

A Case for the Use of Herbal Extracts in Oral Hygiene: The Efficacy of *Psidium guajava*-Based Mouthwash Formulations

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Abstract: Aqueous leaf extract of a tropical variety of *Psidium guajava* L. (Myrtaceae) was used to formulate five batches of herbal mouthwash. The antimicrobial potentials of these herbal mouthwash formulations in oral hygiene were assessed *in vitro* using a modification of the conventional methods for evaluating oral antiseptics. Formulations containing only *Psidium guajava* leaf extract and a standard mouthwash (Minty Brett)[®] served as controls. The mouthwash formulations were screened for antimicrobial activities against cultures of *Staphylococcus aureus*, *Escherichia coli* and *Candida albican*. The extinction time of each formulation batch was determined against each test organism. The five batches containing *Psidium guajava* aqueous extract showed a high level of activity against the test organisms-*E. coli* and *S. aureus* comparable to the activity shown by the standard mouthwash, Minty Brett[®]. The batch containing only aqueous extract of *Psidium guajava* had the least extinction time of 15 and 20 min against *E. coli* and *S. aureus*, respectively. The study encourages further stability and *In vivo* assessment to develop *P. guajava* leaf extract as an ingredient of commercial mouthwashes.

Key words: Antimicrobial, halitosis, herbal mouthwash, herbal formulations, *Psidium guajava*

INTRODUCTION

Mouthwashes are concentrated aqueous solution of antimicrobial preparations, routinely used in the oral cavity, after dilution to counter infections, for cleansing and antiseptics as well as for refreshing the oral cavity (Nairn, 1990). Besides checking halitosis, other possible health benefits of herbal mouthwash include, relieving symptoms of gingivitis, canker sores, inflamed gums, sore mouth, inflamed or ulcerated throat, mouth infections, bleeding gums and teeth sensitivity (Nahir, 1992).

Most mouthwashes contain antimicrobial substances as antiseptic ingredient against germs that commonly cause mouth infections. Safety and palatability are major considerations in the choice of suitable ingredients for mouthwashes. Overtime, plants have been exploited by man for many centuries as sources of chemotherapeutic and other medicinal drugs, due to the presence of some bioactive compounds (Ogunlana and Ramstad, 1975; Ogunyemi, 1979; Iwu, 1984). The use of plant extracts in

mouthwash formulations is attractive for so many reasons among which are biocompatibility and environmental friendliness of such plant substances.

Psidium guajava L. (Myrtaceae) is a shrubby evergreen plant growing up to 10 m high, with spreading branches. The "Guava" as it is commonly known is easy to recognize because of its smooth, thin, copper-colored bark that flakes off, showing the greenish layer behind and is popular for its edible fruits (NTBG, 2007). The leaves, aromatic when crushed, are evergreen, opposite, short-petiole, oval or oblong-elliptic, somewhat irregular in outline; 7-15 cm long to 3-5 cm wide, leathery, with conspicuous parallel veins and more or less downy on the underside (Morton, 1987). *Psidium guajava* abundantly distributed in South-eastern Nigeria the twigs are commonly used as chewing stick in local communities and as medication for teeth and gum infections.

Phytochemical studies of *Psidium guajava* indicated the presence of bioactive substances like tannins, phenols, triterpenes, flavonoids, essential and fixed oils,

saponins, carotenoids, lectins, vitamins, alkaloids, reducing-sugars, glycosides (Osman *et al.*, 1974; Taylor, 1998, Begum *et al.*, 2004). Infusions of the leaves and bark of the plant are variously utilized as medications for treating many infections, including diarrhoea and as wound-cleansing agents to prevent infection by opportunistic pathogens, thereby facilitating healing. Equally, the leaves and other parts of the plant have been reported to possess antispasmodic, astringent, antiseptic, hypoglycemic, cicatrizing, antimicrobial and antihelminthic activities (Jaiarj *et al.*, 1999; Abdelrahim *et al.*, 2002; Lutterodt, 1989).

However, despite the popular ethnomedicinal use in oral hygiene, there is still dearth of information on the use of *Psidium guajava* in herbal mouthwash formulations. This study was therefore carried-out to assess the antimicrobial potency of aqueous extract of *Psidium guajava* leaves as a major excipient in mouthwash formulations.

MATERIALS AND METHODS

Test organisms: Clinical isolates of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* were used in the assay. They were cultured and maintained on slants of the respective growth media containing 0.05 % chloramphenicol and preserved at 4°C.

Reagents/Chemicals: Culture media used were nutrient-agar, NA (Oxoid, England), Sabouraud dextrose agar, SDA (Oxoid, England). Chemicals used were sodium lauryl Sulphate (BDH, England), peppermint emulsion, chloroform-water (double strength).

Collection of plant material: Leaves of *Psidium guajava* were randomly collected from different mature plants growing at the Medicinal Plant Garden of the Department of Botany, University of Nigeria. The collected leaves were identified by a plant taxonomist, Mr. J. M. C. Ekekwe who works in the garden.

Extraction process: The leaves were sun-dried, pulverized and stored in air-tight bottles. About 400 g of the pulverized leaves were cold macerated by soaking in 200 mL of distilled water for 72 h. The aqueous extract was later filtered and stored in sterile universal bottles, preserved at 4°C for subsequent assays.

Preliminary antimicrobial activity of the leave extract: The preliminary sensitivity of the aqueous leave extract against the test-organisms was assessed by the agar-well diffusion method (Baron and Finegold, 1990). Stock

Table 1: Mouthwash formulations of *Psidium guajava* leave extract

Excipient	Composition of mouthwash formulations in mL					
	A	B	C	D	E	F
<i>Psidium guajava</i> extract	25	25	25	25	25	100
Chloroform water (d/s)	25	12.5	8.3	100	50	-
Peppermint emulsion	2.5	2.5	2.5	2.5	2.5	2.5
Sodium lauryl sulphate (0.2 %)	1	1	-	-	1	-
Distilled water to 100 mL	46.5	59.0	63.2	28.5	21.5	-

solution (100 mg mL⁻¹) of the aqueous extract was two-fold serially diluted to obtain graded concentrations of 50, 25, 12.5, 6.25 and 3.125 mg mL⁻¹. Holes of diameter 4 mm were aseptically bored with a sterile cork-borer on NA and SDA plates previously seeded with 0.1ml standardized inocula of the respective test organisms and then 0.2 mL of the graded concentration of the aqueous extract of *psidium guajava* was introduced into them using separate plates for each organism. The assay plates were held at 4°C for 4 h for rapid diffusion of extract into the assay medium and suppression of immediate microbial growth and subsequently incubated at standard growth conditions for each test microorganism. After incubation, plates were observed for the presence or absence of growth inhibition zones.

Formulation of *Psidium guajava* Mouthwash: The batches of mouthwash tested was prepared by adding varying proportions of sodium lauryl sulphate, peppermint emulsion (2.5 mL), double strength chloroform water (8.3-100 mL) and 25 mL of *Psidium guajava* aqueous extract (Table 1). A commercial mouthwash, Minty Brett® designated as formulation G served as the standard treatment.

Antimicrobial Activity of mouthwash preparation: Each batch of mouthwash formulation was diluted two-folds in a serially arranged triplicate test tubes to obtain six graded concentrations used for the assay. The potency of the graded dilutions was evaluated using the agar-well diffusion method as previously described (Bauer *et al.*, 1966).

Determination of killing rate: The killing rate of the formulations was determined by mixing 5.0 mL of the preparations with 0.1 mL of 10⁶ cfu mL⁻¹ suspensions of the test microorganisms and mixture kept for 1 h. At time intervals of 0, 10, 20, 30, 40, 50 and 60 min, a loopful of the mixture was aseptically withdrawn, subcultured on fresh NA plates and incubated at 37°C for 24 h. After incubation, plates were observed for the presence or absence of growth. The extinction time of the formulations against each test organism was taken as the shortest time

after a loopful of culture withdrawn and sub-cultured showed the absence of any microbial growth.

RESULTS AND DISCUSSION

The result of the preliminary antimicrobial sensitivity (Table 2) showed that the aqueous extract of the leaves was highly active against *Staphylococcus aureus* and *Escherichia coli*, but was not active against *Candida albicans* at the concentrations tested. The susceptibility of the organisms to the extract could be a function of their physiology and metabolism, informed by the intrinsic composition of their cell envelopes, particularly the presence of outer membrane and inactivating enzymes (Davies, 1979; Neu, 1984; Nikaido, 1989; Franklin *et al.*, 1989). Thus, the presence of the outer-membrane in the Gram-negative *Escherichia coli* and the refractory nature of *Candida albicans* cell wall to many antimicrobial agents could be possibly linked to their susceptibility profiles in this study.

The antimicrobial potency of the formulations is in the order F>E>B>A>D>C. The formulation F which contains only the aqueous extract of *P. guajava* showed the highest level of antimicrobial activities and could be attributed to the presence of potent bioactive compounds with antimicrobial activities (Arima and Danno, 2002) (Table 3). Thus, the use of aqueous extract of *P. guajava* as mouthwash and gargles against oral infections could be effective. Other excipients used affected the efficacy of the various herbal mouthwash formulations. The potency of formulations A, B and E appears to be potentiated by the incorporation of sodium lauryl sulphate, an anionic surfactant. The absence of sodium lauryl sulphate in formulations C and D could be responsible for the reduced potency irrespective of the strength of chloroform water used as preservative. Generally, the formulations at all concentrations tested were more active against *Staphylococcus aureus* than the *Escherichia coli*.

Antimicrobial properties of mouthwashes is desired as it helps to reduce bacterial flora of the mouth, especially the anaerobic flora which produce the volatile sulfur compounds which are responsible for bad breath. The extinction time studies (Table 4), showed that formulation F recorded the shortest killing time for the test organisms, followed by other formulations in the sequence F>E>B>A>D>C. The biocidal activity of the formulations could be related to the nature and concentration of excipients as well as to dilution effect. Hence, formulations F, containing mainly the aqueous guava extract being the most lethal; followed by formulation E which has high concentration of preservative and surfactant. Formulations A and B which have lower preservative strength is not as lethal as the formulation E. Formulations C and D which do not contain the surfactant but contain similar levels of the preservative is not as lethal as the formulations containing the sodium lauryl sulphate. Surfactants have been shown to increase the antimicrobial activity of mouthwash formulations (Filip *et al.*, 1973; Jenkins *et al.*, 1991).

Guava aqueous extract is also attractive for use as a mouthwash because of some its active ingredients which has been demonstrated to possess anti-oxidant properties which are attributed to the polyphenols found in the leaves (Qian and Nihorimbere, 2004; NBTG, 2007). These compounds are capable of neutralizing Volatile Sulfur Compounds (VSC's) and/or the compounds from which they are formed. Since volatile sulfur compounds are the

Table 2: Preliminary antimicrobial sensitivity of aqueous extract of *Psidium guajava* leaves

Test organisms	concentration of aqueous extract (mg mL ⁻¹)				
	100	50	25	6.25	3.125
<i>Escherichia coli</i>	-	-	-	+	+
<i>Staphylococcus aureus</i>	-	-	-	-	+
<i>Candida albicans</i>	+	+	+	+	+

(+) = presence of growth, (-) absence of growth

Table 3: Antimicrobial activity of mouthwash formulations

Mouthwash formulations	Test Microorganism	-----Inhibition zone diameter (mm) (IZD) ± SEM-----					
		Dilutions of mouth wash					
		1:1	1:2	1:4	1:8	1:16	1:32
A	SA	12±0.58	9±0.50	7±0.00	+	+	+
	EC	14±0.33	11±0.50	9±0.50	7±0.50	+	+
B	SA	13±0.50	10±0.58	8±0.33	+	+	+
	EC	13±0.58	11±0.33	9±0.58	8±0.58	+	+
C	SA	9±0.00	+	+	+	+	+
	EC	7±0.33	+	+	+	+	+
D	SA	9±0.50	8±0.58	+	+	+	+
	EC	9±0.50	9±0.33	+	+	+	+
E	SA	16±0.58	14±0.50	11±0.50	9±0.33	+	+
	EC	13±0.33	11±0.33	10±0.33	8±0.00	+	+
F	SA	16±0.33	13±0.50	11±0.33	10±0.50	+	+
	EC	14±0.00	12±0.33	10±0.33	7±0.33	+	+

+ = Presence of growth, SA = *staphylococcus aureus*, EC = *Escherichia coli*, n = 3

Table 4: Extinction time of *P. guajava* mouthwash formulations

Mouthwash formulations	Killing time (mins)	
	SA	EC
A	30	40
B	40	>60
C	55	>60
D	>60	>60
E	20	30
F	15	20
G	10	15

SA = *Staphylococcus aureus*; EC = *Escherichia coli*, > = lethal above 60 min. The Executive Managing Editor, Research Journal of Applied Science Medwell Journals, Submission of manuscript

malodorous substances that actually cause halitosis. Anti-oxidant constituents of guava extract-based mouth wash could decrease the concentration of these compounds in a person's breathe thereby reducing mouth odour.

From the results of this study, formulations F, E and A are effective and could be explored further for possible commercialization and use as mouthwashes. Thus, there is a need for further stability, safety and *In vivo* efficacy studies to validate and corroborate the result of this studies.

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Psidium guajava L. is a small medicinal tree that is native to South America. It is popularly known as guava (family Myrtaceae) and has been used traditionally as a medicinal plant throughout the world for a number of ailments. There are two most common varieties of guava: the red (*P. guajava* var. *pomifera*) and the white (*P. guajava* var. *pyrifera*) [9,10]. Singh and Marar [74] studied the effects of *Psidium guajava* leaves on the inhibition of the activity intestinal glycosidases related with postprandial hyperglycemia, suggesting its use for the treatment of individuals with type 2 diabetes. Other studies have demonstrated that guava leaf and peel extracts also had hypoglycemic effects on experimental models drug-induced to severe conditions of diabetes [17,75,76]. Wu et al.