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IN-VITRO ANTIBACTARIAL ACTIVITY OF COMMONLY USED CHEWING STICK ON SELECTED ORAL MICROBES

AZEEZ, E.M.,1 & ADEYEMI, S.O2
Department of Biology, Emmanuel Alayande College of Education, Oyo

ABSTRACT
This study investigates the antibacterial effect of commonly used chewing stick. The three chewing sticks selected for this study are *Prosopis Africana, Sarcocephalus latifolius and Zanthoxylum zanthoxyloides.* Extracts were made from 150g each of finely grounded powder of the chewing sticks soaked in 150ml of 70% ethanol and water for 48 hours. The ethanol and aqueous extract were then used on *Streptococcus mutans and Pseudomonas aeruginosa* from clinical origin. Antibacterial effect was measured by on the diameter of the zone of inhibition. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the extracts were also determined. All the medicinal plants studied were active against the test organism at varying concentrations. 19mm was the maximum zone of inhibition observed in ethanol extract of *Zanthoxylum zanthoxyloides* at concentration of 200mg/ml against *Pseudomona aeruginosa* and the minimum zone of inhibition in ethanol extract is 4mm of *Prosopis africana* against *S.mutans* The ethanol extract of *Zanthoxylum zanthoxyloides* had the lowest minimum inhibitory concentration on *Pseudomonas aeruginosa* at 25mg/ml while aqueous extract of *Prosopis africana* and *Sarcocephalus latifolius* had the highest value of MIC on *Pseudomona aeruginosa* and *Streptococcus mutans* at 200mg/ml each. The ethanol extract of *Prosopis africana* had the highest MIC at 200mg/ml on *Streptococcus mutans* and aqueous extract of *Sarcocephalus latifolius* have the lowest MIC at 100mg/ml. The MBC shows that *Prosopis africana, Sarcocephalus latifolius* and *Sarcocephalus latifolius* of ethanol extract had bactericidal effect on *Pseudomonas aeruginosa* and *Streptococcus mutans* at 200mg/ml and 100mg/ml respectively. Based on the findings, it was therefore

recommended that efforts should be made to purify active component of the plants extract so as to standardize it in recommendable dosage.

Keywords: *Prosopis Africana, Sarcocephalus latifolius, Zanthoxylum zanthoxyloides, Anticonvulsant, and anxiolytic.*

INTRODUCTION

In recent years, drug resistance to human pathogenic bacteria has been reported all over the world, with continuous use of antibiotics, microorganisms have become resistant (Piddock and Wise, 1989). Adverse effects have been associated with the use of antibiotics which has created immense clinical problem in the treatment of oral diseases leading to the demand of alternative antibacterial drugs to combat infectious diseases.

Scientists are working to find an alternative by using plants to produce new antibacterial drugs and to confirm the claims of indigenous people that use plants as antibacterial agents. Plant materials still remain an important source to combat world serious diseases (Davis, 1994). Examples of plants reported as antibacterial agents include *prosopis africana, Sarcocephalus latifolius*, *Zanthoxylum zanthoxyloides, Abutilon indicum, Allium cepa, Allium sativum, Azadirachta indica, Carica papaya, Alangium salvifolium, Acorus calamus, Bergenia ligulata,* and *Tinospora cordifolia,* among others. (Pamar and Rawat, 2012). Prosopis africana is a multipurpose tree of great economic value among the rural communities in the Guinea savanna of Nigeria. The fruit of the tree is used as feed for animals, while the seeds are fermented to make ukpehe, a highly proteinaceous condiment. The tree is not cultivated. The products from the hard wood, such as some wooden farm implements, kitchen utensils, and planks for construction, are extensively traded. The tree is a good source of firewood and charcoal. The secondary roots are used as medicine, Agboola, (2004). P. africana is a species of great socioeconomic importance but threatened with extinction in Niger because of overuse and regeneration problem. The study conducted in the Maradi (Niger) area, precisely at El Gueza in the south of Gazaoua department, evaluated the vegetative propagation capacity of P. africana by air layering under the Sudano-Sahelian climate of the south-central Niger. A ring of bark was taken on each selected branch and the wound was

covered with a black plastic filled with a damp mixture of soil and wood debris. The chosen parameters were the diameter class and the position on the branch. In all, 60 branches were treated and followed for 130 days: 28.33% produced shoots and there was no significant difference between the diameter classes and between the positions. These results showed that propagating trees of the species by air layering is possible and this technique can be used to multiply and keep this species, which will reduce the regeneration problem linked to a low seed germination rate, Laouali, et al., (2015).

Sarcocephalus latifolius (African peach) is one of the numerous plant species believed to have medicinal value. For example, it is among those medicinal plants strongly associated with antimalarial potency and widely used as such (Zirihi et al., 2005; Asase and Mensah, 2009). Sarcocephalus latifolius is a widely distributed straggling shrub mostly found in the tropical areas of Africa and Asia. The shrub has multiple stems with numerous irregular branches and dense foliage. In Nigeria, the plant is widely distributed in the South-east and South-south regions of the country. Sarcocephalus latifolius is reported to have a wide range of medicinal properties and its medicinal uses vary from one traditional setting to another; some common traditional uses which have been mentioned in different literatures include fever, pain, dental caries, septic mouth, malaria, hypertension, dysentery, diarrhea and diseases of the central nervous system such as epilepsy (Amos et al., 2005; Bum et al., 2009; Abbah et al., 2010).

Anticonvulsant, anxiolytic and sedative properties of Sarcocephalus latifolius roots decoction had also been reported (Bum et al., 2009). Enemor and Okaka, (2013) worked on the effect of ethanol extract of the root of the plant on some serum electrolytes was studied. A total of thirty Wistar albino rats were used to determine serum concentrations of K+, Ca2+, Cl^{-} and $HCO3^{-}$. The animals were divided into six groups of five rats each. Five groups labeled A, B, C, D and E, were administered orally with graded doses of root extract of Sarcocephalus latifolius at concentration of 300, 350, 400, 450 and 500 mg kg-1 body weight, respectively. The sixth group (Group F) was used as the control and its animals were simply sustained on normal diet and water. Administration of the extract lasted for twenty-one days after which the animals were sacrificed by cardiac puncture. K+, Ca2+, Cl^{-} and $HCO3^{-}$ were determined from each sample and the mean concentration was calculated for each dose and the control.

Potassium, calcium and chloride determination were done by colorimetric methods while determination of bicarbonate concentration was done by simple titration. Na+ was separately assayed, by flame photometer, from a set of 18 rats of six animals in each of three groups. For K+, non dose dependent increases were observed which was non-significant ($p>0.05$), for A, D and E but significant ($p<0.05$) for B and C. Ca2+ showed a dose dependent and significant ($p<0.05$) decreases, except for A ($p>0.05$). Decreases ($p< 0.05$) for C, D, E and ($p>0.05$) for A and B were observed for Cl^{-}. Serum bicarbonate appeared almost completely unaffected by the extract, showing no significant changes. Na+ levels were depressed for the two test groups, A and B compared with the control (group C), with test group B showing a significant decrease ($p<0.05$). From the analysis, it could be concluded that Sarcocephalus latifolius has the capacity to influence various electrolytes to physiologically important degrees. Significant reductions in sodium and calcium levels indicate the usefulness of the plant in treatment of hypertension and pain/fever, respectively. However, significant reductions in chloride may negatively affect the normal balance of fluid in the body.

Zanthoxylum zanthoxyloides Lam also known as Fagara zanthoxyloides, is an indigenous plant used widely as chewing stick for tooth cleaning in West Africa (Adebiyi et al., 2009; Adegbolagun and Olukemi, 2010). Several studies on the various effects of its extracts have been reported. For example, Kassim et al. (2005) reported the anti-malarial activity attributed to benzophenanthridine alkaloid, fagaronine from F. zanthoxyloides' root extracts. Anti-malarial activity was also reported in a study using extracts from trunk barks of F. zanthoxyloides (Gansane et al., 2010). On the other hand, Patel et al. (2010) named the compound nitidine as the agent in F. zanthoxyloides' anticancer capabilities while an anti-inflammatory property due to ortho-hydroxymethyl benzoic acid made F. zanthoxyloides useful in the management of pain in sickle cell crisis (Oyedapo and Famurewa, 1995; Folasade et al., 2006). More recently, the potential of Z. zanthoxyloides leaf, bark and root extracts as a biopesticide for stored food protection has been reported (Udo, 2011). The natural phytochemicals from plant could offer and effective alternative to antibiotics and represent a promising approach in prevention and therapeutic strategies for oral diseases and other pathogenic infections. Due to the reported biological

activities present in these plants, the aims of the present study were to investigates the antibacterial effect of commonly used plants as chewing sticks.

MATERIALS AND METHODS

This study was conducted from February 2020 - March 2021. Sample collections and plant extractions were done at the School of Sciences, Departments of Biology and Chemistry, Emmanuel Alayande College of Education, Oyo while the antibacterial activity assays were done at the Department of Botany, University of Ibadan, Ibadan.

Plants Indentification and Collection

Information about the commonly used chewing sticks was gathered through the use of semi-structured mode of interview, which involved one-one interview with the herb sellers in four herbal markets located in Ilorin metropolis. The stem of the plant used as chewing sticks were purchase from Oja-Oba market in Ilorin, Kwara state. The chewing sticks were identified and authenticated at the Department of Plant Biology, University of Ilorin, Nigeria.

Preparation of plant extracts

The stems of the three chewing sticks were chopped in to small pieces and ground into fine powder. 150g each of finely grounded powder of the chewing sticks were weighed and soaked in 150ml of 70% ethanol and water for 48 hours with occasional shaking the different extracts was then filter using Whatman No 1.Filter paper through a funnel into sterile universal bottles. Plant extracts were stored in beakers covered with aluminum foil paper and were stored in the refrigerator at 4OC prior to use.

Collection and maintenance of test organisms

The test organisms that were used were all human oral organisms from clinical origin. These isolates include *Streptococcus mutans and Pseudomonas aeruginosa.* The organisms were collected on sterile agar slants and incubated at 37°c for 48hours. They were then kept as stock cultures in the refrigerator at 4°c.

Determination of Antibacterial Activity of the Extracts

Sterile nutrient agar plates were prepared and allowed to solidify. Standardized organism of 0.1ml of a day old were introduced into the plate and inoculating loop was used to streak. The plate was left on the bench for 1hour so that the inoculums will diffuse into agar. A sterile cork borer was used to make 5 ditches on the plates. Varying concentration of the extracts, i.e. 200mg/ml,100mg/ml,50mg/ml,25mg/ml were made and 0.5ml of the extract was dropped in each of the appropriate labeled plate and controls were setup for each plate by adding 0.5ml of streptomycin into the 5thditch. The plates where duplicated and left on the bench for few minutes for the extract to diffuse into the agar and incubated at 370c for 24hours.After incubation the zone of clearance around each ditch was measure using a ruler by taking measurement from the edge of the plate to the point where the growth of the organisms started. The diameter of the zone of inhibition which represents antibacterial activity was measured.

Determination of Minimum Inhibitory Concentration (MIC)

For determination of Minimum Inhibitory Concentration (MIC), the broth dilution method of Albert *et al*., (1991) was used. The MIC helps to measure more exactly the concentration of an antibiotic necessary to inhibit growth of inoculums under defined conditions. Varying concentrations of the extracts were used. An arithmetic series of dilution was prepared between the concentrations. One milliliter of each concentration was added to each 9 milliliters of sterile nutrient broth containing one milliliter of standardized organism. Controls were also prepared; a total of 12 test tubes were used per extract. The tubes were incubated aerobically at 37◦C for twenty-four hours. The least concentration of the plants extracts that did not permit any visible growth of the inoculated test organism in broth culture was taken as Minimum Inhibitory Concentration in each case.

Determination of Minimum Bactericidal Concentration (MBC)

Samples from the tubes used in the Minimum Inhibitory Concentration (MIC) assay which did not show any visible growth after the period of incubation were streaked out using an inoculating loop each on an Agar plate to determine the Minimum Concentration required to kill the organisms. These concentrations

were indicated by the failure of the organism to grow on subsequent transfer nutrient Agar plates. However, the plate growing after 24hours of incubation period indicated a bactericidal effect. The minimum concentration of the plant extract that kill the bacterial is the Minimum Bactericidal Concentration (MBC) (Sani and Banso, 2003).

Statistical Analysis

All analyses were carried-out using the general linear model facility of the software Statistical Packages for the Social Sciences version 18.0 (SPSS, Chicago, Illinois). To identify which of the extracts mean were or were not significantly different, a Duncan's Multiple Range Test (DMRT) was employed (Field and Miles, 2010). To compare the 12 interaction means, 95% confidence intervals for each of the microorganism-extract combinations were calculated whereby overlapping intervals meant no significant difference

RESULTS AND DISCUSSION

Plant profiles

S/N	Botanical Name Family	Common name	Part used	Habit
1	*Prosopis africana* (Gull and Perr)	Ayan (Y)	Stem	Tree
2	*Sarcocephalus latifolius* (smith)Bruce (Syn.Nauclea latifolia smith)	Egbesi (Y) Africa peach (E)	Stem	Shrub
3	*Zanthoxylum zanthoxyloides* (Lam) (Zepern and Timler)	Orin Ata (Y) Candle wood	Stem	Tree

The profile of the plants used is presented in Table 1. The plants used were mostly trees. The percentage yield of the studied plants revealed that the ethanol extract is of higher yield. *Prosopis africana* yield 25%, *Sarcocephalus latifolius* yield 15% and *Zanthoxylum zanthoxyloides* yield 28%. The aqueous extracts were lower in yield *Prosopis africana* yield 10%, *Sarcocephalus latifolius* yield 10% and *Zanthoxylum zanthoxyloides* yield 15%.

Table 2: Diameter of zone of inhibition of the Ethanol Extract of the Chewing Sticks

Extract	Extract concentration/control (mg/ml)	*Pseudomonas aeruginosa*	*Streptococcus mutans*
Prosopis africana	200	13.50±0.25a*	11.50±0.23a*
	100	10.50±0.18b*	09.25±0.19b*
	50	09.25±0.12c*	07.25±0.10c*
	25	06.50±0.09d*	04.00±0.03 d*
	Control	09.50±0.18e*	09.00±0.13e*
Zanthoxylum zanthoxyloides	200	19.00±0.70a*	17.00±0.38 a*
	100	17.50±0.50 b*	15.25±0.35 b*
	50	16.25±0.40 c*	13.75±0.28 c*
	25	12.55±0.24 d*	12.25±0.23 d*
	Control	09.50±0.16 e*	10.05±0.17 e*
Sarcocephalus latifolius	100	09.25±0.15 b*	08.00±0.11 b*
	50	06.85±0.10 c*	06.50±0.09 c*
	25	05.62±0.07 d*	05.25±0.06d*
	Control	10.00±0.18 e*	09.75±0.13 e*

Legend: Values are means of 3 replicates ± standard error of mean in mm. Values with the same letter down the column are not significant ($P \leq 0.05$).

Table 3: Diameter of zone of inhibition of the Aqueous Extract of the Chewing Sticks

Extract	Extract concentration/control(mg/ml)	*Pseudomonas aeruginosa*	*Streptococcus mutans*
Prosopis africana	200	05.50±0.09 a*	06.15±0.09 a*
	100	05.00±0.06 b*	05.50±0.08 b*
	50	02.14±0.01c*	05.25±0.07 c*
	25	03.50±0.02 d*	02.25±0.02 d*
	Control	06.50±0.10 e*	07.50±0.12 e*
	200	05.45±0.08 a*	05.00±0.06 a*

Zanthoxylum zanthoxyloides	100	04.50±0.04 b*	05.25±0.08 b*
	50	04.25±0.03 c*	03.75±0.04 c*
	25	03.14±0.03d*	02.45±0.01 d*
	Control	07.75±0.12 e*	06.50±0.10 e*
Sarcocephalus latifolius	200	04.23± 0.03 a*	06.45±0.09 a*
	100	05.25±0.07 b*	04.00±0.02 b*
	50	04.85±0.04 c*	03.50±0.03 c*
	25	04.25±0.03 d*	02.50±0.01 d*
	Control	08.00±0.13 e*	08.45±0.14 e*

Table 4: The Minimum Inhibitory Concentration Plants Extracts (MIC)

S/N	TEST Organisms	Solvents	MIC *Prosopis africana*	MIC *Sarcocephalus latifolius*	MIC *Zanthoxylum zanthoxyloides*
1	*Pseudomonas aeruginosa*	Ethanol	100	100	25
		Water	200	200	100
2	*Streptococcus mutans*	Ethanol	200	100	50
		Water	200	200	100

Table 5: The Minimum Bactericidal Concentration Plants Extracts (MBC)

S/N	TEST Organisms	Solvents	MBC *Prosopis africana*	MBC *Sarcocephalus latifolius*	MBC *Zanthoxylum zanthoxyloides*
1	*Pseudomonas aeruginosa*	Ethanol	200	200	100
		Water	-	-	-
2	*Streptococcus mutans*	Ethanol	-	-	200
		Water	-	-	-

DISCUSSION

Antibacterial Activity of the Extracts

All the medicinal plants studied were active against the test organism at varying concentrations. 19mm was the maximum zone of inhibition observed in ethanol extract of *Zanthoxylum zanthoxyloides* at concentration of 200mg/ml against

Pseudomona aeruginosa and the minimum zone of inhibition in ethanol extract is 4mm of *Prosopis africana* against *S.mutans* (Table 2). The aqueous extract of *Sarcocephalus latifolius* shows maximum zone of inhibition against *S.mutans* (6.45mm) at 100mg/ml and the minimum zone of inhibition is (2.14mm) at 50mg/ml of *Prosopis africana* against *Pseudomonas aeruginosa* (Table 3).

MIC values obtained on the test organism varied from one organism to another. The ethanol extract of *Zanthoxylum zanthoxyloides* had the lowest minimum inhibitory concentration on *Pseudomonas aeruginosa* at 25mg/ml while aqueous extract of *Prosopis africana* and *Sarcocephalus latifolius* had the highest value of MIC on *Pseudomona aeruginosa* and *Streptococcus mutans* at 200mg/ml each. The ethanol extract of *Prosopis africana* had the highest MIC at 200mg/ml on *Streptococcus mutans* and aqueous extract of *Sarcocephalus latifolius* have the lowest MIC at 100mg/ml. (Table 4). The MBC shows that *Prosopis africana, Sarcocephalus latifolius* and *Sarcocephalus latifolius* of ethanol extract had bactericidal effect on *Pseudomonas aeruginosa* and *Streptococcus mutans* at 200mg/ml and 100mg/ml respectively.

The percentage yield varies for each plant used; the stem of *Zanthoxylum zanthoxyloides* in ethanol had a higher yield than *Prosopis africana* and *Sarcocephalus latifolius* for both ethanol and aqueous extract this may be due to its components.The phytochemical screening of the plants revealed the presence of saponin, flavonoids, phenol, glycoside, tannin, alkaloid and terpenoid while glycoside is absent in *Zanthoxylum zanthoxyloides.* The compounds were also reported by (Kaufman *et al*., 1999). To be indicative of the potential medicinal value of the plants in which they appear. One of the molecular actions of tannins is to form complex proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as covalent bond formation resulting in the inhibition of cell protein synthesis of the organisms (Stern *et al*., 1996), herbs that have tannins as their major components are used for wood healing. Phenols are known to have antimicrobial activity possibly due to enzyme inhibition in the oxidized forms or through more interactions with non-specific proteins (Mulla *et al.,* 2010). Thus the antibacterial activity of the extracts on the test organisms may be due to the presence of the aforementioned phytochemical components.

The result of this research work shows that the medicinal plants studied have antibacterial effect by inhibiting the growth of *Pseudomonas aeruginosa* and *S mutans*. They showed varying degree of activity based on the concentration, extract and test organism used. This research work shows that an increase in the concentration of the extracts brought about an increase in antibacterial activity as shown in Table 4 and 5. Ethanol extracts showed more activity against the bacterial isolates than the aqueous extracts. This may be due to the higher volatility of the ethanol which tends to extracts more active compounds from the samples than water. This is in concord with the observation of Ibekwe *et al*. (2000).

The marked difference in the effects of the extracts on the organism therefore, is suggestive of the activity against cell wall components of the organism. The antimicrobial substance appears to exert bactericidal activity by inhibiting the growth and by killing the sensitive bacteria. This particular finding was also reported by Emeruwa (1982).

The ethanol extract has the largest zone of inhibition in all. This is probably due to the higher concentrations of active components where aqueous extract had a higher activity from *Sarcocephalus latifoliuss* against *S. mutans*. This is in tandem with the work of Ijeh and Adedokun (2006). One of the factors that affect microbial susceptibility is the concentration of the active component; the more the concentration the higher the activity of the chemical substance.

From the result presented, the different concentrations of the extract have shown varying degree of antibacterial activities against the test organisms the ethanol extract of *Zanthoxylum zanthoxyloides* show a very high antibacterial activity because of the phytochemical constituents. On the whole some aqueous extracts were able to inhibit the growth of the test organisms which is known as bacteriostatic while most ethanol extract exert a killing effect on the test organism and suggest that the extracts were bactericidal.

The minimum inhibitory concentration (MIC) for ethanol extract against *Pseudomonas aeruginosa* are *prosopis africana* 100mg/ml, *Sarcocephalua latifolius* 100mg/ml, *Zanthoxylum zanthoxyloides* 25mg/ml and the aqueous extract are *prosopis africana* 200mg/ml, *Sarcocephalua latifolius* 200mg/ml and *Zanthoxylum zanthoxyloides*100mg/ml. while MIC of ethanol extract against *S mutans* are *prosopis africana* 200mg/ml, *Sarcocephalua latifolius* 100mg/ml and *Zanthoxylum zanthoxyloides* 50mg/ml and the aqueous extract

are *prosopis africana* 200mg/ml, *Sarcocephalua latifolius* 200mg/ml and *Zanthoxylum zanthoxyloides* 100mg/ml. This evidence suggests that the ethanol extract is more potent against *Pseudomonas aeruginosa* while higher concentration will be needed for the aqueous extract.

CONCLUSION AND RECOMMENDATION

Findings of the present study suggested that ethanol extract of the chewing sticks are bactericidal on test organisms. Efforts should be made to purify active component of the plants extract so as to standardize it in recommendable dosage. This is of importance as it will remove the fear of overdose, toxicity and other side effects.

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