Phytochemical screening and antimicrobial activities of ethanolic and aqueous root extracts of *Zanthoxyllum zanthoxylloides* (Lam.) Waterm. on selected dental caries causing microbes

E.O. OSHOMOH, M. IDU*

Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria

*Corresponding author, Tel: +2348050607009

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Abstract: Secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids and volatile oil are abundant in plants and are responsible for their therapeutic activities. Chewing sticks obtained from a variety of selected plants are used as a traditional method of mechanical oral hygiene by up to 80% – 90% of Nigerians. The root of *Zanthoxyllum zanthoxylloides* used in this study revealed that chewing sticks are capable of inhibiting gram-positive, gram-negative bacteria and fungi such as *Staphylococcus aureus*, *Staphylococcus auricularis*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Candida albicans*, *Aspergillus flavus*, *Microsporium gypseum* and *Bacillus subtilis*. The highest zone of inhibition was recorded against *M. gypseum* in 100mg/ml ethanol extract with a sensitivity diameter of 16.10±0.58 mm, while the least sensitive was recorded against *S. auricularis* with a sensitivity diameter of 5.23±0.12 mm. The results from these studies provide evidence for the ethnomedicinal use of the tested plant as chewing stick. The presence of bioactive substances (secondary metabolites) in the plant could be attributed to its medicinal properties.

Keywords: Ethnomedicinal; gingivitis; periodontal disease; zone of inhibition; metabolites.

Introduction

Some plants have been discovered to be rich in secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids and volatile oil. These compounds are responsible for their therapeutic activities (Cowan 1999; Rabe and Vastoden 2000). Also, some plant parts have been used as antimicrobial agents, especially their extracts either as decoctions, infusions, or oral administration (Okemo et al. 2001). Importantly, plants have been known to exhibit medicinal properties on internal organs of animals. If the toxic effect after administration is low, there is a possible chance of introduction of such drugs for therapeutic purpose (Ibeh 1998).

Teeth are usually involved in digestion as they cut or crush large pieces of food into smaller pieces and mix them with saliva a digestive juice which contains the enzyme *amylase*.

There are a number of common mouth diseases that can affect humans at various points in their lives such as hand-foot-mouth diseases, thrush and gingivitis. Hand-foot-mouth diseases are usually seen in babies and children (Grundermann 2000). Chewing sticks are sold all over Nigeria as an aid to mouth hygiene at a minimal cost (Orhiere 1993).

Gingivitis (Gum disease) is due to the long-term effects of plaque deposits. Plaque is a sticky material made of bacteria, mucus and food debris that develops on the exposed parts of the teeth. It is a major cause of tooth decay. If not removed, it turns into a hard deposit called tartar that becomes trapped at the base of the tooth. Plaque and tartar irritate and inflame the gums. Bacteria and the toxins they produce cause the gums to become infected, swollen and tender. Injury to the gums from any cause, including overly vigorous brushing and flossing of the teeth can cause gingivitis. The following al-
so raise the risk for developing gingivitis: General illness, poor dental hygiene, pregnancy (hormonal changes increase the sensitivity of the gums) and uncontrolled diabetes.

In Nigeria, as in other developing countries, a very significant proportion of orofacial diseases are due to microbial infection (Ndukwe et al. 2002). This has led to a wide spread use of antibiotics in dental practice in these regions and this gives microorganisms enhanced opportunities for the development of resistance to a broad spectrum of antibiotics (Ndukwe et al. 2002). The need to conserve antibiotics in order to prevent the selection of antibiotic-resistant organisms has been recognized (Levy 1997) and there is therefore the need to look for non-antibiotic substances with proven antimicrobial activity which can be used in the treatment of microbial infections, including those encountered in dental practice.

Chewing sticks are widely used in Africa and Asia as a means of maintaining oral hygiene (Otuyemi et al. 1996). They are obtained from the roots, twigs, or stems of various plants. The preferred part or parts are cleaned with water to remove dirt, cut to a convenient length, which varies from 15 – 30 cm. The user holds one end directly in his/her mouth and chews it into a fibrous brush-like fringe, which is used to scrub the surfaces of the teeth. A combination of vertical and horizontal strokes of the ‘brush’ on tooth surfaces removes plaque.

Chewing sticks are used in the morning before breakfast and at night after supper for daily oral hygiene maintenance. About five minutes of complete devotion to this exercise is deemed adequate to achieve good cleansing. According to (Sole and Wilson 1995), chewing sticks obtained from a variety of selected plants are used as a traditional method of mechanical oral hygiene by up to 80% – 90% of Nigerians.

Periodontal diseases and dental caries are two main common dental pathologies affecting human kind (Marsh and Martin 1992). These conditions are caused by plaque forming bacteria and yeast which reside in the oral cavity. Periodontal diseases have mainly been associated with Actinomyces, Actinobacillus, Streptococcus and Candida species (VanOosten et al. (1987). Ndukwe et al. (2004) confirmed that chewing sticks have potential of preventing oral ailments. A majority of plants tested in his study revealed that chewing sticks are capable of inhibiting gram-positive and negative bacteria such as Bacillus subtilis, Porphyromonas gingivalis and Fusobacterium nucleatum.

Plants that contain substances which can be used for therapeutic purposes or which can be used as precursors for the synthesis of useful drugs is a medicinal plant (WHO 1977; Sofowora 1982). In spite of the millions of chemical structures currently available for screening for therapeutic value, natural products particularly of plant origin remain a most important source of new drugs (Odugbemi and Akinsulire 2006).

The root of Zanthoxylum zanthoxyloides is used as chewing sticks and mouth wash with a root infusion for toothache. Zanthoxyllum is of the family Rutaceae has membranous leaflets numbering about 5 – 11 and grows in opposite pairs. It possesses axillary flowers with clusters of fruits. The buds are hairy. The leaves are bitter-aromatic green with crenate margins, Duncan and Marion (1988). The berries are initially red which turned deep blue to black on maturity. The flowers have yellow-green petals and dioecious. Z. zanthoxyloides occurs more abundantly in the savannah and dry forest vegetations and is found in the drier parts of South Western Nigeria extending to Northern States.

This study is aimed at testing the phytochemistry, antimicrobial activities, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) levels of the aqueous and ethanol extracts of Z. zanthoxyloides collected from Edo north in Edo State, Nigeria.

Material and methods
Collection and identification of plant material
The roots of Zanthoxylum zanthoxyloides plant were collected from Edo North Senatorial District of Edo State. The plant was identified by Dr J.F. Bamidele of the Department of Plant
Preparation and extraction of plant material

Fresh roots of *Z. zanthoxyloides* were cut from the plants, rinsed in water and spread on trays and dried under the sun. Plant materials were then transferred to the oven set at 45°C for 20-30 minutes before being reduced to fine powder with the aid of a mechanical grinder. The powdered plant materials were then collected and stored in a tightly covered glass jars and kept for further studies.

For ethanol extraction, 100 g of the powdered root materials were soaked in 600 ml of ethanol. The resultant solution was filtered using Whatman filter paper No 1 after 48 hours under room temperature (25°C). For aqueous extraction, 100 g of the powdered root materials were boiled in 600 mls of water for 24 hours after which the resultant solutions were filtered using Whatman filter paper No 1. The two extracts were concentrated through evaporation process using a water bath set at 100°C. The extracts were then stored in a refrigerator until required for use.

Preparation of stock solution of extracts

Fresh stock (known concentration) solution of each extract was prepared for each experiment. To prepare a required concentration of the extract, a specific weighed amount of the concentrated extract was dissolved completely in an appropriate volume of distilled water. To prepare 100 mg/ml concentration of extract, 1 gm of either of the extract was dissolved in 10 ml of distilled water in a sample bottle, corked and shaken vigorously to obtain a homogenous solution.

Phytochemical screening

The phytochemical tests were carried out on the aqueous and ethanol extracts using standard procedures as described by (Trease and Evans 1996; Edeoga et al. 2005).

Antimicrobial investigation

Source of microorganisms

Pure stock cultures of *Staphylococcus aureus*, *Staphylococcus auricularis*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Candida albicans*, *Aspergillus flavus*, *Microsporum gypseum* and *Bacillus subtilis* isolated from patients with dental diseases were obtained from the Department of Medical Microbiology, Department of Dentistry University of Benin, and University of Benin Teaching Hospital (UBTH). These pure isolates were used and maintained in slants of Nutrient Agar (NA), Blood Agar (BA) and Potato Dextrose Agar (PDA) at 4°C until when needed for further studies.

Microbial inoculums preparation for susceptibility testing

The inocula of the bacterial isolates were prepared by growing each pure isolate in nutrient broth at 37°C for 24 hrs. The fungal isolates were grown in Potato dextrose broth at 28±2°C for 48 hrs. After incubation, 1 ml of the diluted cultures of the microbial isolates in normal saline using a Pasteur pipette was inoculated unto the solidified nutrient agar and blood agar at 40°C for bacteria and Potato dextrose agar for fungi.

Antimicrobial Assay

Antimicrobial activity was evaluated by noting the zone of inhibition against the test organisms (Eloff 1998). Two colonies of a 24-hour plate culture of each organism were transferred aseptically into 10 ml sterile normal saline in a test tube and mixed thoroughly for uniform distribution. A sterile cotton swab was then used to spread the resulting suspension uniformly on the surface of oven-dried Nutrient Agar, Blood Agar and Potato Dextrose Agar plates for bacteria and fungi, respectively. Three (3) adequately spaced wells of diameter 4 mm per plate were made on the culture agar surface respectively using a sterile metal cup-borer. 0.2 ml of each extract and control were put in each hole under aseptic condition, kept at room temperature for 1 hour to allow the agents to diffuse into the agar medium and incubated accordingly. Conventional antibiotics were used as positive controls for bacteria and fungi respectively; distilled water was used as the negative control. The plates were then incubated at 37°C for 24 hours.
for the bacterial strains and at 28°C for 72 hours for fungal isolates. The zones of inhibition were measured and recorded after incubation. Zones of inhibition around the wells indicated antimicrobial activity of the extracts against the test organisms. The diameters of these zones were measured diagonally in millimeter with a ruler and the mean value for each organism from the triplicate cultured plates was recorded. Using the agar-well diffusion technique, an already made gram positive and gram negative (Asodisks Atlas Diagnostics, Enugu, Nigeria) standard antibiotic sensitivity disc bought from a laboratory chemical equipment store in Benin city was used as positive control for bacteria while Ketoconazole was used as positive control for fungi. Distilled water was used as negative control for all the test organisms.

**Determination of minimum inhibitory concentrations (MICs) of the extracts**

The lowest concentration of the extracts that will inhibit the growth of test organisms is the Minimum Inhibitory Concentration (MIC). The initial concentration of the plant extract (100 mg/ml) was diluted using double fold serial dilution by transferring 5 ml of the sterile plant extract (stock solution) into 5 ml of sterile Normal saline to obtain 50 mg/ml concentration (Oboh et al. 2007). Different concentrations were prepared from the crude extract by doubling dilution in distilled water. The different concentrations were 50, 25, 12.5, 6.25, 3.125, 0.625, and 0.3125 mg/ml respectively. Each dilution was introduced into nutrient agar plates and potato dextrose agar plates already seeded with the respective test organism. All test plates were incubated at 37°C for 24 hrs for bacteria and 28°C ± 2°C for 72 hrs for fungi. The minimum inhibitory concentration (MIC) of the extracts for each test organism was regarded as the agar plate with the lowest concentrations without growth (Eloff 1998).

**Determination of the antibiotic susceptibility of bacterial isolates**

The disc diffusion method (Anonymous 2003) was used for the determination of microbial sensitivity. The antibiotic discs employed were: septrin, chloramphenicol, sparfloxacin, ciprofloxacin, amoxicillin, augmenting, gentamicin, pefoxacin, ofloxacin, streptomycin, zinacef and recophin. The zones of inhibition were measured and interpretation was in accordance with manufacturer’s instructions.

**Results**

The results of the phytochemical analysis of aqueous and ethanolic root extracts of *Z. zanthoxyloides* revealed the presence of some secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, anthraquinones, phlobatannins and tannins (Table 1). Saponins and cardiac glycosides were absent in the aqueous and ethanol extract of *Z. zanthoxyloides* root while carbohydrates were present in the aqueous and ethanol extract of *Z. zanthoxyloides* root.

**Table 1**: Phytochemical screening of the aqueous and ethanolic extracts of *Z. zanthoxyloides* root used as chewing sticks.

<table>
<thead>
<tr>
<th>Chemical components</th>
<th>Extract</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Anthraquinones</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Cardiac Glycosides</th>
<th>Steroids</th>
<th>Terpenoids</th>
<th>Phlobatannins</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aq</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Et</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key: + = Present, - = Absent, Aq = Aqueous, Et = Ethanol

Oshomoh and Idu

http://www.openaccessscience.com

ijmap@openaccessscience.com
Table 2 shows the antimicrobial properties of the ethanol and aqueous extract of *Z. zanthoxyloides* on the test microorganisms. All the test organisms were sensitive to the ethanol extract at a concentration of 100 mg/ml. The activities of the ethanol extracts on all the tested organisms were significantly different from one another. The highest zone of inhibition was recorded against *M. gypseum* with a sensitivity diameter of 16.10±0.58 mm, while the least sensitive was recorded against *S. auricularis* with a sensitivity diameter of 5.23±0.12 mm.

Plant extracts were more susceptible to *M. gypseum* (fungus) followed by *A. flavus* (fungus), *S. aureus* (gram +ve), *B. subtilis* (gram +ve rod), *C. albicans* (fungus), *S. mutans* (gram +ve), *S. pyogenes* (gram +ve) and *S. auricularis* (gram +ve) respectively.

Table 2 also shows the comparison of the effect of the aqueous and ethanol extracts of *Z. zanthoxyloides* on the test organisms. It was revealed that the ethanol extract has the highest antibacterial and antifungal activity against all the tested oral microorganisms with inhibition diameters of 8.40±0.12 mm and 16.10±0.58 mm respectively at 100 mg/ml.

Table 3 revealed that the activity of the ethanol (Et) extract of *Z. zanthoxyloides* root was significantly different from one concentration to another on each organism. *A. flavus* was not susceptible to the plant extract at any concentration; *S. mutans* was susceptible to all the concentrations of the extracts with a range of inhibition of between 3.20 and 15.07 mm. *S. mutans* revealed the highest susceptibility when compared with other test organisms at all the concentrations used.

Table 4 presents the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) values of the ethanol extracts. The ethanol extract of *Z. zanthoxyloides* showed minimum inhibitory concentration (MIC) at 6.25 mg/ml against *S. aureus*, *S. auricularis* and *S. mutans* and while MBC values for the three organisms was 12.5 mg/ml.

Table 5 presents the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MFC) values of the ethanol extracts. The ethanol extract of *Z. zanthoxyloides* showed minimum inhibitory concentration (MIC) at 3.125 mg/ml against *M. gypseum* and not sensitive to *A. flavus* at all concentration used.

Table 2: Zone of inhibition of aqueous and Ethanol extracts (100 mg/ml) of *Z. zanthoxyloides* root against selected oral pathogens.

<table>
<thead>
<tr>
<th>Extract</th>
<th><em>S. aureus</em></th>
<th><em>S. auricularis</em></th>
<th><em>M. gypseum</em></th>
<th><em>S. pyogenes</em></th>
<th><em>S. mutans</em></th>
<th><em>B. subtilis</em></th>
<th><em>A. flavus</em></th>
<th><em>C. albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Aq</td>
<td>7.07±0.03</td>
<td>4.80±0.06</td>
<td>11.0±0.58</td>
<td>5.70±0.58</td>
<td>5.40±0.06</td>
<td>7.67±0.09</td>
<td>8.30±0.06</td>
<td>6.03±0.03</td>
</tr>
<tr>
<td>Et</td>
<td>7.23±0.09</td>
<td>5.23±0.12</td>
<td>16.10±0.58</td>
<td>5.70±0.06</td>
<td>7.53±0.09</td>
<td>8.40±0.12</td>
<td>9.97±0.03</td>
<td>8.00±0.06</td>
</tr>
</tbody>
</table>

NB: Means ± S.E.M; n=3, Means ± S.E.M within a row are significantly different, P< 0.01

Table 3: Zone of inhibition in mm of various concentrations of the ethanol extract of *Z. zanthoxyloides* root on test organisms.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Concentration of extract (mg/ml)</th>
<th>Sterile distilled water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.125</td>
<td>6.5</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>–</td>
<td>3.40 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. auricularis</em></td>
<td>–</td>
<td>2.40 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>–</td>
<td>3.30 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. mutans</em></td>
<td>3.20 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.30 ± 0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>M. gypseum</em></td>
<td>–</td>
<td>3.77 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

NB: Means ± S.E.M; n=3; Means ± S.E.M within a row with different alphabet are significantly different, P< 0.01. – = No inhibition.
Table 4: Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) in mg/ml of the ethanol extracts of Z. zanthoxyloides root against the test bacteria.

<table>
<thead>
<tr>
<th>Test Bacteria</th>
<th>Z. zanthoxyloides (root) (mg/ml)</th>
<th>S. aureus</th>
<th>S. auricularis</th>
<th>S. pyogenes</th>
<th>S. mutans</th>
<th>B. subtilis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC</td>
<td>6.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>MBC</td>
<td>12.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Means ± S.E.M; n=3, Means ± S.E.M within a column with different alphabet are significantly different, P< 0.01. – = No inhibition.

Table 5: Minimum Inhibitory Concentrations (MICs) and Minimum fungicidal Concentrations (MFCs) in mg/ml of the ethanol extracts of Z. zanthoxyloides root against the test fungi.

<table>
<thead>
<tr>
<th>Test fungi</th>
<th>Z. zanthoxyloides (root) (mg/ml)</th>
<th>M. gypseum</th>
<th>A. flavus</th>
<th>C. albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIC</td>
<td>3.125&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>MFC</td>
<td>6.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-= 50.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NB: Values are means ± S.E.M (n=3); Values within a row with different alphabet are significantly different, P< 0.01; – = No inhibition.

Table 6: Sensitivity zone of inhibition of commercial antibiotics (standard sensitivity disc) on the test bacteria.

<table>
<thead>
<tr>
<th>Test isolates</th>
<th>Zone of inhibition (in mm) for commercial antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CN</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>28.3</td>
</tr>
<tr>
<td>Staph. auricularis</td>
<td>27.0</td>
</tr>
<tr>
<td>Strept. mutans</td>
<td>20.6</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>24.8</td>
</tr>
</tbody>
</table>

PEF = Pefloxacin (10 µg/ml), – = No inhibition, CN = Gentamicin (20 µg/ml) APX = Ampiclox (30 µg/ml), OFX= Ofloxacin (10 µg/ml), AM = Amoxacillin (30 µg/ml) R = Rocephin (25 µg/ml), CPX = Ciprofloxacin (10 µg/ml), S = Streptomycin (30 µg/ml) SXT =Septrin (30 µg/ml), E = Erythromycin (10 µg/ml), APX = Ampiclox (10 µg/ml)

Table 7: Sensitivity zone of inhibition of commercial fungi antibiotics (ketoconanzone) on the test fungi.

<table>
<thead>
<tr>
<th>Test fungi</th>
<th>Ketoconanzone (200 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus flavus</td>
<td>17 mm</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>24 mm</td>
</tr>
<tr>
<td>Microsporium gypseum</td>
<td>26 mm</td>
</tr>
</tbody>
</table>

Discussion

The result of qualitative phytochemical screening tests carried out on the aqueous and ethanol extracts of the roots of Z. zanthoxyloides, revealed the presence of alkaloids, flavonoids, terpenoids, steroids, anthraquinones while cardiac glycoside and saponins were absent in aqueous and ethanol extracts of Z. zanthoxyloides and carbohydrate absent in aqueous extract (Table 1).

The presence of bioactive compounds has been known to show medicinal activity as well as exhibit and regulate some physiological activity (Sofowora 1993; Harborne 1998). Saponins have been reported to be an antifungal agent, while tannins prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable to them (Ogunleye and Ibitoye 2003) and tannins have been traditionally used on inflamed
Antimicrobial activities of root extracts of Z. zanthoxylloides on dental microbe

surfaces of mouth and treatment of catarrh, (Sodipo et al. 1991; Stephen et al. 2009) also reported that tannins have antioxidant properties. Okwu (2001) gave the importance of steroids as potent starting material in the synthesis of sex hormones. Some of these bioactive compounds could be said to be responsible for the antimicrobial activity observed in this study. The aqueous and ethanol extracts of the plant used showed inhibitory activities against all the test organisms (Table 2). Of all the tested organisms, S. auricularis recorded the highest resistance ranging from 0.00±0.00 mm to 5.23±0.12 mm for ethanol extract; S. auricularis also recorded the highest resistance ranging from 0.00±0.00 mm to 4.80±0.06 mm for aqueous extract both at concentration of 100 mg/ml.

It was observed that susceptibility increased with increased concentration of the extracts (Table 3). The ethanol extracts exhibited more activity, potency and consistency than the aqueous extract. The ethanol extract recorded the highest inhibitory effect against the test organisms compare to that recorded for the aqueous extract (Table 2 and 3). These results support earlier studies which observed that plant extracts in organic solvent provided more consistent antimicrobial activity compared with those extracted in water (Parekh et al. 2005; Ahmad et al. 1998).

The most sensitive test bacterium was B. subtilis in ethanol with zone of inhibition of 8.40±0.12 mm at the highest concentration of 100 mg/ml. The aqueous extract was most active against B. subtilis with zone of inhibition of 7.67±0.09 mm at 100 mg/ml (Table 2).

Ethanol extract recorded the highest antifungal activity of 16.10±0.58 against M. gypseum at 100 mg/ml, while A. flavus recorded no antifungal activity even at a concentration of 100 mg/ml (Table 3).

It was also observed that the extracts were active when compared with the negative control (sterile distilled water) against all the test organisms (Table 3). The control recorded no visible activity. The positive control (standard sensitivity disc) used on the test bacteria revealed that gentamycin, perflaxacin, ampcilox, ofloxacin, ciprofloxacin, perflaxacin and erythromycin, had inhibitory effects on all the test bacteria (Table 6). Rocephin, streptomycin and septrin showed no inhibition zone on any of the tested organisms. In addition amoxicillin showed inhibition against all organisms except S. aureus and S. mutans.

Comparatively, the ethanol and aqueous extracts of the plant can be said to possess better activity than the antibiotics since they contain both pharmacological and non-pharmacologically active substances as oppose to the pure active substances contained in the control antibiotics. The effect of the commercial antifungal drug (Ketoconanzone) tested at a concentration of 200 mg/ml against the test fungi (Table 7) can be considered not better in activity when compared with the extracts, particularly at the highest tested concentration of 100 mg/ml which was two times lower in concentration than that of the fungal antibiotics. This probably implies that if the concentrations of the extracts were increased, it could lead to increased activity.

Okeke (2003) confirmed the efficacy of Baphia nitida herb as used by the local inhabitants in Eastern Nigeria for the management of dental caries. Leitao et al. (2004) reported that Newbouldia leavis, Brazilian green propolis and Bracchis drancuncufolia has anti caries effect. It has been reported that Africans that use chewing sticks have fewer carious lesions than those that use toothbrushes. In a related development, Enwonwu (1997) posited that chewing sticks, in addition to providing mechanical stimulation of the gums, also destroy microbes; these advantages of the chewing stick over conventional toothpaste and brushes has been attributed to the strong teeth of Africans (Ugoji et al. 2000).

Conclusion

While toothpaste and toothbrushes are widely used by a sector of the population with a high level of formal education, toothpaste consumption is still low and chewing sticks are still in common use in many parts of Africa. Even when people prefer to use toothbrushes, access to toothpaste may be limited due to high cost. The practice of dental hygiene is very important. Therefore, continued access to popular and ef-
fective sources of chewing sticks with antibacterial and anti-fungal properties is important as a primary health care measure. The results from these studies provide evidence for the ethnomedicinal uses of the tested plants as chewing sticks. This implies that the plant used for this study possesses both antibacterial and antifungal properties and is recommended as chewing sticks for the reduction of dental caries caused by antimicrobial agents.

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