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RESEARCH ARTICLE

In-vitro Screening of Some Selected Nigerian Medicinal Plants (*Fagara zanthoxyloides, Vernonia amygdalina, Prosopis africana and Azadirachta indica*) for Antibacterial activity

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ABSTRACT
Fagara zanthoxyloides, Vernonia amygdalina, Prosopis africana and Azadirachta indica are some of the medicinal plants used as chewing sticks in Africa.. The antimicrobial activities of the extracts of these plants in the treatment of human oral diseases were investigated in five treatments. These include the hot water extract, cold water extract, hot ethanol extract and essential oils. Clinical samples were collected from a dental clinic at Ijebu – Ode General Hospital, Ogun State. These were swabbed and plated directly on McConkey agar, blood agar and chocolate agar. The organisms implicated were Streptococcus viridians, Staphylococcus albus, Pseudomonas aeruginosa, Klebsiella pneumoniae and Proteus vulgaris. It is concluded in this study that the various extracts of these plants have strong antimicrobial efficacies against human oral pathogens in varying degrees. The essential oils of these plants have proven to be most efficacious. The active ingredient obtained from these plants could be modernized and used conveniently by both rural and urban communities for the treatment of oral infections.
KEYWORDS: Chewing sticks, extracts, caries, oral pathogens, tooth decay, hygiene.

INTRODUCTION

Good oral hygiene is necessary for healthy teeth, gum and fresh breath. A number of methods are used in oral hygiene to prevent and cure oral diseases. It is pertinent to look at the role plants play in oral hygiene as a number of them have medicinal properties [1]. In many African homes, teeth are cleaned in the morning by chewing the root or slim stem of certain plants until they acquire brush-like ends [2].

The Babylonians recorded the use of chewing sticks in 7000 BC and its use ultimately spread throughout the Greek and Roman Empires 3. It is also used by Egyptians, Jews and in the Islamic empires. It is believed that the counterpart of the modern day toothbrush was unknown in Europe until about 300 years ago. Presently, chewing sticks are used in Africa, South America, the Middle East and Asia. Chewing stick has different names depending on different societies. For instance, *Miswak, Siwak* or *Arak* is used in Middle East, *Miswaki* in Tanzania, *Datan* in India and Pakistan [3]. A number of plants are used as chewing sticks in West Africa these include: Neem (*Azadirachta indica*), *Zanthoxylum zanthoxyloides* waterman (*Fagara zanthoxyloides* lam) root and *Vernonia amygdalina* (Deli root). Others include the lime tree (*Citrus aurantifolia*) and the orange tree citrus (*Citrus sinensis*) sometimes provides chewing sticks. The roots of senna (*Cassia vinnea*) have been used by American Negroes and those of African Laburnum (*Cassia sieberiana*) and those of Sierra Leone. Neem (*Azadirachta indica*) has also been widely used as chewing sticks in the Indian sub-continent; these are just few of the plants used as chewing sticks [4].

Chewing sticks impact varying taste sensations, a tingling, peppery taste and numbness is provided by *Zanthoxylum zanthoxyloides* waterman (*Fagara zanthoxyloides* lam) root, a strong bitter taste and frothing from *Masularia accuminata* (G. Don*)* Bullox ex Hoyle stem and initial bitterness becoming sweet later from *Vernonia amygdalina* (Deli root). The most popular chewing sticks are ones having good flavour and texture and a recognized effect on teeth and supporting tissues. Freshly cut

specimens are always desirable because they are more easily chewed into a brush. Some of them possess such tough fibers that penetrate the gum during use thus causing some discomfort [5].
In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms have been developed due to the indiscriminate use of commercial antibacterial drugs/chemical commonly used in the treatment of infectious diseases [6]. This situation forced scientists to the search for new antibacterial substance from various sources like medicinal plants [7].
For years, Man has used various parts of plants in the treatment and prevention of various ailments [8]. Plant based natural constituents can be derived from barks leaves, flowers, roots, fruits, seeds of plants [9] which in most cases contain active components 10. Since the use of certain plants as traditional chewing sticks has been neglected and, which could be attributed to the failure of researchers to establish concrete facts as regards the antimicrobial efficacies of these plants to combat dental diseases, it becomes necessary to investigate which plants could be recommended for this purpose. This study presents *Fagara zanthoxyloides, Azadirachta indica, Vernonia amygdalina* and *Prosopis africana* with their chemotherapeutic properties in order to establish their antimicrobial efficacies against human oral pathogens.

MATERIALS AND METHODS

The experiment involved four types of chewing sticks; five categories of extracts and 32 isolates obtained directly from infected teeth.

Collection of plants: *Azadarachta indica* (Dongoyaro), *Fagara zanthoxyloides* (Orin ata), *Proposis Africana* (Pako ayan) and *Vernonia amygdalina* (Ewuro) barks were collected from cultivated sources in Ago-Iwoye and Ijebu-Igbo communities, South-Western Nigeria. These plants were identified in the department of Plant and Applied Zoology of Olabisi Onabanjo University and Voucher specimen deposited at the Elkaf Herbarium of the same University. The four different chewing stick samples were investigated for their antibacterial effects.

Processing and Extraction of Plant Material

The plant parts were cut into small pieces and shade-dried at room temperature for two weeks. The parts were then ground into fine powder so as to enable ease of extraction of the active compound. The ground powder were weighed and soaked in predetermined volume of solvent. As listed below:

Hot ethanol extract: One gram of each species was extracted on a sorxhelet apparatus using ethanol as solvent. The concentrated extract was recovered and stored in sterile McCartney bottles until ready for use.

Cold ethanol extract*:* Two grams of each species was poured into McCartney bottles and filled with 20 ml of ethanol. The bottles were placed on a shaker for about 4 h. The extracts were centrifuged at 2000 rpm for 20 minutes. The supernatants were decanted, labeled and stored in the refrigerator.

Hot water extract*:* Two grams of each species was poured into sterile McCartney bottles, filled with 20 mls of sterile distilled water and placed in a water bath at 100^0C for 3 hours. The bottles were then cooled and the extracts were centrifuged at 2000 rpm for 20 mins. The supernatants were decanted, labeled and stored in the refrigerator.

Cold water extract: Two grams of each species was poured into McCartney bottles, filled with 20 ml of distilled water and left to soak overnight. The supernatants were centrifuged at 2000 rpm for 20mins. The supernatants were decanted, labeled and stored in the refrigerator.

Extraction of the volatile oils: The volatile oil of each plant was extracted by distillation method using Clavenger-type apparatus for 4 hours. After collection, the volatile oils were measured to determine their yields and each was kept in a refrigerator maintained at 4°C until needed.

Isolation of bacteria:

Ethical clearance was sought and granted from the authorities of dental clinic at Ijebu-Ode General Hospital, Ogun State. The test organisms were isolated directly from infected teeth of twenty (20) patients comprising of males and females. Subjects were known to have had history of dental ailments. The infected areas of the teeth were swabbed three times with sterile cotton wool to remove debris and saliva, and was used to inoculate three different media namely blood agar, chocolate agar and MacConkey agar plates. The plates were aerobically incubated at 37°C for 24-48 hour for the organisms to grow. Characteristics colonies were picked from the plates and purified by repeated sub-culturing. Pure colonies were streaked on nutrient agar slopes in McCartney bottles incubated at 37^0C for 24 h. These slants were used as stock cultures and were stored in the refrigerator.

Characterization of bacterial isolates: The bacterial colonies were differentiated first on basis of colonial morphology followed by microscopic examination after Gram staining [11]. Pure cultures of bacterial isolates were characterized and identified on the basis of their biochemical properties and by reference to Bergey's Manual of Determinative Bacteriology [12, 13].

RESULTS AND DISCUSSION

The morphological and biochemical characteristics of the isolated bacteria showed that the isolates from decay teeth belong to five genera namely *Streptococcus, Staphylococcus, Pseudomonas, Klebsiella* and *Proteus.* At the end of the extraction period, 7.4ml of the volatile oil was obtained from 4.20 kg of the barks of *Azadirachta indica* (Dongoyaro) and *Fagara zanthoxyloides* (Orin ata) while 10 ml of volatile oil was obtained from 1.78 kg of *Prosopis africana* (Pako ayan) and *Vernonia amygdalina* (Ewuro).

Table 1: Percentage frequency of bacterial isolates with associated dental diseases

Bacterial species	Dental diseases	Occurrence of the Bacteria	% occurrence of the bacteria
Streptococcus viridans	Dental caries, periodontitis and gingivitis	17	53.13%
Staphylococcus albus	dental caries and aveola abscess	8	25%
Proteus vulgaris	dental caries	2	6.25%
Klebsiella pneumonia	dental caries	3	9.34%
Pseudomonas aeruginosa	dental caries	2	6.25%

Table 1 shows the percentage frequency of the bacterial isolates with associated dental diseases. It is noticeable that *Streptococcus viridans* is a common organism associated with the four dentals diseases investigated in this study, thus it has the highest percentage frequency of 53.13%. This is followed by *Staphylococcus albus* (25%), *Klebsiella pneumoniae* (9.34%). However, *Proteus vulgaris* and *Pseudomonas aeruginosa* showed the same percentage frequency of 6.25% indicating that they are seldom associated with dental diseases (Table 1).

Table 2: Inhibitory properties of *Azidarachta indica* and *Fagara zanthoxyloides* extracts on isolates

Isolates	Diameter of Inhibition zone (mm)± SD									
	Azidarachta indica (100% concentration)				*Fagara zanthoxyloides* (100% concentration)				D. M. S. O.	Sodium Fluoride
	HW	CW	HE	CE	HW	CW	HE	CE		
Streptococcus viridans	16.0 ± 1.0	17.0 ± 2.0	27.0 ± 2.0	28.0 ± 2.0	22.0 ± 2.0	17.0 ± 2.0	28.0 ± 2.0	28.0 ± 2.0	6.0±0.0	30.5 ± 2.0
Staphylococcus albus	11.0 ± 1.0	17.5 ± 2.0	27.0 ± 2.0	24.5 ± 2.0	22.5 ± 2.0	12.5 ± 1.0	27.0 ± 2.0	28.0 ± 2.0	6.0±0.0	27.0 ± 2.0
Pseudomonas aeruginosa	14.0 ± 2.0	13.0 ± 1.0	21.0 ± 2.0	6.0±0.0	26.0 ± 2.0	23.0 ± 2.0	28.0 ± 2.0	27.5 ± 3.0	6.0±0.0	26.5 ± 2.0
Klebsiella pneumoniae	15.0 ± 2.0	12.0 ± 1.0	19.0 ± 2.0	28.0 ± 2.0	28.0 ± 2.0	22.5 ± 2.0	27.5 ± 2.0	28.0 ± 2.0	6.0±0.0	28.5 ± 2.0
Proteus vulgaris	13.0 ± 2.0	11.0 ± 1.0	23.5 ± 2.0	26.0 ± 2.0	28.0 ± 2.0	25.0 ± 3.0	28.0 ± 2.0	27.0 ± 2.0	6.0±0.0	29.0 ± 2.0

Key:
HW = hot water, CW = cold water, HE = hot ethanol, CE = cold ethanol,
SD =Standard Deviation,D.M.S.O = Dimethylsulfoxide

The hot and cold water extracts of *Azadirachta indica* recorded low inhibitory activity against most of the bacterial isolates. However, the ethanol extracts (hot and cold) also recorded good inhibitory activity against the tested bacterial isolates except *Pseudomonas aeruginosa* which was not inhibited by the cold ethanol extract of *Azadirachta indica* (table 2). The hot water extract of *Fagara zanthoxyloides* has higher inhibitory effect than cold water extract on the isolates. It is, however, noticeable that hot and cold ethanol extracts have the most effective inhibitory effects on the bacterial isolates (Table 2).

Table 3: Inhibitory properties of *Prosopis africana* and *Vernonia amygdalina* extracts on isolates

Isolates	Diameter of Inhibition zone (mm)± SD									
	Prosopis africana (100% concentration)				*Vernonia amygdalina* (100% concentration)				D. M. S. O.	Sodium Fluoride
	HW	CW	HE	CE	HW	CW	HE	CE		
Streptococcus viridans	6.0±0.0	6.0±0.0	26.0 ± 3.0	16.0 ± 2.0	24.0 ± 2.0	7.5 ± 1.0	24.0 ± 3.0	16.5 ± 2.0	6.0±0.0	30.5 ± 2.0
Staphylococcus albus	6.0±0.0	13.5 ± 2.0	23.0 ± 2.0	21.0 ± 2.0	14.5 ± 2.0	9.0 ± 2.0	24.0 ± 2.0	22.0 ± 2.0	6.0±0.0	27.0 ± 2.0
Pseudomonas aeruginosa	16.5 ± 3.0	17.0 ± 2.0	24.5 ± 1.0	22.0 ± 3.0	19.5 ± 2.0	8.5 ± 2.0	23.5 ± 2.0	23.0 ± 1.0	6.0±0.0	26.5 ± 2.0
Klebsiella pneumoniae	17.5 ± 2.0	16.0 ± 2.0	13.5 ± 2.0	25.0 ± 2.0	22.0 ± 2.0	9.5 ± 2.0	25.0 ± 2.0	23.0 ± 2.0	6.0±0.0	28.5 ± 2.0
Proteus vulgaris	22.0 ± 2.0	16.5 ± 2.0	26.5 ± 2.0	25.5 ± 2.0	23.0 ± 3.0	20.0 ± 2.0	23.5 ± 2.0	22.0 ± 3.0	6.0±0.0	29.0 ± 2.0

Keys: HW = hot water; CW = cold water; HE = hot ethanol; CE = cold ethanol,
SD =Standard Deviation, D.M.S.O = Dimethylsulfoxide

The hot and cold water extracts of *Prosopis africana* showed little activity against *Streptococcus viridians* while hot and cold ethanol extracts showed stronger and better inhibitory activity against the bacteria tested in this study, This observation is at variance with the reports of [14] , who reported good activity for both the aqueous (hot and cold) and ethanol extracts of *Prosopis africana* against *Streptococcus mutans*. The obvious disparity between these reports could be as a results of the different strains of *Streptococcus* sp tested, the part and the age of the plants used. Except for the inhibitory activity shown against *Proteus vulgaris* (20.0 ± 2.0), the cold water extract of *Vernonia amygdalina* did not show any appreciable inhibitory property against any of the tested bacteria (Table 3). This is in agreement with study of [15], who reported a narrow spectrum of activity for *Vernonia amygdalina.* In their report, it was stated that the plant lack efficacy against *Pseudomonas aeruginosa* and *E. coli.* However, we have discovered that most of the bacteria tested, in these study showed susceptibility against the hot ethanol extract of *Vernonia amygdalina* when compared to others (Table 3).

Table 4: Susceptibility of bacterial isolates to the effective concentrations (%) of the essential oils *Azidarachta indica* and *Fagara zanthoxyloides* species

Isolates	Diameter of Inhibition zone (mm)± SD											
	Azidarachta indica (% concentration)					*Fagara zanthoxyloides* (% concentration)					D. M. S. O.	Sodium Fluoride
	0.5%	1.0%	2.0%	5.0%	10.0%	0.5%	1.0%	2.0%	5.0%	10.0%		
Streptococcus viridans	14.0± 2.0	16.0± 2.0	22.0 ± 2.0	23.5± 2.0	25.0 ± 2.0	13.5 ± 2.0	18.0 ± 2.0	23.0 ± 2.0	26.0 ± 1.0	25.0 ± 2.0	6.0±0.0	30.5 ± 2.0
Staphylococcus albus	13.0± 2.0	15.5 ± 2.0	25.5± 2.0	22.0 ± 2.0	24.0 ± 3.0	16.0 ± 2.0	17.0 ± 2.0	20.0 ± 1.0	25.0 ± 3.0	24.5 ± 3.0	6.0±0.0	27.0 ± 2.0
Pseudomonas aeruginosa	26.0± 3.0	22.0 ± 2.0	25.0± 2.0	22.5 ± 2.0	23.0 ± 2.0	19.0 ± 2.0	21.0 ± 2.0	23.0 ± 2.0	25.0 ± 2.0	25.0 ± 2.0	6.0±0.0	26.5 ± 2.0
Klebsiella pneumoniae	14.0± 2.0	14.5.0 ± 2.0	22.5 ± 2.0	22.5 ± 2.0	22.5 ± 2.0	27.0 ± 1.0	27.5 ± 2.0	22.0 ± 2.0	25.0 ± 2.0	25.0 ± 2.0	6.0±0.0	28.5 ± 2.0
Proteus vulgaris	24.0± 2.0	24.5 ± 2.0	23.0 ± 2.0	23.0 ± 2.0	23330 ± 3.0	27.5 ± 2.0	27.5± 2.0	22.0 ± 1.0	24.5 ± 2.0	24.0 ± 3.0	6.0±0.0	29.0 ± 2.0

Keys:SD =Standard Deviation, D.M.S.O = Dimethylsulfoxide

Table 4 shows the susceptibility of the bacterial isolates to the effective concentrations (%) of the essential oils of *Azadirachta indica* and *Fagara zanthoxyloides* species. At lower concentrations of the essential oils of *Azidirachta indica* i.e between 0.5 to 1.0% concentrations, *Streptococcus viridans*, *Staphylococcus albus* and *Klebsiella pneumoniae* showed some degrees of resistance as indicated by their notable smaller zones of inhibition, while at higher concentrations (between 2.0% to 10.0%), the isolates showed some high level of sensitivity comparable with the standard. The essential oils of *Fagara zanthoxyloides* were able to inhibit the growth of *Klebsiella pneumoniae* and *Proteus vulgaris* even at the minimal concentration of 0.5%.

Table 7: Susceptibility of bacterial isolates to the effective concentrations (%) of the essential oils of *Prosopis africana* and *Vernonia amygdalina* species

Isolates	Diameter of Inhibition zone (mm)± SD											
	Prosopis africana (% concentration)					*Vernonia amygdalina* (% concentration)					D. M. S. O.	Sodium Fluoride
	0.5%	1.0%	2.0%	5.0%	10.0%	0.5%	1.0%	2.0%	5.0%	10.0%		
Streptococcus viridans	23.0 ± 2.0	22.0 ± 2.0	24.5 ± 3.0	26.0 ± 2.0	28.0 ± 2.0	20.0 ± 1.0	22.0 ± 3.0	22.0 ± 3.0	28.5 ± 2.0	27.0± 2.0	6.0±0.0	30.5 ± 2.0
Staphylococcus albus	21.0 ±1.0	22.0 ± 2.0	20.0 ± 2.0	25.0 ± 3.0	25.5 ± 2.0	21.0 ± 2.0	22.0 ± 1.0	23.0 ± 2.0	27.0 ± 3.0	26.0 ± 1.0	6.0±0.0	27.0 ± 2.0
Pseudomonas aeruginosa	21.5 ± 2.0	22.0 ± 2.0	20.0 ± 3.0	26.0 ± 2.0	27.0 ± 2.0	21.0 ± 1.0	22.0 ± 1.0	24.0 ± 3.0	27.0 ± 1.0	27.5 ± 1.0	6.0±0.0	26.5 ± 2.0
Klebsiella pneumoniae	23.0 ± 1.0	23.0 ± 2.0	23.0 ± 2.0	26.0 ± 2.0	26.0 ± 1.0	22.0 ± 1.0	21.0 ± 2.0	22.0 ± 2.0	27.0 ± 1.0	28.0 ± 2.0	6.0±0.0	28.5 ± 2.0
Proteus vulgaris	24.0 ± 2.0	24.0 ± 2.0	23.0 ± 3.0	28.0 ± 2.0	23.0 ± 2.0	23.0 ± 2.0	20.5.0 ± 2.0	22.0 ± 3.0	27.0 ± 2.0	28.0 ± 2.0	6.0±0.0	29.0 ± 2.0

Keys: SD =Standard Deviation, D.M.S.O = Dimethylsulfoxide

Table 7 shows the susceptibility of bacterial isolate to the effective concentrations (%) of the essential oils of *Prosopis africana* and *Vernonia amygdalina* species. It is interesting to see that, at all percentage concentrations of the essential oil of *Prosopis africana* and *Vernonia amygdalina*, the bacterial isolates were all susceptible as shown by clear inhibition zones in the plates.

It is concluded in this study that *Prosopis africana* and *Vernonia amygdalina*, extracts possess very strong efficacies in the treatment of various dental infections as described by the capability of their extracts to inhibit the growth of *Streptococcus viridians, Staphylococous albus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus vulgaris* which are the organisms directly associated with the establishment of dental infections. This is in agreement with the finding of [16] , who revealed that in addition to the mechanical cleansing action of the essential oils and aqueous extracts of chewing sticks, they are also potent against bacterial colonization and plaque formation, thereby inhibiting caries formation, stimulate oral tissues and control bacterial growth in the oral cavity. It is, however, worthy of note that the essential oils of these two plants (*Prosopis africana* and *Vernonia amygdalina*) possess outstanding efficacy in combating oral pathogens and, thus carry a good template for the large scale production of the new generation synthetic toothpastes. It is also possible to conclude that the essential oils of these plants possess stronger efficacy than other extracts whose antimicrobial activities were investigated in this study.

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