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# The Antibacterial Effect of some Selected Chewing Sticks on Bacteria Isolated from Decayed Tooth

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## Abstract
The study assessed the antibacterial efficacy of different chewing sticks purchased from market in Sokoto, on selected bacteria isolated from decayed tooth. 10 decayed tooth specimens were collected from Usmanu Danfodiyo University Teaching Hospital Dental Clinic, in Sokoto State. The specimens were processed for isolation of bacteria following standard bacteriological. The bacterial isolates were *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aureginosa.* The chewing sticks (*Azadirachta indica* (Neem plant), *Massularia acuminata* (kako stick) and *Salvadora persica* (kirya stick) showed antibacterial activity against all the bacterial isolates. techniques. Phytochemical and antibacterial properties of aqueous extracts of the chewing sticks were investigated in this study. This Phytochemical analysis of the extracts showed the presence of carbohydrate, alkaloids, flavinoids, tannins and saponins. At concentrations ranging from 50 to 400 mg/ml the aqueous extracts showed activity against the three bacteria species, with zone of inhibition ranging from 20 to 25mm. Minimum inhibitory concentrations of the extracts were 62.5, 100 and 125 mg/ml and the minimum bactericidal concentration ranged from 12.5 to 200mg/ml. It can be concluded from this present study that overall antibacterial activity of the extracts was quite reasonable against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aureginosa* and provide data that may be supportive point about its medicinal values.

***Keywords****: Antibacterial, Chewing stick, Bacteria and Decayed tooth*

## Introduction
Chewing sticks have been used for recorded periods of time. Approximately 7000 years ago, the Babylonians practiced this type of tooth brushing [1]. Instead of using plastic-bristle brushes, many traditional societies frequently employ chewing sticks made of herbs to treat tooth issues. Chewing sticks are believed to have cleansing properties due to the mechanical action of their fibers, the release of beneficial compounds, or a combination of both [2]. According to Evans [3], there have been reports of fluoride ions, silicon, tannic acid, sodium bicarbonate, and other naturally occurring compounds that inhibit plaque formation and limit bacterial colonization found in several African chewing sticks. Dental caries is among the most prevalent mouth infections affecting people in the modern world. It is an extremely dangerous dental condition [4]. Due to the breakdown of the acids produced by the fermentation of food particles left on the teeth after eating,

it results in tooth decay [5]. Through their combination with food remnants and saliva, oral bacteria such as *Lactobacillus acidophilus, Eschrichia coli, Staphylococcus aureus* Marsh *et al.* [6] build up to produce a thick, white coating on teeth known as dental plaque. According to Verkaik *et al.* [7], the organism creates acid from fermented meals, which erodes the tooth's surface and results in cavities and holes in the teeth.

Due to a lack of knowledge about dental caries, poor oral hygiene is one of the main causes of tooth decay. As a result, dental caries is becoming more commonplace worldwide. Treatment for it is costly and necessitates the expertise of highly qualified specialists [8]. It is crucial to educate people about dental care in developing nations where the majority of people lack access to dental facilities in order to encourage using traditional methods of cleaning teeth in addition to conventional toothpastes. As an abrasive, toothpaste works to maintain dental health, remove food particles and plaque from teeth, reduce bad breath, and its active component, fluoride, helps to prevent gum disease and tooth decay [9]. However, miswak was used by early humans as a natural mouth hygiene tool before toothpaste was invented. Nonetheless, a number of investigations have been conducted to clarify the overall impact of miswak on dental health as well as to clarify specific naturally occurring chemical components found in miswak that are crucial for upholding proper hygiene [10]. As of right now, toothpaste contains miswak extract as a flavoring and active ingredient [11]. In order to prevent dental caries, the purpose of this study is to evaluate the effectiveness of various chewing sticks as well as their antibacterial properties against certain bacteria that have been isolated from the oral cavity.

## Materials and Methods

### Plant Material and Tooth

Chewing sticks were obtained from Sokoto Main market and was authenticated at the herberium unit, Department of Biological Sciences Usmanu Danfodiyo University. Decayed tooth which are removed and to be discarded was obtained from dental clinic in Usmanu Danfodiyo University Teaching Hospital Sokoto.

### Aqueous Extraction

A 100g of chewing sticks was cut into pieces and ground into powder with a commercially available food blender. 100ml of distilled water was added to 10g of the powder in a sterile well capped flask, left for 7 days at room temperature and then filtered using number 1 Whatman filter paper. The extract then evaporated in a mantle heater at 40℃ until distilled water was removed while the aqueous extract was centrifuged at 2000pm for 10 minutes [12]. The supernatant was passed through a 0.45mm membrane filter. The extracts were preserved in sterile screw-capped vials in the refrigerator for further use [13].

### Bacterial Isolate

The test organism was isolated directly from an infected tooth obtained from dental clinic, the tooth was swabbed with sterile cotton wool swab and immediately streak on a sterile blood agar and MacConkey in duplicate, the plates were incubated at 37℃ for 24 hours. Characteristics colonies were picked from the plate and purified by repeated sub culturing. Pure isolates obtained were characterized using gram staining technique and biochemical tests which include catalase, oxidase, coagulase and indole. Pure colonies were streaked on nutrient agar plates and was incubated at 37℃ for 24 hours.

### Gram Staining

Smear was prepared by emulsifying a colony from the culture plate in a drop of normal saline solution and a thin film will be made on a slide. The smear was allowed to air dry and fixed under flame. The smear was stained using the appropriate technique. The smear was examined under oil immersion objectives [14].

**Catalase Test**

Procedure: A drop of hydrogen peroxide was place on clean grease free glass slide. Using a sterile applicator stick, the colonies of the test organism was place on the hydrogen peroxide and emulsify. Formation of bubbles was observed [15].

**Coagulase Test**

Procedure**:** Two separate drops of saline was placed on a clean grease free glass slide. Using sterile applicator stick, the colony of the test organism was emulsified on the saline to make two thick suspensions. A drop of plasma was added on one of the two drops of saline. The mixture was observed for clumping of the organism within 10 seconds [16].

**Oxidase Test**

Procedure: A piece of filter paper was place in a petri dish. Two drops of freshly prepared oxidase reagent were added. Using a sterile applicator stick, a colony of test organism was smeared on the filter paper looking out for development of blue purple color within a few seconds [17].

**Indole**

Procedure: A sterilized test tubes containing 4ml of tryptophan broth. The tube was innoculated aseptically by taking the growth from 18 to 24 hours culture. The tube was incubated at 37C for 24 to 28 hours. 0.5ml of Kovac's reagent was added to the broth culture. Presence or absence was observed [18].

**Kliger's Iron Agar (KIA)**

Procedure: Using an inoculating needle, KIA was inoculated with a light inoculum from a pure, 18–24-hour culture growing on solid medium. The slants were streaked back and forth across the entire surface and stabbed to the bottom of the agar butt. The tubes were then incubated in ambient air at 33-37°C for 18-24 hours and with cap loosened [17].

**Urease Test**

Procedure of Urease Test: the surface of a urea agar slant was streaked with a portion of a well-isolated colony. The cap was loosely leave on and the tube was incubated at 35°-37°C in ambient air for 48 hours to 7 days, the development of a pink color for as long as 7 days was examined [19].

**Citrate Test**

Procedure of Citrate Utilization Test: the slant was streaked back and forth with a light inoculum picked from the center of a well-isolated colony. It was then Incubated aerobically at 35 to 37C for up to 4-7 days. color change from green to blue along the slant was observed [20].

**Preparation and Standardization of the Innoculum**

An overnight broth culture of the test bacterium was used to prepare a standard innoculum of 3.30 x 106cfu/ml. This value was arrived at appropriate dillution of the broth culture in 0.85% sodium chloride solution to match with standard turbidity of 1% barium sulphate suspention [14].

**Preparation of Concentration for the Chewing Stick Extract**

About 2ml of the plant extract was measured using a 2ml syringe and were transferred into a test tube containing 3ml of distilled water to give a concentration of 500µg/ml; these was further diluted in distilled water to give three different concentrations of 250, 125 and 62.5mg/ml respectively.

**Preparation of Macfarland Standard**

A 0.5 McFarland standard was prepared by mixing 0.05ml of barium chloride dehydrate, with 9.95ml of 1% sulfuric acid [21]. The standard (MACFARLAND) was compared visually to a suspension of bacteria isolate in sterile normal saline.

**Preliminary Screening for Phytochemicals in Crude Extract**

Phytochemical screening was carried out on the chewing sticks extract to determine the presence of the following constituents: flavonoids, tannins, saponins, terpenes, basic alkaloids and glycosides, using the method described by Thilagavati *et al*. [22].

**Test for Alkaloids**

The extracts were evaporated to dryness and the residue was heated on boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and heated with a few drops of Mayer's reagent the sample was then be observed for the presence of turbidity [23].

**Test for Tannin**

A total of 1g of the extract was dissolved in 20ml of distilled water and filtered. 3drops of Fecl was added to 2ml of the filtrate. The production of blackish-blue or blackish-green was indicative of the presence of tannin [24].

**Test for Flavonoid**

About 4mg of the extract was dissolved in water and will be treated with 2drops of Fecl and blue-black coloration was observed for the presence of Flavinoid [24].

**Test For Reducing Sugar**

About 1ml of Fehling's solution I and II was added to 2ml of the aqueous extract solution and was heated for 5minute in a heated boiling water bath. The production of a brick red colour will indicate the presence of reducing sugar [25].

**Test For Glycosides**

Two (2) ml of test solution, 3ml of glacial acetic acid and 1 drop of 5% ferric chloride was integrated in a test tube,0.5ml of concentrated sulphur acid was integrated by the side of the tube. Formation of blue colour on the acetic acid layer indicates the presence of cardiac glycosides [26].

**Determination of Minimum Inhibitory Concentration**

Ability of isolate to grow in extract: This was established if the different extracts had inhibitory properties. A total of 0.25 ml of the isolate obtained from a 24 hours broth culture was introduced into sterile test tubes containing 2.5 ml undiluted extracts. The tubes were incubated at 37℃ for 24 hours. The broth culture was sub cultured onto agar plates by the streaking method to observe for growth [27].

**Determination of Minimum Bactericidal Concentration**

Minimum bactericidal concentration was gotten from minimum inhibitory concentration in which plates that showed no growth on the MIC were sub cultured onto nutrient agar individually, and the concentration that shows no growth was marked as MBC [28].

**Results**

The identified bacteria from clinical specimens are shown in Table 1. These organisms were isolated and identified using the standard microbiological methods. 4 organisms were isolated and identified from 10 samples analyzed and include *E. coli*, two *Staphylococcus aureus* and *Pseudomonas aureginosa.* The biochemical characteristics of the bacteria isolated from decayed tooth which include gram, catalase, coagulase, oxidase and KIA (Kliegler iron agar) are shown in Table 2. The Phytochemical compounds present from the extracts include carbohydrate, alkaloids, Flavinoid, tannins and glycoside and are shown in Table 3 The screening antibacterial activity pattern of the aqueous extracts of *Azadirachta indica*, *Massularia acuminata* and *Salvadora persica* shows that the aqueous extracts have all antibacterial activity against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aureginosa* with the zone of inhibition of 22mm, 25mm and 20mm respectively against 3000mg of extract in 12ml of distilled water as shown in Table 4 The Minimum Inhibitory Concentration of aqueous extracts of *Azadirachta indica*, *Massularia acuminata* and *Salvadora persica* against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aureginosa* were found to be 62.5mg/ml, 100mg/ml and 125mg/ml each as shown in Table 5 The Minimum Bactericidal Concentration of aqueous extracts of *Azadirachta indica*, *Massularia acuminata* and *Salvadora persica* against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aureginosa* were found to be 200mg/ml each Table 6.

**Table 1: Bacteria Isolated from decayed tooth**

| **Bacteria** | **Number isolated n = 10** |
|---|---|
| *E. coli* | 1 |
| *Staphylococcus aureus* | 2 |
| *Pseudomonas aureginosa* | 1 |

**Table 2: Biochemical characteristics of the bacteria isolated from decayed tooth**

| | | **Biochemical Reactions** | | | |
|---|---|---|---|---|---|
| **Bacteria isolated** | **Gram reaction** | **Catalase** | **Coagulase** | **Oxidase** | **KIA** |
| ***E. coli*** | **Gram –ve bacilli** | * | * | - | + |
| ***Staphylococcus aureus*** | **Gram +ve cocci** | + | + | * | * |
| ***Pseudomonas aureginosa*** | **Gram –ve bacilli** | - | * | + | - |

**Key + = positive**
**-= negative**
*** = not applicable**

**Table 3: Phytochemical screening of the Extracts of *Azadirachta indica, Massularia acuminata* and *Salvadora persica***

| **S/N** | **Phytochemical** | ***Azadirachta indica* (Neem)** | ***Massularia acuminata* (kako)** | ***Salvadora persica* (kirya)** |
|---|---|---|---|---|
| **1.** | **Carbohydrate** | + | - | - |

| | | | | |
|---|---|---|---|---|
| **2.** | **Alkaloid** | **+** | **+** | **+** |
| **3.** | **Flavinoid** | **+** | **+** | **+** |
| **4.** | **Tannin** | **+** | **+** | **+** |
| **5.** | **Glycoside** | **+** | **-** | **+** |
| **6.** | **Saponin** | **+** | **+** | **-** |

**Key + = Present - = Absent**

**Table 4: Screening for Zone of Inhibition of Aqueous Extracts of *Azadirachta indica*, *Massularia acuminata* and *Salvadora persica***

| **Organisms** | **Diameter of zone of Inhibition (mm)** | | |
|---|---|---|---|
| | ***Azadirachta indica*** | ***Massularia acuminata*** | ***Salvadora persica*** |
| ***E. coli*** | **22** | **20** | **20** |
| ***Staphylococcus aureus*** | **25** | **30** | **10** |
| ***Pseudomonas aureginosa*** | **20** | **17** | **15** |

**Table 5 Minimum Inhibitory Concentration of Extracts *Azadirachta indica, Massularia acuminata* and *Salvadora persica***

| **Organisms** | ***Azadirachta indica* (mg/ml)** | ***Massularia acuminata* (mg/ml)** | ***Salvadora persica* (mg/ml)** |
|---|---|---|---|
| ***E. coli*** | **62.5** | **12.5** | **100** |
| ***Staphylococcus aureus*** | **15.6** | **62.5** | **62.5** |
| ***Pseudomonas aureginosa*** | **62.5** | **62.5** | **100** |

**Table 6: Minimum Bactericidal Concentration of the Extracts of *Azadirachta indica, Massularia acuminata* and *Salvadora persica***

| **Organisms** | ***Azadirachta indica* (mg/ml)** | ***Massularia acuminata* (mg/ml)** | ***Salvadora persica.* (mg/ml)** |
|---|---|---|---|
| ***E. coli*** | **200** | **100** | **125** |
| ***Staphylococcus aureus*** | **200** | **100** | **100** |
| ***Pseudomonas aureginosa*** | **200** | **100** | **200** |

## Discussion

In this study, both gram negative and gram-positive bacteria were isolated and identified using standard microbiological technique. The bacterial isolates seen were *Escherichia coli*, *S. aureus* and *Pseudomonas aureginosa*. The presence of these bacteria shows they are pathogenic and could

be responsible for tooth decay. These bacteria were subjected to different chewing sticks extract to test for their efficacies which correspond to the work carried out by Sudhir *et al*. [11].

The result of the current research clearly showed that the chewing sticks (*Azadirachta indica*, *Massularia acuminata* and *Salvadora persica)* inhibited the growth of bacteria causing tooth decay. However, the range of effectiveness is concentration-dependent and varied against the different tested bacteria [29].

The phytochemical compound of the aqueous extracts of *Azadirachta indica*, *Massularia acuminata* and *Salvadora persica* were investigated. The results revealed the presence of saponin, flavonoid, tannin, alkaloid and glycoside. Similar studies [30- 32] consistently reported phytochemical constituents of *Azadirachta indica*, *Massularia acuminata* and *Salvadora persica* to be alkaloids, tannins, saponins and flavonoids. Therefore, the result of the phytochemical analysis of the extracts of *Azadirachta indica*, *Massularia acuminata* and *Salvadora persica* obtained in this study confirms to the previous reports. However, the phytochemical result of this study contradicts the work of Chinsembu [33] who reported flavonoids to be absent in the phytochemical screening. These phytohemical compound have been known to play important roles in bioactivityof medicinal plants. Flavinoids are known to have a wide range of antimicrobial activities which has been linked to their ability to form complex with extracellular and soluble proteins and with bacterial cell walls while tannins are known to exert antimicrobial activity by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in micro-organisms [34].

In all, the antibacterial activity of the extracts of *Azadirachta indica*, *Massularia acuminata* and *Salvadora persica* were found to be more as the concentration of the extracts increases, which implies that the higher the concentration, the more the activity by the extracts on the organisms. This is also in line with the observations of [30]. The minimum inhibitory concentration (MIC) is the smallest concentration that visibly inhibits growth. The MIC is useful in determining the smallest effective dosage of a drug against bacteria. The MIC result obtained from this study revealed that different concentrations of the extracts served as the MIC values against the organisms. The bacteria (*E. coli, Staphylococcus aureus* and *Pseudomonas aureginosa*) were more sensitive to the aqueous extract with an MIC value of more than 50mg/ml each. This is in line with the findings of [35]. The results of the minimum bactericidal concentration (MBC) of the extracts yielded a higher MBC value ranging from 50 to 200 mg/ml which implies that very high concentration of the extracts is required to exert a bactericidal effect on the organisms. This conforms to the work of Abdalbaqi *et al.* [35].

**Conclusion**

The result of this study indicated that the aqueous stick extracts of *Azadirachta indica*, *Massularia acuminata* and *Salvadora persica* were effective against *E. coli, Staphylococcus aureus* and *Pseudomonas aureginosa* and a high concentration of the plant is required to act on the bacteria.

Further research is therefore recommended to isolate, purify and characterize these chemical constituents with a view to supplementing conventional drug development especially in developing countries.

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