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The Antimicrobial Properties of Terminalia Glaucescens and Zanthoxylum Zanthoxyloides Extracts and the Spectrum of their Activity

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Abstract

The antimicrobial activity of the ethanol extracts of two Nigerian chewing sticks (Terminalia glaucescens and Zanthoxylum zanthoxyloides) against oral bacteria and fungi isolates implicated in dental caries were assessed. The aim of the study is to isolate microbial organisms associated with dental caries and to assess the antimicrobial activity of the ethanol extracts of Zanthoxylum zanthoxyloides and Terminalia glaucescens stem used as chewing sticks. The dental extracts from 20 (twenty) patients with clinical features of dental caries were collected from School of dental technology and therapy clinic, Trans-ekulu, Enugu. The patients comprised of 8 males and 12 females with ages ranging from 16-40 years. The disc diffusion method was used in evaluating sensitivity to the extracts and it was observed that all the isolates were sensitive to T. glaucescens at different levels of concentration with a MIC and MBC of 6.25mg/ml for S. pyogenes. The isolated and tested dental pathogens were Escherisia coli, Pseudomonas aerugenosa, Streptococcus mutans, Streptococcus pyogenes, Staphylococcus aureus and Candida albicans. However, of the all the isolates E. coli proved resistant to Z. zanthoxyloides extract. Conventional antibiotics were used as positive control while distilled water was used as negative control.

The ability of T. glaucescens and Z. zanthoxyloides extracts to inhibit the growth of the bacteria and fungi in this study is an indication that the plant can be used as a source for antimicrobial agent in the formulation of toothpaste and mouth wash, thus justifying the use of the plant locally as chewing sticks.

Keywords: *Dental Caries, Zanthoxylum zanthoxyloides and Terminalia glaucescens.*

1.0 Introduction

Dental caries is an infectious microbial disease that results in localized dissolution and destruction of calcified tissues of the teeth. The human oral cavity harbours species of bacteria, fungi and protozoa. The oral cavity causes major dental diseases such as caries and periodontitis. Dental problem is known to be one of the most common health problem in the importance throughout the world. (Almas, 2001) .The food debris, acid, bacteria, and saliva combine in the mouth to form a sticky substance called “plaque” that adheres to the teeth and this plaque leads to tooth decay. Ethnomedicinal plants in form of chewing sticks used for the cure of dental problems in this work include *Zanthoxylum zanthoxyloides* (Oriata / tejovati) and *Terminalia glaucescens* (Idiodan / arjun).

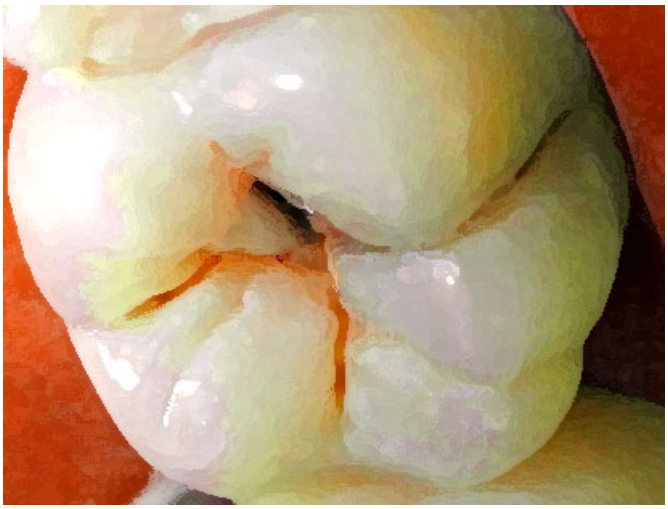

Figure 1: Images of dental caries

1.2 How dental caries occurs and developed.

The pathogenic organisms will first colonize on tooth surfaces, synthesize insoluble polysaccharides from sucrose. This synthesis allows adhesion to smooth surfaces and appears to be important in the formation of smooth surface caries and then**,** ferment sucrose to form lactic acid. (Holt *et al*., 1994)

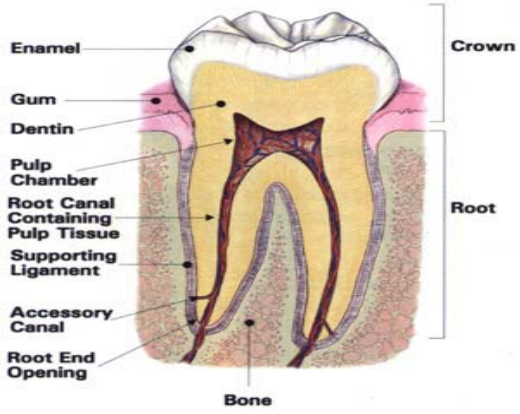

Figure 2: Cross section of a tooth illustrating the various structural regions susceptible to colonization or attack by microbes.

Significance of the Study

This study is designed with the intent that it will serve as an available reference source and be of good value to researchers in this field; thus updating the existing literature in this subject matter. Moreover, the study will assist government and medical personnel in using these extracts as a source for antimicrobial agent in the formulation of toothpaste, thus justifying the use of the plant locally as chewing sticks and implementation for better service delivery.

Statement of Problem

- Dental caries is steadily increasing in the underdeveloped and developing countries. Treatment is expensive and not a realistic option for the poor.
- Also, there are diseases that spread from the oral cavity to other vital organs, for example infective endocarditis that leads to severe morbidity and motality.
- Hence, there is an urgent need to promote traditional preventive measures that are acceptable, easily available, and cost effective.

The main objectives of this work therefore is:

1. To isolate microbial organisms associated with dental caries.
2. To assess the antimicrobial activity of the ethanol extracts of *Zanthoxylum zanthoxyloides* and *Terminalia glaucescens* used as chewing stick.
3. Determination of Minimum Inhibitory Concentration (MIC) of the extracts.
4. Determination of Minimum Bactericidal Concentration (MBC) of the extracts.
5. To objectively compare the antimicrobial activity of these extracts with conventional antimicrobial.

Literature Review

Dental caries is a microbial infectious disease that occurs in the localized dissolution and destruction of the calcified tissues of the teeth. Streptococcus group *(S. salivarius, S. mitis, S.mutans* etc) are known as the causative bacteria and fungi in the formation of dental plaque and dental caries (Pretorius *et al*., 2003). The acid producing *S. mutans* inhabiting the mouth causes damage by dissolving tooth structures in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose. When food debris, acid, bacteria, and saliva combine in the mouth and form a sticky substance which adheres to the teeth called plague. If not removed thoroughly and routinely, the plague will result to tooth decay. (Wolinsky and Sote, 1984).

Persistent or chronic dental disease has suggestively linked to diabetes, high blood pressure and heart disease later in life. Heat, cold or sweet foods and drinks worsen the dental disease pain. Treatment often prevents further infection. Dental caries also lead to the cause of bad breath and foul tastes etc. Infection can progress aggressively and spread from the tooth to surrounding soft tissues which may lead to an edentulous mouth (Kleinberg, 2002).

Erythromycin and penicillin are the two antibiotics reported to prevent dental caries effectively in animals and humans but has great adverse effect that prevented their used clinically, Recent natural remedies has been introduced with the use of medicinal plants, which has a good reservoirs of chemo-therapeutants and has been contributed as an alternative for antibiotic effects such as hypersensitivity reaction, supra infections, and teeth staining.

It has been well documented that medicinal plants confer antimicrobial activity against oral bacteria. The literature survey of the folklore medicine reveals the use of *Zanthoxylum zanthoxyloides* (Oriata Yoruba and Tejovita- English) leaves maintain oral hygiene and stem of *Terminalia glaucescens* (Idiodan- Yoruba and Arjun- English) for the treatment of tooth-ache. (Ogundiya *et al*., 2014).

Though recent reports show the antibacterial activity of *Zanthoxylum zanthoxyloides* and *Terminalia glaucescens* against the cariogenic bacteria (Fufulu, 1975), its antibacterial and antifungal activity with ethanolic solvent extracts are screened in this study. This study is focused on assessing the plant extracts with good ethanol solvent and the present investigation on *Streptococcus pyogenes, Streptococcus mutans, Staphylococcus aureus, Escherichia coli, Pseudomonas aerogenosa and Candida albicans* which are the bacterial and fungal strains selected as target organisms from infected patients with caries and screened using ethanol extracts of the stem of *Zanthoxylum zanthoxyloides* and *Terminalia glaucescens* (Barry and Thornsberry 1991). *In vivo* trials is been carried out for the treatment of dental caries by external application on the caries tooth once the antimicrobial property of the plant extracts has been screened under '*in vitro*' condition against oral pathogens (Ogundiya *et al*., 2008).

It has been reported that in Nigeria, some of the chewing sticks being used are obtained from the following plants: *Garcinia manni* (aki inu- Igbo), *Masularia accuminata* (Pako-Yoruba), *Terminalia glaucescens* (Idiodan-Yoruba, Arjun- English), *Zanthoxylum zanthoxyloides* (Oriata- Yoruba, Tejovati- English) and *Azadiracta indica* (Neem- English). (Akande and Hayashi (1998) .

T. glaucescens and Z. zanthoxyloides are widely used plants for chewing stick application in Nigeria and this has lead to their *vast* array of studies on its antimicrobial activity against some oral pathogens.

2.0 Methods

2.1 Collection and identification of plant parts and dental caries Pathogens.

- **Plant Materials Collection**

The small branches of locally available *Z. zanthoxyloides* and *T. glaucescens* were collected from local Ogbete main market Enugu and were authenticated by Prof. Okigbo R.N of the department of Botany of Nnamdi Azikiwe University, Awka. These plant materials were dried under the sun for two weeks and also cut into pieces of approximately 15cms and transferred to the oven set at 45°C for 20-30mins before it was reduced to fine powder with the aid of mechanical grinder. The powder plant materials were collected and stored in a tightly covered glass jar for further studies.

- **Collection and Recovery of Caries Sample**

The dental extracts from 20 (twenty) patients with clinical features of dental caries were collected from School of dental technology and therapy clinic, Trans-ekulu, Enugu. The patients comprised of 8 males and 12 females with ages ranging from 16-40 years.

The samples were collected under strict aseptic conditions and patient's consent. Prior to the collection of dental caries sampling, patient was made to rinse the tooth with water. The tooth and the surrounding field were cleaned with 3% hydrogen peroxide and then decontaminated with a 2.5% sodium hypochlorite solution. The food debris on the chewing surface was removed using a dental excavating instrument .The tooth was then extracted by a clinician and then introduced into the 20ml broth of Brain Heart Infusion (BHI) in appropriate sterile screw cap bottles. The dental caries sample was collected from the extracted tooth using an excavator under aseptic conditions. The clinical samples were mixed well using a magnetic stirrer before incubation. The samples were then inoculated using the streak plate technique on to nutrient and sabouraud dextrose agar under various culture conditions- aerobic, microaerophilic, and anaerobic culture conditions for each patient sample (Holding and Colee, 1971).

The organism isolated was identified on the basis of morphological, cultural and biochemical characteristics according to standard procedures (Holding and Colee, 1971).

2.2 Preparation and Extraction of Plant Materials.

- **Ethanol Extraction**

20g of fine-powder stem of *Z. zanthoxyloides* and *T. glaucescens* was weighed and soaked in 200mls of ethanol in a conical flask and kept at room temperature (25°C) in a rotary shaker for 48 hours. After 48 hours, filtered through Whatman No1 filter paper; solvent was allowed to evaporate and stored at room temperature until when required for use.

2.3 Identification of the Isolates

The isolated organisms were identified using sub-culturing, gram staining technique and biochemical test like catalase test, indole test, coagualase test, methyl red test, oxalase test, sugar fermentation test.

2.4 Test Organisms

- **ISOLATION OF THE TEST ORGANISMS.**

The dental specimen collected was streaked out on Nutrient agar and Sabouraud dextrose agar plates. The plates were incubated at 37^0C for 24 hours for bacterial isolates and 31°C for 48 hours for fungi. Colonies that developed were respectively sub-cultured into freshly prepared Nutrient agar and Sabouraud dextrose agar.

2.5 Preparation of Sensitivity Disc:

Disc of 6mm in diameter was punched out using Whatman No1 filter paper. Placed in bijou bottles, then sterilized the disc by autoclaving at 121°C for 15mins, and allowed to cool.

- **PREPARATION OF SENSITIVITY DISC WITH ETHANOL EXTRACTS OF *Z. zanthoxyloides and T. glaucescens* :**

The stock solutions of the ethanolic crude extracts (i.e. that were recovered) of these two plants were prepared by dissolving 0.5g (i.e. 500mg) of each of the two plant extracts in 5ml Dimethyl sulphoxide (DMSO). Therefore, each stock solution had a concentration of 100mg/ml.

Different concentrations of each of the plant extract were prepared from this stock. These are 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, and 3.13mg/ml by serial double dilution, followed by introducing the disc in each concentration. The disc was allowed to absorb the solution for 10mins and kept for further analysis. Each paper disc is capable of absorbing 0.01ml.

2.6 Determination of Antimicrobial Activities of Extracts.

Disc Diffusion Assay: Antibacterial activity of the ethanolic extracts of the plant sample was evaluated by noting the zone of inhibition against the test organisms. (Schaeken *et al*., 1986).

Antimicrobial activity was carried out using disc-diffusion method.

Two colonies of 24-hour plate culture of each organism was transferred aseptically into 10ml sterile normal saline in a test tube and mixed thoroughly for uniform distribution. A sterile cotton swab was used to spread the resulting suspension uniformly on the surface of oven-dried Nutrient agar and Sabouraud dextrose agar plates for bacteria and fungi respectively. The disc containing each concentration were impregnated on the culture plates and incubated at 37°C for 24hours and 31°C for 48hours for bacterial and fungal isolates respectively.

Conventional antibiotics were used as positive controls for bacteria and fungi respectively; distilled water was used as negative control. The plates were then incubated accordingly. The zones of inhibition were measured and recorded after incubation. The inhibition around the extracts indicated antimicrobial activity of the extracts against the test organisms. The diameters of these zones were measured diagonally in millimetre with a ruler and the mean value for each organism from the triplicate cultured plates was recorded. Using the disc 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 Enugu state was used as positive control for bacteria while ketoconanzole was used as positive control for fungi. Distilled water was used as negative control for all the test organisms.

2.7 Determination of Minimum Inhibitory Concentration (MIC) of the extracts:

The MIC for bacteria was determined as the lowest concentration of the extracts inhibiting the visual growth of the test cultures on the agar plate. The initial concentration of the plant extracts (100mg/ml) was diluted using double fold serial dilution by transferring 5ml of the sterile plant extract (stock solution) into 5ml of sterile normal saline to obtain 50mg/ml concentration. Different concentrations were 50, 25, 12.5, 6.25 and 3.13 mg/ml respectively. Each dilution was introduced into nutrient agar plates and Sabouraud dextrose agar plate already seeded with the respective test organism. All test plates were incubated at 37°C for 24hrs for bacteria and 31°C for 72hrs 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.

2.8 Determination of Minimum Bactericidal Concentration (MBC) of the extracts:

The Minimum Bactericidal Concentration (MBC) of the plant extracts were determined by the method described by Holding and Colee (1971). Samples were taken from plates with no visible growth in the MIC assay and subcultured on freshly prepared nutrient agar plates and Sabouraud dextrose agar plate and later incubated at 37°C for 24 hrs and 31°C for 48hrs for bacteria and fungi respectively. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

2.9 Statistical Analysis

- Mean value: The mean value for each organism from triplicate cultured plates was recorded.
- $M = \Sigma x/n$ (where M=mean, x= recorded values, n=number)

Result:

The microorganisms isolated from the teeth of patients with clinical features of dental carries were: *Escherichia coli, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus pyogenes, Staphylococcus aureus and Candida albicans (Table 1).*

The organism isolated was identified on the basis of morphological, cultural and biochemical characteristics according to standard procedures (Holding and Colee, 1971).

The sex distribution of dental caries is demonstrated by table 2 , fig.3 & 4 and it was observed that of the 20 specimens evaluated, 8 were from male and 12 from female patients. *Streptococcus mutans* and *Candida albicans* were predominantly more common in females than males. Table 3 and fig. 5 shows the age distribution of dental caries. It was observed that *Streptococcus mutans* and *Candida albicans* which are common dental caries pathogens were predominately found in the younger age group i.e. 16-30 years.

The antimicrobial activity of extracts of the stem of *T. glaucescens* was significant and varied with concentration on each of the isolated organism

(table 4). *S. pyogenes* and *S. aureus* were particularly sensitive to *T. glaucescens* even at a low concentration of 6.25mg/ml. Other isolates inhibited by *T. glaucescens* in descending order included: *S. mutans, P. aeruginosa, E. coli* and *C. albicans.*

The extract of *Z. zanthoxyloides* also showed good antimicrobial activities against *C. albicans, S. mutans, S. pyogenes, S. aureus* as well *P. aeruginosa* in that order (Table.5).

The implication of this data is that *C. albicans* and *S. mutans* which are implicated in dental caries were significantly inhibited by *Z. zanthoxyloides* even at fairly low concentration of 6.25mg/ml. Fig 6,Tables 6 & 7 show the Minimum Inhibitory Concentrations(MICs) and Minimum Bactericidal Concentrations(MBCs) values of both *T. glaucescens* and *Z. zanthoxyloides* extracts respectively.

It was observed that *T. glaucescens* also had the lowest MIC of 6.25mg/ml for *S. aureus* and *S. pyogenes* . *S. Pyogenes* also maintained 6.25mg/ml as MBC but *S. aureus* needed a concentration of 12.5mg/ml for bactericidal effect to occur.

The extract of *Z. zanthoxyloides* also showed good antimicrobial activities against *P. aeruginosa, S. aureus, S pyogenes*, as well as *C. albicans* even at a low concentration of 6.25mg/ml.

This implies that *Z. zanthoxyloides* has a higher potency than *T. glaucescens* against dental caries pathogens*(S.mutans* and *C.albicans).*

Table 1:- Isolated Organism from Dental Carries sample

ISOLATED ORGANISMS
Streptococcus pyogenes, *Staphylococcus aureus,* *Pseudomonas aeruginosa,* *Escherichia coli* *Streptococcus mutans,* *Candida albicans*

Table 2. Sex distribution of Isolated organisms.

ISOLATED ORGANISM	**MALE**	**FEMALE**	**TOTAL**
E. coli	8	12	20
P. aeruginosa	5	9	14
S. mutans	6	12	18
S. aureus	7	11	18
S. pyogenes	6	11	17
C. albicans	6	10	16

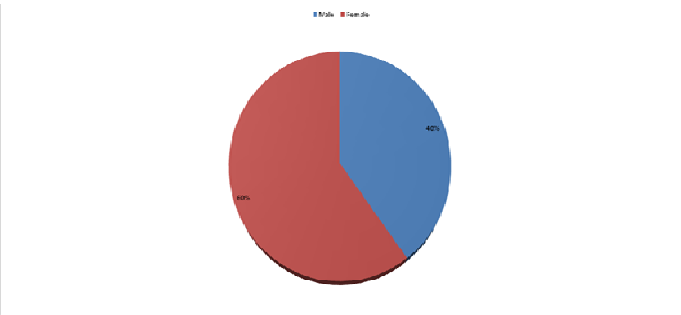

Fig 3. Pie chart showing the sex distribution of the patients in the study.

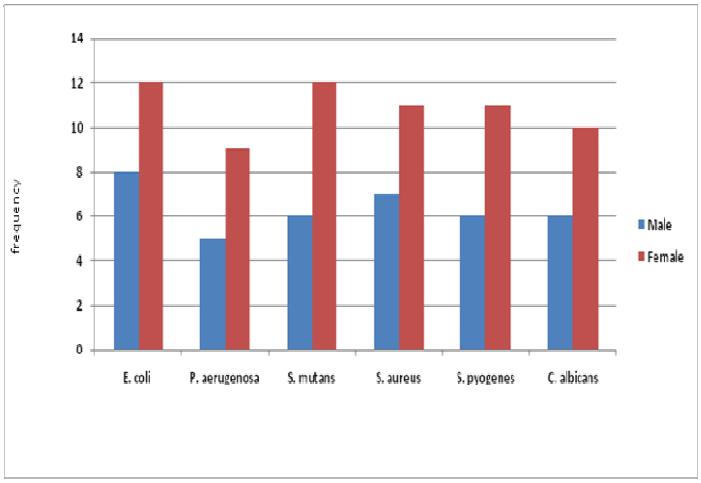

Fig. 4 Bar chart showing the sex distribution of the patients with dental pathogens.

Table 3. Age distribution of patients with dental pathogens.

Organisms	**16-20yrs**	**21-25yrs**	**26-30yrs**	**31-35yrs**	**36-40yrs**
E. coli	5	6	4	3	2
P. aeruginosa	3	4	4	3	-
S. mutans	5	6	4	2	1
S. aureus	4	6	4	2	2
S. pyogenes	4	7	4	-	2
C. albicans	4	5	5	1	1

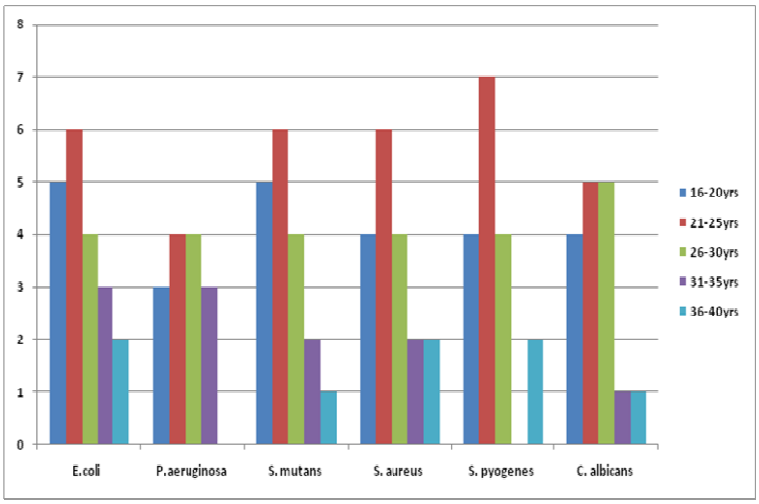

Fig. 5. Bar chart showing the Age distribution of the patients with dental pathogens.

Table 4: Zone of inhibition in mm of various concentration of the ethanol extract of T. glaucescens stem on test organisms.

Test organisms	Concentration of extract(mg/ml)						Sterile distilled water
	3.13	6.25	12.5	25 50	100		
E. coli	0.0	0.0	0.0	9.0	16.0	20.0	No inhibition
P. aeruginosa	0.0	0.0	0.0	10.5	14.5	22.0	No inhibition
S. mutans	0.0	0.0	7.5	9.0	15.0	18.0	No inhibition
S. aureus	0.0	5.0	8.5	10.0	14.5	17.5	No inhibition
S. pyogenes	0.0	4.5	7.5	10.5	11.5	17.0	No inhibition
C. albicans	0.0	0.0	0.0	0.0	5.5	18.5	No inhibition

Table 5: Zone of inhibition in mm of various concentration of the ethanol extract of Z. zanthoxyloides stem on test organisms.

Test organisms	Concentration of extract(mg/ml)						Sterile distilled water
	3.13	6.25	12.5	25 50	100		
E. coli	0.0	0.0	0.0	0.0	0.0	0.00	No inhibition
P. aeruginosa	0.0	0.0	8.0	16.5	20.0	27.5	No inhibition
S. mutans	0.0	5.0	9.0	15.0	20.5	26.0	No inhibition
S. aureus	0.0	4.5	8.0	13.0	19.0	25.0	No inhibition
S. pyogenes	0.0	5.0	9.0	15.0	19.0	22.0	No inhibition
C. albicans	0.0	10.0	11.0	12.5	13.0	16.5	No inhibition

Table 6. Minimum Inhibitory Concentrations (MICs) in mg/ml of the ethanol extracts T. glaucescens and Z. zanthoxyloides plant against the isolated organisms.

ISOLATED ORGANISM	*T. glaucescens*	*Z. zanthoxyloides*
E. coli	25	-
P. aerugenosa	25	12.5
S. mutans	12.5	6.25
S. aureus	6.25	6.25
S. pyogenes	6.25	6.25
C. albicans	50	6.25

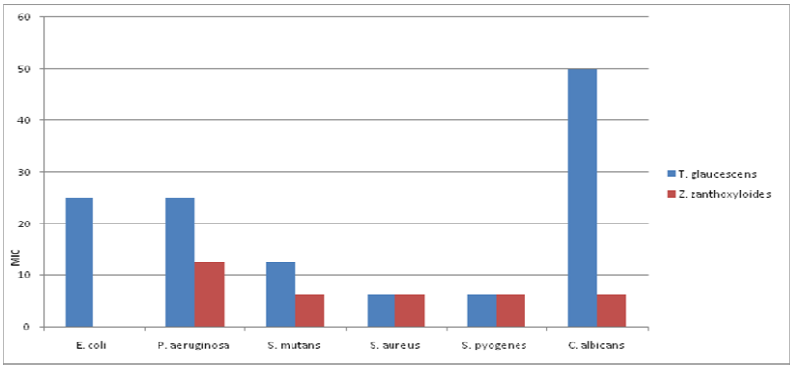

Fig 6. Bar chart showing the Minimum Inhibitory Concentrations (MICs) in mg/ml of the ethanol extracts T. glaucescens and Z. zanthoxyloides plant against the isolated organisms.

Table 7. Minimum Bactericidal Concentrations (MBCs)/Minimum Fungicidal Concentrations (MFC) in mg/ml of the ethanol extracts of T. glaucescens and Z. zanthoxyloides plants against the isolated organisms.

ISOLATED ORGANISM	*T. glaucescens*	*Z. zanthoxyloides*
E. coli	25	-
P. aerugenosa	25	12.5
S. mutans	25	6.25
S. aureus	12.5	6.25
S. pyogenes	6.25	12.5
C. albicans	50	6.25

Table 8: Zone of inhibition of Conventional Antibiotic in mm.(Positive control)

Test Isolates	Ciprofloxacin	Genticin	Ampiclox	Septrin	Ketoconazole
E.coli	-	-	-	-	not applicable
P. aeruginosa	21	18	-	-	not applicable
S. mutans	16	14	20	-	not applicable
S. aureus	25.5	27	7.5	-	not applicable
S. pyogenes	20	19	7.0	-	not applicable
C. albicans	not applicable	not applicable	not applicable	not applicable	22
N.B: - = No Inhibition.					

Discussion

The microorganisms isolated from the teeth of patients with clinical features of dental carries were: *Escherichia coli, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus pyogenes, Staphylococcus aureus and Candida albicans.* These organisms were characterised as shown in table 1 and it agrees with the predominant oral pathogens as isolated by Barry and Thornsberry (1991).

However, in the case of dental caries *Streptococcus mutans* and *Candida albicans* are the microorganisms that have been commonly implicated.

The sex distribution of dental caries is demonstrated by table 2 and it was observed that of the 20 specimens evaluated, 8 were from male and 12 from female patients. *Streptococcus mutans* and *Candida albicans* were predominantly more common in females than males. This statistics may be related to the gender differences in eating habits. Females consume more chocolates, sweets, ice creams, cake icing etc than males and may be therefore more prone to caries.

Table 3 showed the age distribution of dental caries. It was observed that *Streptococcus mutans* and *Candida albicans* which are common dental caries pathogens were predominately found in the younger age group i.e. 16-30 years. This may also be reflective of the eating habits of young people in terms of the consumption of earlier listed carries causing snacks. There is also possibility of use of chewing sticks with good antimicrobial activity by the older age group. *E. coli* which is one of the most common oral flora was seen in all the specimens irrespective of sex or age.

The antimicrobial activity of extracts of the stem of *T. glaucescens* was significant and varied with concentration on each of the isolated organism

(table 4).*S. pyogenes* and *S. aureus* were particularly sensitive to *T. glaucescens* even at a low concentration of 6.25mg/ml. Other isolates inhibited by

T. glaucescens in descending order included: *S. mutans, P. aeruginosa, E. coli* and *C. albicans.*

The extract of *Z. zanthoxyloides* also showed good antimicrobial activities against *C. albicans, S. mutans, S. pyogenes, S. aureus* as well *P. aeruginosa* in that order (Table.5).The implication of this data is that *C. albicans* and *S. mutans* which are implicated in dental caries were significantly inhibited by *Z. zanthoxyloides* even at fairly low concentrations. This agrees with the study done by (Akande and Hayashi 1998)

Table 6. Showed the Minimum Inhibitory Concentrations (MICs) Concentrations (MFCs) values of *T. glaucescens* and *Z. zanthoxyloides* extract.

For *T. glaucescens*, it was observed that *S. aureus* and *S. pyogenes* also had the lowest MIC of 6.25mg/ml. *S. mutans* and *C. albicans* which are the main causative pathogens implicated in dental caries showed a MIC of 12.5mg/ml and 50mg/dl respectively. This study agreed with a similar study done by (Ogundiya *et al*., 2008)

For *Z. zanthoxyloides,* the degree of antimicrobial activity is also shown by a low MIC of 6.25mg/ml for *S. mutans* and *C. albicans* which are responsible for dental caries. It was however observed that *Z. zanthoxyloides* extract did not show any appreciable antimicrobial activity against *E. coli* which is a common oral pathogen, though not implicated in dental caries.

The comparison also further showed that the extract of *Z. zanthoxyloides* had lower MICs against almost all the pathogens when compared to *T. glaucescens.* This implies that *Z. zanthxyloides* has a higher potency than *T. glaucescens* against dental caries pathogens- *S. mutans* and *C. albicans.*

The tables also showed that sterile water which was used as negative control had no inhibition of the isolated pathogens.

Table 7. showed the Minimum Bactericidal /Fungicidal Concentrations MBC/MFC of the extract of *T. glaucescens* and *Z. zanthoxyloides.* For *Z. zanthoxyloides,* this indicated a low concentration of 6.25mg/ml was required for bactericidal and fungicidal effect on *S. mutans* and *C. albicans* respectively. For *T. glaucescens* higher concentrations of 25mg/ml and 50mg/ml was required for bactericidal and fungicidal effect on *S. mutans* and *C. albicans* respectively. This obviously makes *Z. zanthoxyloides* more potent.

Table 8. demonstrated varying degree of inhibition of the isolated bacteria by conventional antibiotics which was used as positive control. All the bacteria were found to be resistant to septrin. Ketoconazole was used and seen to inhibit *C. albicans.*

Conclusion

This study has proved the *T. glaucescens and Z. zanthoxyloides* extracts possess very strong potency in the treatment of various dental infections.

This is illustrated by the capability of their extracts to inhibit the growth of *Escherichia coli, Pseudomonas aeruginosa, Streptococcus mutans, Streptococcus pyogenes, Staphylococcus aureus and Candida albicans* which are the common oral pathogens. It can also be inferred that these dental pathogens are more prevalent in females and the younger age group which should therefore be the targets for education on healthier eating habits and oral hygiene.

S. mutans and *C. albicans* which are the main organisms directly involved in dental caries were significantly inhibited by the two extracts even at fairly low concentrations of 6.25mg/ml for *Z. zanthoxyloides*.

From the lower MICs of *Z. zanthoxyloides* compared to that of *T. glaucescens*, it was observed that the former is generally more potent against dental pathogens implicated in dental caries.

It is therefore concluded that since *T. glaucescens* and *Z. zanthoxyloides* extracts has inhibitory effects against the growth of oral pathogens implicated in dental caries, it is an indication that the plants can be used as a source for antimicrobial agent in the formulation of toothpaste and mouth wash. Therefore the use of their stem locally as chewing stick is justified.

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