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AQUEOUS METHANOL EXTRACT OF THE ROOT BARK OF *ZANTHOXYLUM ZANTHOXYLOIDES* PROVIDES NATURAL REMEDY FOR DENTAL CARIES AND TOOTHACHE

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ABSTRACT

Zanthoxylum zanthoxyloides has a wide ethnopharmacological relevance and has been used for curing stomachache, tooth-ache, mouth fresh, coughs, urinary and veneral diseases, leprous ulcerations, rheumatism and lumbago. The extracts of the plant were tested against clinical four isolates: *Streptococcus mutans, Lactobacillus spp., Staphylococcus aureus and Sarcina lutea*. Toxicity test was carried out using the Lorke's method. Antimicrobial assay was done using Agar-diffusion method. The Norciceptive evaluation was carried out using the acetic acid-induced and hot plate methods. The aqueous methanol extract of the root bark of *Z. zanthoxyloides* exhibited significant activity ($p<0.005$) against *Streptococcus mutans, Lactobacillus spp., Sarcina lutea, Candida albican* and *Aspergellus niger* but was resistant to *Staph aureus.* It also demonstrated excellent norciceptive effect. The LD_{50} was found to be > 5000 mg/kg. The aqueous methanol extract of *Z. zanthoxyloides* has demonstrated an excellent antimicrobial activity against key microorganisms implicated in dental caries and oral thrush. It also has significant norciceptive activity. This study has clearly shown why this plant has been used for treatment of dental caries, toothache and as a mouthwash. The

antibacterial and the analgesic properties play synergistic role in alleviating dental caries and toothache.

KEYWORDS: *Zanthoxylum zanthoxyloides,* dental caries, toothache, *Streptococcus mutans, Lactobacillus spp,* natural remedy.

INTRODUCTION

Oral diseases like dental caries, periodontitis, oral tissue lesions, infections and oral cavity cancers are major health problems worldwide.[1] Dental caries is a term used by healthcare and dental professionals to describe the disease more commonly known as tooth decay. Dental caries (tooth decay) results from a progressive destruction of tooth enamel by bacteria and bacterial products within the oral environment. There is the accompanying pains (toothache) as a result of bacterial activity in the pulp of a carious tooth. Dental caries is the single most common chronic disease of childhood.[2]

Despite advancements in oral disease science, dental caries continues to be a worldwide health concern, affecting humans of all ages, especially children where caries disease is on the rise. Globally, children miss 51 million hours of school each year because of dental caries.[3] Its negative effects on human population are far reaching. These range from loss of teeth to serious health challenges. Globally, about 30% of people aged 65–74 have no natural teeth.[4] Only 41% of Europeans still have all their natural teeth.[5] Researchers have found that people with periodontal disease are almost twice as likely to suffer from coronary artery disease as those without periodontal disease.[6] Generally, the quality of life of the sufferers are greatly reduced due to the debilitating pains associated with dental caries.

Bacteria have been implicated in dental caries. The two most notable ones are *Streptococci mutans* and *Lactobacilli*. The most virulent of these species is Streptococcus mutans, which has been found to be the initiator of most dental caries and which is a transmissible bacterium that can be transmitted both horizontally and vertically.[7,8,9] *Streptococci mutans* produces an enzyme dextran-sucrase, which converts the sucrose of food to dextrin, and dextrin combines with salivary proteins to create a sticky, colorless film (plaque) on tooth surfaces. Plaque provides the haven for the activities of *Lactobacilli* and these produce of lactic acid, which attacks the enamel by decalcifying it.[10] While *Streptococcus mutans* have been strongly associated with the initiation of dental caries, studies show that *lactobacilli* may be more significant in the progression of caries lesions and have found to be responsible for the

initiation of a low percentage of coronal caries.[11] *Streptococci mutans* and *lactobacilli* are strong acid producers and hence cause an acidic environment, creating the risk for cavities.[12] Several factors, such as adherence to enamel surfaces, production of acidic metabolites, the capacity to build up glycogen reserves and the ability to synthesize extracellular polysaccharides are present in dental caries.[13] Simple sugars like sucrose, fructose, lactose, galactose, and glucose foster colonization and growth of bacteria linked to caries, particularly *Streptococci mutans*.[14]

There are overwhelming evidences of the influences and the dependence on modern medicine and tremendous advances in synthetic drugs. However, large segments of the world population depend on drugs from plants.[15] Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs, and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plants as sources of medicines for a wide variety of human ailments.[16, 17, 18, 19] According to World Health Organization (WHO), over three-quarters of the world population rely on plants and their extracts for healthcare needs.[20] Hollist (2004) reported that about 10 different oral/dental conditions are treatable with plants in traditional health practice, namely, toothache/decay, gingivitis, ulcerative gingivitis, angular stomatitis, mouth ulcers, swollen tonsil, oral thrush, tonsillitis, and black tongue.[21]

Traditionally, the roots of *Zanthoxylum zanthoxyloides* are externally applied to ulcers, swellings, haemorrhoids, abscesses, snake bites, yaws, wounds leprosy and syphilitic sores as well as rheumatic and arthritic pain and hernia.[22] The roots and stem bark (commonly used as chew-sticks) give a warm, pungent and benumbing effect on the palate when chewed, and are widely used in the treatment of sore gums, toothache and dental caries. A decoction of the roots is used as a mouthwash and against a sore throat. The roots bark and leaves of many species are used in various medicinal preparations for curing stomachache, tooth-ache, coughs, urinary and veneral diseases, leprous ulcerations, rheumatism and lumbago.[23] The fruits are used as digestive appetizer, to cure asthma and bronchitis, eliminate pain, and use to treat heart diseases, piles, diseases of mouth, teeth and throat disorder, also prescribed in dyspepsia and diarrhea.[24] *Zanthoxylum* has been studied for several types of biological activities such as larvicidal, anti-inflammatory, analgesic, antinociceptive, antioxidant, antibiotic, hepatoprotective, antiplasmodial, cytotoxic, antiproliferative, anthelminthic, antiviral, anticonvulsant and antifungal.[25,26,27,28,29,30,31,32,33,34,35,37,38,39,40] In Nigeria,

Zanthoxylum zanthoxyloides plant is located in the south east, south west and northern part of Nigeria.

The current study aimed at evaluating the antimicrobial activity of the aqueous methanol extract of the root bark of Zanthoxylum *zanthoxyloides* against the clinical isolates of bacteria implicated in dental caries and the analgesic effect of the plant.

MATERIALS AND METHODS

Ethics statement

The ethical clearance to use rats was in accordance with the University of Nigeria Ethics Committee on the use of laboratory animals, registered by the National Health Research Ethics Committee (NHREC) of Nigeria, with the number; NHREC/05/01/2008B.

Housing and handling of the animals

The animals were kept in a controlled environment inside a cage and had free access to food and tap water throughout the study. All experiments were conducted in accordance with the guidelines by ethics committee for animal welfare.

Plant material

The root bark (Fig. 3) of the plant *Zanthoxylum zanthoxyloides (*common name: Fagara) was gotten from the Uburu forest in Ohaozara Local Government Area of Ebonyi State Nigeria. Figures 1, 2 and 3 showed the leaves, stem and root barks respectively of the plant. These parts were use for identification and authentication by Mr. Onukwu of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka.

Preparation of aqueous methanolic extract

A 600 g of the powered root bark of Fagara was measured into a container; 1 L of n-hexane was poured into the container and left for 12 hours with intermittent shaking. This was decanted to obtain the filtrate. Fresh solvent was introduced into the marc and the above procedure was repeated until exhaustive extraction had occurred within 48 hours. The filtrate was roughly clarified using clean muslin cloth and finally filtered using Whatman filter paper. The solvent was allowed to evaporate and the extract, nHZZ was obtained. The marc was kept for further use.

Using the marc after defatting, 70% methanol was used to extract the aqueous components of the plant following the same method as in defatting process. The solvent was allowed to evaporate and crude extract, AMZZ was obtained.

Microorganisms

An informed consent was sought and gotten from patients with dental caries at Dental Clinic Department of I.C.O.M Hospitals Limited, Trinity Plaza Odenigbo Road Nsukka, Enugu State. The swab was collected from the infected teeth using a cotton wool and transferred into a freshly prepared normal saline. It was cultured in blood nutrient agar medium and incubated for 48 hours at 37 °C. The organisms that grew were further sub-cultured in Mueller Hinton Agar and subsequently isolated and purified. Four main clinical isolates were gotten namely: *Streptococcus mutans, Lactobacillus spp, Staphylococcus aureus and Sarcina lutea*. The fungi, *Candida albican and Aspergillus niger* used for the antifungi study were obtained from Department of Pharmaceutics University of Nigeria Nsukka.

EXPERIMENTAL

Acute toxicity study

Intraperitoneal median lethal dose (LD_{50}) estimation was conducted in mice using the method of Lorke. The LD_{50} of the aqueous methanol extract of *Zanthoxylum zanthoxyloides* (AMZZ) has been determined and reported. The LD_{50} of the n-hexane fraction (nHZZ) was evaluated. Briefly, the method was divided into two phases. In the initial phase, three groups of three mice each were treated with nHZZ at doses of 10, 100, and 1000 mg/kg body weight i.p. and observed for signs of toxicity and death for 24 hours. In the second phase, four groups each containing three mice was injected with four more specific doses of nHZZ. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived.

Antimicrobial study

Twenty suitable McCartney bottle were washed sterilized in the autoclave at 121°C for 15 minutes. The sterile medium that is Mueller Hinton agar was cooled at 45-50 °C and then mixed uniformly with measured volume of a standard suspension of the test organisms in sterile Petri dishes. Each plate is allowed to stand in a horizontal position until the agar solidifies with 3.5mm thickness. This was done separately for all the microorganisms to be tested. Ten Petri dishes were divided into six different segments at the back using marker. Disc of agar were removed from each segment of the agar layer with sterile cork borer of

about 8mm diameter in order to produce wells in each segment of the agar plates. Five different concentrations (100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) of the n-hexane extract of fagara dissolved in DMSO were introduced into five of the wells which were labeled 1-5 while the vehicle DMSO was introduced into the sixth well. A disk of standard antibiotic (Amoxicillin clavulanic acid) was placed at the middle of each plate. This method was also repeated for the aqueous methanol extract using ten Petri dishes containing agar and the seeded microorganism. Then the twenty plates were then incubated at 37 °C for 24 hours. The diameters of the zone of inhibition were carefully measured with a suitable instrument such as caliper.

Nociceptive behavioral tests

Acetic acid-induced writhing test

Swiss albino mice weighing between 15-35g were used for evaluation of analgesic activity. Swiss albino mice of either sex were divided into five different groups each containing five animals, the animals were marked individually. A solution of acetic acid (1% v/v) in distilled water was prepared. Group I was given distilled water (10ml/kg), group II diclofenac (20mg/kg), groups III, IV and V received AMZZ at the doses of 100, 500 1000 mg/kg respectively. After 30 minutes, writhing was induced by intraperitonial injection of 1% acetic acid in volume of 0.1 ml/kg body weight. The writhing episodes were recorded for 10 minutes; stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted. Percentage inhibitions of writhes were calculated for the extract and the standard agent using the formula:

$$\text{Inhibition (\%)} = \frac{\text{mean number of writhes (control)} - \text{mean number of writhes (test)}}{\text{mean number of writhes (control)}} \times 100$$

The result of the experiment was represented in table 2

Hot plate test

Swiss albino mice of either sex weighing between 15-35g were also divided into five different groups each containing five animals, the animals were marked individually.

A 600 ml test beaker was placed on thermostat hot plate (Gallenkamp thermostat). The temperature was regulated to 50 ± 1°C. Each mouse was placed in the beaker (on the hot plate) in order to obtain its response to electrical heat-induced nociceptive pain stimulus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's

response to heat-induced nociceptive pain stimulus. The latency (reaction time) until mice showed first signs of discomfort (hind paw lifting, hind paw licking, or jumping) was recorded, before (baseline), and response was determined at 30, 60, 90 and 120 min after the administration of normal saline, AMZZ (100, 500, and 1000 mg/kg), and diclofenac (20 mg/kg) subcutaneously. The percentage thermal pain stimulus or protection by applying the formula:

$$\% \text{ Protection against thermal stimulus} = \frac{\text{Test mean} - \text{Control mean}}{\text{Control mean}} \times 100$$

Data analysis

Data were analyzed using statistical software SPSS version 20. One-way analysis of variance (ANOVA) test was used to ascertain the significance of variations between the test and control groups. Data are shown as mean ± S.E.M. All data were considered significant at $p < 0.05$.

3. RESULTS

The plant, *Zanthoxylum zanthoxyloides,* showing different parts

Fig. 1: Leaves of *Zanthoxylum zanthoxyloides*

Fig. 2: Stem parts of *Zanthoxylum zanthoxyloides*

Fig. 3: Root parts of *Zanthoxylum zanthoxyloides*

Isolated microorganism

a. *Streptococcus mutan*

b. *Lactobacillus*

c. *Sarcina lutea*

d. *Staph aureus*

Toxicity studies

LD_{50} of nHZZ = 5000 mg/kg body weight

LD_{50} of AMZZ = 5000 mg/kg body weight.[41]

Table 1: Antimicrobial result of the aqueous methanol extract of *Zanthoxylum zanthoxyloides*

	IZD (mm)					
Test organism	***Streptococcus mutan***	***Lactob acillus***	***Sarcina lutea***	***Staph aureus***	***Candida albican***	***Aspergillus niger***
100mg/ml	20	56	32	R	25	26.5
50mg/ml	19	54	30	R	23	25
25mg/ml	18	53	28	R	20	24
12.5mg/ml	15	52	26	R	16	23
6.25mg/ml	13	50	24	R	13	22
Amoxicillin clavulanic acid (10µg)	R	50	R	22	-	-
DMSO	0	0	0	0	0	0

R = Resistant

Table 2: The effect of the aqueous methanol of *Zanthoxylum zanthoxyloides* root bark on acetic acid-induced writhing in mice

Treatment	Dose (mg/kg)	Mean number of writhes ±SEM	Percentage Inhibition
Normal saline	10 mL/kg	55±1.52	0
AMZZ	100	35±0.71	36.36
	500	25±0.63*	54.56*
	1000	20±0.71*	63.64*
Diclofenac	20	21±1.22*	61.82*

n = 5, *p < 0.05.

Table 3: The effect of the aqueous methanol of *Zanthoxylum zanthoxyloides* root bark on hot plate latency in rats

		Reaction time in mins (Mean±SEM)			
Treatment	**Dose (mg/kg)**	**30**	**60**	**90**	**120**
Normal saline	10 mL/kg	4.19±0.17	5.10±0.30	5.22±0.28	5.20±0.18
AMZZ	100	4.49±0.13	6.33±0.15	10.03±0.25*	11.08±0.79*
	500	6.08±0.18	7.51±0.26	9.08±0.30*	12.32±0.25*
	1000	7.10±0.31*	11.44±0.56*	16.51±0.41*	18.32±0.25*
Diclofenac	20	7.34±0.25*	11.09±0.27*	17.46±0.39*	18.52±0.13*

n = 5, *p < 0.05.

DISCUSSION

Toxicity test

The LD_{50} of AMZZ[41] and nHZZ were found to be 5000 mg/kg body weight respectively. This indicates that the root bark of *Zanthoxylum zanthoxyloides* is relatively safe and can be used by humans without any outward effects.

Isolation of microorganisms

Four different microorganisms namely: *Streptococcus mutans, Lactobacillus spp, Staphylococcus aureus and Sarcina lutea* were isolated from the teeth of dental caries patients. Notably, *Streptococcus mutans* and *Lactobacillus spp* have been implicated in dental caries whereas *Staphylococcus aureus and Sarcina lutea* are normal flora but could cause opportunistic infections.

Antimicrobial activity

The antimicrobial result in table 1 revealed that the aqueous methanol extract of *Zanthoxylum zanthoxyloides* **AMZZ** were found to be significantly active against all the tested microorganisms except *Staphylococcus aureus.* The two notable culprits of dental caries and

the associated toothache – *Streptococcus mutans* and *Lactobacillus spp*. AMZZ had significant activity against *Streptococcus mutans* in dose-dependent response when compared to the standard drug – amoxicillin clavulanic acid (10µg), in which the organism has developed resistance. Antibiotic resistance to bacteria is a global concern. AMZZ against *Lactobacillus spp* was significantly activity at all the concentrations tested, and were comparable to the standard drug. At concentration of 100 mg/ml, AMZZ activity against *Lactobacillus spp* was significantly higher than that of standard drug. In the same vein, AMZZ showed significant activity against *Sarcina lutea, Candida albican and Aspergillus niger*. However, AMZZ and amoxicillin clavulanic acid were found to be resistant against *Staph aureus* and *Sarcina lutea* respectively.

The oral cavity contains many microbial entities and S. aureus and *Sarcina lutea* are part of the normal flora. These normal flora provides many benefits to its human host by inhibiting and killing non-indigenous species of microbes, colonizing throughout to prevent the establishment of non-indigenous species of microbes which may be pathogenic in nature.[42] Therefore, the resistance observed with AMZZ against *S. aureus* can be advantageous in a way. The antibacterial and antifungi activities of the AMZZ indicate its dual applications in the treatment of dental caries and oral thrush at the same time. This has scientifically confirm the ethnobotanical uses as a mouthwash and in the treatment of sore gums, toothache and dental caries.

NOCICEPTIVE BEHAVIORAL TESTS

Acetic acid-induced writhing test

At the dose 1% v/v of acetic acid given to the animals i.p., writhing episodes were established. The number of writhings were highest in the group that received normal saline only and decreases with increase in the dose of AMZZ. There was significant reduction in the number of writhings and the corresponding increase in the percentage inhibitions at the concentrations of 500 and 1000 mg/kg of the AMZZ as showed in table 2. There was no significant difference in the percentage inhibitions of AMZZ at these two concentrations and that of the standard drug, diclofenac (20 mg/ml), used. However, at 1000 mg/kg of AMZZ, the percentage inhibition was higher than that of the standard drug. It was also observed that the response was dose-dependent.

Hot plate

In the hot plate assay, all the tested doses of AMZZ after 90 mins of administration produced significant increase in the latency of the animals as showed in table 3. At the highest dose used, the effect became significant after 30 mins. This show that the extract at large doses act like strong analgesics since the hot plate test is a model that is used for determining strong and centrally acting analgesics.[43, 44] There was no significant difference in the effect produced by the standard drug and the extract at the highest dose used. These observations justify the use of the plant in the treatment of toothache.

CONCLUSION

The aqueous methanol extract of *Z. zanthoxyloides* has demonstrated an excellent antimicrobial activity against key microorganisms implicated in dental caries and oral thrush. It also has significant norciceptive activity. This study has clearly shown why this plant has been used for treatment of dental caries, toothache and as a mouthwash. The antibacterial and the analgesic properties play synergistic role in alleviating dental caries and toothache. This has given ascientific evidence to the ethnobotanical uses of *Z. zanthoxyloides* in the treatment of dental caries, toothache and oral thrush.

Conflict of interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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