extracts against *E. coli* and *S. mutants* ranged between 3.125 and 12.5 mg/ml. Secondary metabolites present in extracts included Saponins, Reducing sugar, Tannins. Flavonoids and Triterpenes were only present in 70% ethanol extract of *P. biglobosa*. However, Alkaloids, Anthracenocides, Phytosterols and Cyanogenic glycosides were absent. The aqueous and 70% ethanol extracts of guava and dawadawa exhibited good antibacterial activity against *E. coli* and *S. mutants* but ineffective against *candida albicans*.

Keywords: *Parkia biglobosa*, antimicrobial, phytochemical, dawadawa, *Candida albicans*

Introduction
Plants have been used for treatment for thousands of years, wide attention for their role in the treatment of mild and chronic disease (Venkata, 2014) [8]. According to world health organization (WHO), 70-80% of world population uses the plant or herb derived traditional methods for the treatment of various health problems, and the availability of medicinal plants and their cheaper cost in comparison to modern therapeutic agents makes them more attractive as therapeutic agents (Sharma, 2015) [7].

Guava stem have been used medically as an astringent and to treat diarrhea in children, while the flowers have been used to treat bronchitis and eye sores and to cool the body. The fruit has been used as a tonic and laxative and for treatment of bleeding gums. The plant is widely used in Africa and Asia in the prevention and treatment of scurvy. The guava plant is of great medicinal and economic importance. The stem of the plant contains tannins (12% to 30%) and calcium oxalate crystals, while the seeds contain glycine-rich proteins, starch, and phenolic and flavonoid compounds (Weni, 2011) [9].

The locust bean is a perennial tropical plant legume, predominantly dispersed within the Savannah region of the West Africa (Campbell-Platt, 2010) [3]. Locust bean is said in local dilate as 'dawadawa'. Dawadawa is of economic importance and is highly rich in protein and mostly used as condiment in local soups and as a dietary protein source (Wokoma, 2001) [10]. Besides the domestic uses of locust bean, it also has different medicinal properties. In traditional medicine practices, the bark have been explored to be used as a hot mouth wash to relieve toothaches (Ajaiyeoba, 2002) [1].

Dental plaque is due to the dissolution of the tooth mineral (primarily hydroxyapatite) by acids derived from bacterial fermentation of sucrose and other dietary carbohydrates. These bacteria live in bacterial communities known as dental plaque which accumulates on the tooth surface. For almost a century it was believed that any bacterial community on the tooth surface could cause dental decay, and treatment was almost exclusively the mechanical cleaning of these surfaces by tooth brushing, using some type of mild abrasive. Such treatment based on debridement and in extreme cases, upon dietary carbohydrate restriction, were singularly unsuccessful in reducing dental decay (Keyes, 2001) [5].

Materials and Methods

Sample Collection

The guava and dawadawa stems were collected randomly from trees in Kumasi and Navrongo respectively on the 12th and 13th of February, 2018. It was then authenticated at the Department of Applied Biology, UDS, Navrongo Campus.

Sample Preparation

The samples were washed with tap water and then air dried for about one weak to be free from moisture. The dried samples were then broken down into homogenous sizes in a process of size reduction to expose it to easy extraction. The mashing and homogenizing was done using a mortar and pestle.

Sample Extraction

Aqueous Extraction of the Samples (*Psidium guajava* and *Porkia biglobosa*)

The roughly homogenized guava and dawadawa stems were extracted by a process hot maceration. 150 g of the sample was weighed and kept in a container with 1000 ml of distilled water added and shaken. The mixture was then boiled on a stove for about 45 mins to allow removal of the active ingredients in the guava and dawadawa stem. The mixture was then filtered with the filtrate kept in a diff container and the residue discarded. The filtrate in the container was then put in an oven at 75 ℃ and left overnight for about 3 days to obtain the dried form of the extract. After the 3 days, it was removed from the oven and scooped out into a 50 ml falcon tube, weighed and then stored in a freezer until use.

70% Ethanol Extraction of the Samples (*Psidium guajava* and *Porkia biglobosa*)

The roughly homogenized guava and dawadawa stems were extracted by a process cold maceration. 150 g of the sample was weighed and in kept in a container. A 70% ethanol was prepared by weighing 700 ml of alcohol and adding 300 ml of distilled water to it. The 70% ethanol was then added to the weighed sample in the container and left for 3 days. After the 3 days, the mixture was filtered with filtrate kept in a container and residue discarded. The filtrate was then evaporated by a rotary evaporator to remove the ethanol and then keep the fluid content of the mixture. The mixture left was then kept in an oven for 3 days at 75 ℃ to obtain the dried form of the extract. After the 3 days, it was removed from the oven and scooped out into a 50 ml falcon tube, weighed and then stored in a freezer until use.

Phytochemical Analysis Test for Alkaloids

Two (2) ml of the sample extract solution was heated with 4 ml of 2% HCl on a steam bath. 2 ml each were measured into a test tube and then treated with a Wagner reagent (iodine potassium- iodide solution) to give a brown color precipitate that indicated the presence of alkaloids.

Test for Flavonoids

One (1) ml of the extract solution was taken into a test tube and a few drop of dilute NaOH solution were added. An intense yellow color appeared in the test tube. On addition of dilute HCl, the yellow color turned into colorless and indicated the presence of flavonoids.

Test for Saponins

One (1) ml of the sample was put into a test tube with 20 ml of distilled water added. It was shaken for about 10 minutes. A foamy layer appeared on the top of the mixture in the test tube which indicated the presence of saponins.

Test for Tannins

One (1) g of the powdered stem was weighed, transferred into a cornical flask. 10 ml of distilled water was added to it and shaken. The mixture was then boiled for 5 minutes with constant shaking. Two drops of 5% FeCl3 was added to the mixture. A greenish precipitate indicated the presence of tannins.

Test for Glycosides

Five (5) ml of dilute H2SO4 was mixed with the extract and then boiled for 15 minutes in a water bath. After cooling, 20% of Potassium hydroxide was used to neutralize the solution. 10 ml of equal parts of a mixture of Fehling's A and B solution was added after 6 minutes of boiling. The formation of a very dense red precipitate indicated the presence of glycosides.

Test for Steroids and Terpenoids

Nine (9) ml of pure ethanol was mixed with 3 ml of the extract solution. The solution was concentrated to 5 ml in water bath. Eight (8) ml of distilled water was added to the solution. One (1) hour after standing and the waxy matter was sieved off, extracted with 3 ml chloroform using a separating funnel. To one (1) ml of the chloroform extract in a test tube was carefully added 2 ml of conc. sulphuric acid. A lower layer was formed alongside an upper layer with a reddish-brown interface that indicated the presence of steroids. One (1) ml of the chloroform extract was evaporated on a water bath to dryness, 2 ml of conc. sulphuric acid to it and the resultant mixture was then heated for 10 minutes. Red precipitate formation affirmed the presence of terpenoids (Omojola, 2011) [6].

Preparation of Extract Concentration (Serial Dilution)

A stock solution of 200 mg/mL was prepared by dissolving 400 mg of the extract in 2 mL of distilled water. 1 mL of the distilled water was pipetted into four different tubes and arranged. 1 ml of the stock solution was pipetted into the first tube, shaken and another 1mL from the second tube into a third tube containing the distilled water and continuously for the other tubes.

Antimicrobial Test (Agar Well Diffusion Method)

The media for the culturing of the organisms was first prepared, with the *S. mutans* and *E. coli* on a Mueller-Hinton agar and the *C. albicans* on a Malt Extract agar. The plates were than stored in an incubator overnight at 37 ℃. The plates were brought out after an overnight growth and then inoculated into a 5 mL peptone water for activation of the

microbes. The sterile MHA and MEA medium (25 mL) in petri dishes were uniformly smeared using sterile cotton swabs moistened with pure cultures suspension (0.1 mL) of the microbes. Wells were created in the agar with a 6 mm sterile cork borer and 0.8 mL of various concentrations of the extracts (200, 100, 50, 25 and 12.5) mg/mL alongside the positive and negative controls were put in the wells. The plates were left for about an hour to allow for proper diffusion after which the plates were incubated at 37 ℃ for 18 hrs. The plates were examined for zone of inhibition which was measured and the values recorded.

Minimum Inhibitory Concentration (Plate Diffusion Method)

0.1 mL of peptone was first loaded into respected wells on the microtiter plate. 0.1 mL of the least concentration of the extract was added to the 1st column of the wells containing peptone. A serial dilution was then done from the extract in the 1st column until the last column on the microtiter plate. 0.1 mL of the bacteria was added to one row containing the extract and peptone. Each plant sample had a duplicate well with same peptone and extract but no bacteria to serve as control in reading the results. The microtiter plate containing the samples were kept in an incubator at 37 ℃ and left overnight. After incubating for overnight, the plate was taken out of the incubator and an iodonitrotetrazolium chloride dye added. A colour change was observed for the presence or absence of the bacteria upon addition of the dye. (Andrews, 2001) [2].

Results

The tables below display results for phytochemical constituents of the aqueous and 70% ethanol extracts of *Psidium guajava* and *Porkia biglobosa* stems respectively, the antimicrobial activities and MIC test results of the various extracts.

Table 1: Phytoconstituents of aqueous and ethanolic extracts of *p. Guajava* and *p. Biglobosa* stems

Phytochemical	Aqueous (*P. Guajava*)	Etoh (*P. Guajava*)	Aqueous (*P. Biglobosa*)	Etoh (*P. Biglobosa*)
Saponins	+	+	+	+
Reducing sugar	+	+	+	+
Polyuronides	-	-	-	-
Tannins	+	+	+	+
Alkaloids	-	-	-	-
Flavonoids	-	-	-	+
Terpenoids	-	-	-	+
steroids	-	+	-	-
glycosides	-	-	-	-

Key: "+" = positive, "-" = negative

Table 2: Growth inhibitory activity of *p. guajava* and *p. biglobosa* stem extracts on *S. mutans*

Extract Conc. (mg/mL)	Zone of Inhibition/mm (mean±SE)			
	Aqueous Guava	EtOH Guava	Aqueous Dawadawa	EtOH Dawadawa
200	16.00±0.58	18.67±0.33	14.67±0.33	18.67±0.33
100	14.00±0.58	16.00±0.00	13.67±0.33	16.67±0.33
50	12.67±0.67	14.67±0.33	12.33±0.33	15.00±0.00
25	11.67±0.88	12.67±0.33	11.00±0.00	14.33±0.33
12.5	11.00±0.58	12.00±0.00	9.33±0.33	12.67±0.33
-ve control	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
+ve control	15.00±0.00	16.00±0.00	16.00±0.00	16.67±0.33

Key: +ve control: Chlorhexidine, -ve control: distilled water for aqueous and DMSO for ethanol, Number of replicates: 3

Table 3: Growth inhibitory activity of *p. guajava* and *P. biglobosa* stem extracts on *E. coli atcc*

Extract Conc. (mg/mL)	Zone of Inhibition/mm (mean±SE)			
	Aqueous Guava	EtOH Guava	Aqueous Dawadawa	EtOH Dawadawa
200	28.00±1.00	20.33±0.33	21.67±0.67	17.00±0.00
100	26.67±1.20	18.67±0.67	20.67±0.67	15.33±0.33
50	23.67±1.33	16.67±0.67	19.67±0.67	13.33±0.67
25	22.00±1.53	14.67±0.33	14.67±0.67	10.00±0.00
12.5	19.00±1.00	12.33±1.20	10.00±1.00	6.00±0.00
-ve control	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
+ve control	14.33±0.33	11.33±0.33	14.67±0.67	15.67±0.67

Key: +ve control: Chlorhexidine, -ve control: distilled water for aqueous and DMSO for ethanol, Number of replicates: 3

Table 4: Growth inhibitory activity of *p. guajava* and *p. biglobosa* stem extracts against *c. albicans atcc*

Extract Conc. (mg/mL)	Zone of Inhibition/mm (mean±SE)			
	Aqueous Guava	EtOH Guava	Aqueous Dawadawa	EtOH Dawadawa
200	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
100	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
50	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
25	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
12.5	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
-ve control	6.00±0.00	6.00±0.00	6.00±0.00	6.00±0.00
+ve control	15.33±0.33	15.33±0.33	15.33±0.33	15.33±0.33

Key: +ve control: Chlorhexidine, -ve control: distilled water for aqueous and DMSO for ethanol, Number of replicates: 3

Table 5: Minimum inhibitory concentration (MIC) values (mgml-1) of *P. guajava* and *P. biglobosa* stem extracts on *s. mutans* and *E. coli atcc*

Bacteria strain	Aqueous Guava	EtOH Guava	Aqueous Dawadawa	EtOH Dawadawa
S. mutans	6.25	6.25	12.5	3.125
E. coli ATCC	6.25	12.5	12.5	12.5

Discussion

The aqueous extracts for both *P. guajava* and *P. biglobosa* stems showed the presence of saponins, reducing sugar, and tannins. Ethanolic stem extracts of *P. guajava* and *P. biglobosa* showed the presence of saponins, reducing sugar and tannins. In addition, the ethanolic extract of *P. guajava* showed the presence of steroids whereas that of *P. biglobosa* showed the presence of flavonoids and terpenoids.

S. mutans was susceptible to both the aqueous and ethanolic extracts of *P. guajava* and *P. biglobosa* stems. Comparatively, the positive control, chlorhexidine and the aqueous extracts of the two plants at a concentration of 50 mgmL-1 exhibited similar bacterial activity against *S. mutans* (Table 2). MIC values of aqueous extracts of the two plants indicated the stem extract of *P. guajava* had a higher antibacterial activity than that of *P. biglobosa* (Table 5).

On the contrary, the ethanolic extract of *P. biglobosa* stem exhibited a higher activity than the ethanolic extract of *P. guajava* (Table 5). The positive control at 50 mgmL-1 exhibited a higher activity than the two ethanolic extracts (Table 2).

Of all the tested extracts, ethanolic extracts exhibited the highest potencies against *S. mutans* (Table 2). The flavonoids found present in the ethanolic extracts that have been reported in literature to possess antibacterial activity could have been responsible for the higher activity (Chah, 2006) [4].

Similarly, the positive control, chlorhexidine at the concentration of 50 mgmL-1 exhibited a comparable activity to the aqueous extracts of the two plants against *E. coli ATCC* (Table 3). The aqueous extract of *P. guajava* exhibited a higher activity than the aqueous extract of *P. biglobosa* (Table 3), whereas the ethanolic extracts of stems of the two plants exhibited same activity (Table 3). Comparing the aqueous extracts to the ethanolic extracts of stems of both plants, the *E. coli ATCC* was more susceptible to the aqueous extracts than the ethanolic (Table 5).

C. albicans ATCC was found resistant to both the aqueous and the ethanolic stem extracts of the two plants, indicating *P. guajava* and *P. biglobosa* stems have no antifungal activity.

Conclusion

Both *Psidium guajava* and *Porkia biglobosa* stems contain phytoconstituents that inhibited growth of *Streptococcus mutans* and *Escherichia coli*. The stems of the two plants therefore possess bacterial activity against *S. mutans* and *E. coli ATCC*, and no antifungal activity on *C. albicans ATCC*. Thus, data in the present report support the local use of *Psidium guajava* and *Parkia biglobosa* stems to prevent tooth decay.

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