EFFECT OF GUAVA (*Psidium guajava*), BITTER-LEAF (Vernonia *amygdalina*) CHEWING STICKS AND HERBAL TOOTHPASTES ON *Streptococcus mutans*

**Oviasogie**¹, F. I., **Ogofure**²*, A. G., **Beshiru**¹, A., **Ologbosere**¹, O. A., **Omeje**², F. I. and **Raphael**¹, P.

**ABSTRACT**

¹Department of Microbiology, Faculty of Life sciences, University of Benin, Benin City, Nigeria. ²Department of Biological Sciences, Faculty of Sciences, University of Owoke, Bayelsa State, Nigerian. *Corresponding author: Tel: 08100490847 Email: abraham.ogofure@uniben.edu*

The comparative study on the antimicrobial activity of aqueous and ethanolic extracts of guava and bitter-leaf chewing sticks and herbal toothpastes (Darbur and Macleans) against *Streptococcus mutans*, (an oral flora of the mouth responsible for dental caries and plaque) was evaluated in this study. The preliminary phytochemical analysis of ethanolic extract of guava and bitter-leaf chewing sticks revealed the presence of alkaloids, steroids and carbohydrates respectively. Aqueous extract revealed the presence of saponin and carbohydrates for Bitter leaf chewing sticks and flavonoids, steroids, alkaloids and carbohydrates for guava chewing sticks. There was no antibacterial activity observed against *Streptococcus mutans* by both extractants of bitter leaf chewing sticks. The aqueous extract of guava had a mean zone of inhibition of 15.6±1.2 mm at 40 mg ml⁻¹ compared to ethanol extract 12.3±0.7 mm at same concentration. There was however a significant difference (p<0.05) in the activity of both the aqueous and ethanolic extract of guava with the aqueous extract being more potent. Macleans exhibited a better activity against the bacterium than Darbur herbal toothpaste with mean zone of inhibition being 27.3±0.9 mm to 25.7±1.5 mm. There was a significant difference between herbal toothpaste and natural chewing sticks (p<0.05) with herbal toothpaste being much better than the chewing sticks used in this study. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration values observed for ethanolic extract of Guava was 10mg/ml and that of herbal toothpaste examined, was 0.313 mg ml⁻¹ respectively.

Keywords: *Streptococcus mutans*, chewing sticks, extractants, herbal toothpastes.

**INTRODUCTION**

The microorganisms present in the mouth consist of a diverse and populous collection of bacteria, fungi, and transient viruses. Bacteria make up the largest number of varieties (Akinyele et al., 2014). More than 350 cultivable bacterial species have been identified in the oral cavity and molecular analyses suggest that an equal number of non-cultivable flora are also present (Marcotte and Lavoie, 1998). Bacterial accumulation on oral surfaces is a major factor in the development of most of the common dental diseases such as dental caries, plaque and periodontal disease (Vohra et al., 2012). The largest numbers of microorganisms are found on the tooth surfaces (Bankole et al., 2012). Dental plaque is a complex biofilm found on the tooth surface which forms the major reason of the development of dental caries. The accumulation and development of plaque depends upon the outcome of the interactions between the adhesiveness of plaque to the tooth surface and the physical shear forces which serve to dislodge and remove the plaque (Vohra et al., 2012). Tooth brushing with toothpaste is the most widely practiced form of oral hygiene in most countries (Pannuti et al., 2003) and the success of any toothpaste, in part, lies on its ability to eliminate pathogenic oral microflora. A wide range of chemicals, mainly antimicrobial agents, have been added to toothpastes in order to produce a direct inhibitory effect on plaque formation (Fine et al., 2006). Fluoridated toothpastes have been proven to protect teeth against attack from bacteria (Vohra et al., 2012).

Nigerians basically employ two methods to remove debris (plaque) from the mouth which are either by use of tooth brush and paste or by use of parts of various plants native to West Africa, referred to as “African chewing sticks” (Bankole et al., 2012). Meanwhile, in absence of toothpaste, a large proportion of persons living in rural areas and some who are also in the urban areas make use of the natural toothbrush known as “chewing sticks. Ojo et al. (2007) defined chewing sticks as parts of higher plants which are cut into suitable lengths and used for the maintenance of oral hygiene. They are important Non Timber Forest Product (NTFP) widely used for dental cleaning in the tropical West Africa (Akande and Hayashi, 1998). Plants from which chewing sticks are derived are abundant and diverse in Nigeria rural communities. Almost the entire rural population in Nigeria use chewing sticks for orodental hygiene (Bankole et al., 2012). This is due to the fact that the Nigerian chewing sticks, are readily available, cheap and efficacious (Kareem et al., 2012). Chewing sticks are recommended for oral hygiene by the World Health Organization, and some of them, or their extracts, are also used in ethno-medical treatment of oral infections (Ndulko, et al., 2005). Several studies have demonstrated the antiplaque and antibacterial...
activities of extracts of these Nigerian Chewing Sticks (NCS) against oral bacteria, such as *Streptococcus mutans* and *Streptococcus mitis* which are organisms commonly implicated in dental caries and orodental infections. The plants used as chewing sticks are carefully selected for certain properties such as foaminess, hardness, or bitterness and certain species are more popular than the others, in which a great number of these plant species have related medicinal properties that may be antibacterial and are as important today as they were thousands of years ago (Hullins, 2003). In addition, Hooda *et al.* (2010) reported that extracts of chewing sticks can be incorporated into tooth pastes or used as mouth washes. The choice of chewing sticks to be used in most cases depends on its cleansing action of the teeth; the therapeutic value, or preferred taste or flavour. The sticks (which may be stem or root with bark removed or retained) are cut to convenient lengths and washed thoroughly with fresh water to get rid of any dirt (Ogundiya *et al.*, 2006). The usefulness of chewing sticks in oral hygiene maintenance has been considered comparatively effective as tooth brush (Van-Palentstein *et al.*, 2002). In certain parts of Nigeria where chewing sticks are used, dental caries are not usually very rampant. Elujioja *et al.* (2005) opined that Bitter leaf (*Vernonia amygdalina*) has its origin in Nigeria and for the fact that it taste bitter, it is popularly called bitter leaf. It used in traditional medicine as tonic and remedy against different and diverse illnesses and various infectious diseases. It has found relevance in traditional folk’s medicine as anthelmint and serves one of the major commonly expressed plants both locally and in most urban areas to save human from the ordeals of bacterial activities (Afolabi *et al.*, 2014).

Imaga and Bamigbetan, (2013) and Mensah *et al.* (2009) evaluated the phytochemical constituents of bitter leaf extract and found that it contains tannins, saponins, flavonoids and alkaloids. Nwinyi *et al.* (2008) described guava as a tropical tree which grows up to 35 feet tall, widely cultivated for its fruit, a member of the Myrtaceae family, with about 133 genera and more than 3,800 species. The leaves and bark of *Psidium guajava* has been found to possess certain bioactive compounds which have made it useful in herbal and traditional medicine as well as orthodox medicine. Several studies have reported guava to contain antioxidant, used for the treatment of cough, possess antimicrobial activity (Abdelrahim *et al.*, 2002; Arima and Dano, 2002), anti-diarrhoeal activity, (Ojewole *et al.*, 2008), for the treatment of plaque (Prabu *et al.*, 2006), spermatoprotective activity (Akinola *et al.*, 2007), anti-cancer activity (Chen *et al.*, 2010), antifungal activity (Sato *et al.*, 2000) and also have found use in oral care (Okwu and Ekeke, 2003). Guava has also been found to contain broad spectrum of phytochemicals including polysaccharides, vitamins, essential oils, minerals, enzymes, proteins (Deo and Shastri, 2003), sesquiterpenoid alcohols and triterpenoid acids (Begum *et al.*, 2002), alkaloids, glycosides, steroids, flavonoids, tannins, saponins. The aim of this work is to compare the efficacy of guava and bitter leaf chewing sticks and herbal toothpastes against *Streptococcus mutans*.

**MATERIALS AND METHODS**

**Materials**

All materials such as glass wares were manufactured by pyrex in England. The chemical used for extraction (absolute ethanol manufactured in England) was obtained from the Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City. The experiment (phytochemical analysis, antimicrobial activity and characterization of organism) was carried out at Pharmacognosy and Microbiology Laboratories in the same citadel of learning.

**Sample collection**

Ready-to-use guava and bitter leaf chewing sticks (miswaks) were also plucked from trees in Ugbowo area and herbal toothpastes, were obtained from Uselu market, Benin City, Nigeria. The chewing sticks were taken to the Department of Plant Biology and Biotechnology taxonomy laboratory for authentication and identification. Reference *Streptococcus mutans* isolate was obtained from University of Benin Teaching Hospital and characterized as delineated by Chessbrough (2000).

The typed isolate were stored on Nutrient agar (NA) slants in the refrigerator at 4 °C prior to use.

**Extraction process**

Ethanolic and aqueous extracts were prepared using the methods of Ogundiya *et al.*, (2006) but with few modifications. The stems/sticks of the test plants were well dried and pulverized. About 200 g of the powder were separately soaked in 400ml of 95% ethanol in a 500 ml reagent bottle and stoppered. This was allowed to stand for 7 days to permit full extraction of the active ingredients. The fluids were then filtered using Whatman No. 1 filter paper. The extracts were concentrated using rotary evaporator at 65 °C. It was then kept in the refrigerator (4 °C) prior to use. A 2.0 g l⁻¹ solution of each extract was prepared and fractionated into 0.4 g l⁻¹, 0.2 g l⁻¹ and 0.1 g l⁻¹ concentrations needed for the bioassay.

**Test for alkaloids**

5 g of evaporated extract was boiled with 5 ml of 2% HCl on a steam bath for 5 minutes, the mixture was filtered after cooling, and the filtrate was shared into 3 test tubes A B and C. 1ml portion of filtrate was treated with 2
drops of Mayer’s reagent. To confirm this result, 1 ml portion of the filtrate was treated with Dragendorff’s reagent.

**Test for flavonoids**

5 g of extract was introduced into a test tube containing 10 ml ethyl acetate solution and was heated in boiling water for a minute. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml of 1% aluminum chloride solution and left to stand for 10 minutes. The formation of a yellow coloration in the presence of 1 ml of dilute ammonia solution, indicate the presence of flavonoids.

**Test for saponins**

1 g of extract was boiled with 5 ml of distilled water for 5 minutes; the mixture was filtered while hot. To 1 ml of filtrate, two (2) drops of olive oil was added, the mixture was shaken and observed for the formation of emulsion. 1 ml of the filtrate was diluted with 4 ml of distilled water. The mixture was shaken and then observed for the formation of stable frothing on standing.

**Test for tannins**

To 2 g of the sample, 5 ml of 45% ethanol was added and boiled for 5 minutes. The mixture was cooled and filtered. To 1 ml of the filtrate, three (3) drops of lead acetate solution was added. The formation of gelatinous precipitate indicates the presence of tannins. Also as a confirmation test, 1 ml of filtrate was treated with 0.5 ml of bromine water and the formation of a pale brown precipitate indicates the presence of tannins.

**Test for carbohydrate**

2 ml of the plant extract was added to 2 ml of Fehling’s solution A and B for 3 minutes. Observation of a deep blue to green coloration is indicative of a positive result.

**Test for steroids and sterols**

5 g of extract was dissolved in 2 ml of chloroform and equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound, in the extract.

**Antimicrobial sensitivity bioassay**

The antimicrobial assay was performed by using the agar well diffusion method (Habamu et al., 2010; Perez et al., 1990). Wells of 10 mm in diameter were made into previously seeded Nutrient agar plates. Each well was filled with (1.0 ml) of the extract. The same quantity of sterile distilled water and 50% ethanol both without plant extract served as controls. The plates were pre-incubated for 2 hours. to allow diffusion of extract before incubating overnight at 37°C. The diameter of clear zone was measured in mm using a well calibrated meter rule. Triplicate plates were prepared for each extract and controls.

All agar incorporation was carried out aseptically.

**Determination of minimum inhibitory concentration**

The MIC of the extracts were determined using the method described by Vinothkumar et al. (2010) by diluting the extracts double fold (beginning with 40 mg/ml) with nutrient broth in a series of test tubes and to each of the tubes, equal volume of the test organism was added and incubated at 37°C for 24 hours. Controls were prepared by inoculating tubes without the extracts but with the cell suspensions. The tubes were then examined for the presence of turbidity after the incubation period. The least concentration with no observable bacterial and fungal growth when compared with the control was considered as the minimum inhibitory concentration (MIC).

**Statistical analysis**

The Statistical Package for Social Scientists (SPSS, version 16.0) was used for the analysis of the data obtained. Two way ANOVA test was used to determine the level of significance of the test organisms at 5% level of significance.

**RESULTS**

The preliminary qualitative phytochemicals screening of guava and bitter leaf chewing sticks is shown in table 1. Alkaloids, flavonoids, carbohydrates and steroids were present in both ethanol and aqueous extracts of guava chewing sticks while tannins and saponins were absent. Saponins and carbohydrates were the only phytochemicals present in the aqueous extracts of bitter leaf chewing sticks. The sensitivity (mean zones of inhibition) of guava, and bitter leaf chewing sticks against *mutans* species of *Streptococcus* are shown in table 3 and 4 respectively. Both extracts of bitter leaf chewing sticks, showed no zone of inhibition against the tested isolate hence no activity. Table 5 shows the sensitivity (zones of inhibition) of Darbur herbal and Macleans herbal toothpaste against *Streptococcus mutans*. Macleans exhibited a better activity against the bacterium than Darbur herbal toothpaste with mean zone of inhibition being 27.3±0.9mm to 25.7±1.5mm. The Minimum Inhibitory Concentration (MIC) as well as Minimum Bactericidal Concentration (MBC) of the chewing sticks and herbal toothpaste are shown in tables 6, 7 and table 8 respectively. The highest MIC value was that of ethanol extracts of guava chewing stick (10 mg ml⁻¹) while that of herbal toothpaste was 0.313 mg ml⁻¹.
Table 1: Qualitative phytochemical screening of guava and bitter-leaf chewing sticks

<table>
<thead>
<tr>
<th>Phytochemical test</th>
<th>Guava</th>
<th>Bitter-leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol extract</td>
<td>Aqueous extract</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*+= present, -*= absent

Table 2: Sensitivity of Streptococcus mutans to aqueous and ethanol extract of guava and bitter-leaf chewing sticks and herbal toothpastes

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Concentration (mg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Psidium guajava</td>
<td>Aqueous</td>
<td>6.0±0.6</td>
</tr>
<tr>
<td>Psidium guajava</td>
<td>Ethanol</td>
<td>2.3±0.7</td>
</tr>
<tr>
<td>Talinum triangulare</td>
<td>Aqueous</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Talinum triangulare</td>
<td>Ethanol</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>Herbal toothpaste</td>
<td>Darbur</td>
<td>3.7±0.7</td>
</tr>
<tr>
<td>Herbal toothpaste</td>
<td>Macleans</td>
<td>14.0±1.2</td>
</tr>
</tbody>
</table>

Means zones of Inhibition ± Standard Error of Mean (SEM)

Table 3: Minimum inhibitory concentration and minimum bactericidal concentration (mg ml⁻¹) of guava and bitter-leaf chewing sticks, and herbal toothpastes against Streptococcus mutans

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>MIC value (mg ml⁻¹)</th>
<th>MBC (mg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psidium guajava</td>
<td>Aqueous</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Psidium guajava</td>
<td>Ethanol</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Talinum triangulare</td>
<td>Aqueous</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Talinum triangulare</td>
<td>Ethanol</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Herbal toothpaste</td>
<td>Darbur</td>
<td>0.313</td>
<td>0.313</td>
</tr>
<tr>
<td>Herbal toothpaste</td>
<td>Macleans</td>
<td>0.313</td>
<td>0.313</td>
</tr>
</tbody>
</table>

DISCUSSION

The antimicrobial activity of guava and bitter leaf chewing sticks and herbal toothpaste against Streptococcus mutans, was comparatively evaluated in this study. The preliminary phytochemical analysis of guava revealed similar bioactive components when both solvents (ethanol and water) are used for extraction. Psidium guajava, demonstrated high potential antiplaque activity by inhibiting the growth of Streptococcus mutans (Prabu et al., 2006). Ethanol was able to extract 66.7% of the phytochemicals tested for bitter leaf while aqueous extract could only muster 33.3% of the bioactive components. The resistance of Streptococcus mutans to aqueous and ethanolic extract of bitter leaf chewing sticks could be attributed to the absence of certain phytochemical components such as flavonoids which Praba et al. (2006) reported to be very active against the tested isolate. However, alkaloid, steroids, saponins and carbohydrates were present in ethanolic extract while saponins and carbohydrates are the only visible phytochemicals in aqueous extract of bitter-leaf chewing sticks. This result is in consonance with the report of Alo et al. (2012) who opined that ethanol is a better extraction solvent than water. The absence of zones of inhibition for bitter leaf chewing sticks indicates resistance of S. mutans to both aqueous and ethanolic extracts. It was also observed that there was a significant difference (p<0.05) between using herbal toothpastes and both extractants of Psidium guajava and Vernonia amygdalina. It was also observed that the aqueous extract of guava was more potent than the ethanolic extract. The herbal toothpastes used in this study showed significant antibacterial activity against the tested isolate than guava and bitter-leaf chewing sticks. Macleans herbal toothpaste showed a significant antibacterial activity against the tested bacteria than its Darbur herbal counterpart.

CONCLUSION

This study has been able to establish that the use herbal toothpaste is better than the use of guava and bitter-leaf chewing sticks chewing sticks against Streptococcus mutans (a plaque causing bacteria). The antibacterial activity of guava and bitter leaf chewing sticks has also been established in this study. Guava chewing sticks has been found to be much better than bitter leaf chewing sticks in situations where a choice has to be made between both.
However, herbal toothpastes have demonstrated better antibacterial activity than both guava and bitter leaf chewing sticks and as such is a better alternative to the use of certain plant chewing sticks. Nevertheless, hundreds of chewing sticks exist whose antibacterial properties are much better than herbal toothpastes and as such, these chewing sticks should be characterized and properly charted for man’s use.

REFERENCES


