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In vitro Antibacterial Screening of Aqueous and Methanolic Extracts of *Prosopis africana* (Guill., Perrott. & Rich.) Taub, against three Clinical Strains of Orodental Pathogens Isolated from sub – Gingival Crevices of Patients that Presented Periodontitis or Caries at Hospitals in Katsina State

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ABSTRACT
The aqueous and methanolic extracts of the leaves, stem and root barks of *Prosopis africana* (Guill; Perr. & Taub) were assayed for their *in vitro* antimicrobial activity against *Escherichia coli*, *Streptococcus mutans*, and *Staphylococcus aureus* isolated from sub – gingival crevices of patients that presented periodontitis and dental caries, by the agar well – diffusion method. The results show that crude *P. africana* extracts showed concentration – dependent activity against *E. coli* (meanZI = 14.69mm, maxZI = 21mm, lowest MIC = 0.391mg/ml), *S. mutans* (meanZI = 14.27mm, maxZI = 23mm, lowest MIC = 0.391mg/ml), and *S. aureus* (meanZI = 16.31mm, maxZI = 22mm, lowest MIC = 0.391mg/ml). The mean antibacterial activity index (AI) of all extracts were significantly ($P \leq 0.05$) higher than that of chlorhexidine gluconate – containing commercial toothpaste (at 10mg/ml), but significantly ($P \leq 0.001$) lower than that of ciprofloxacin® antibiotic at 100µg/ml. Both *E. coli* and *S. mutans* show their highest susceptibility to the aqueous root and methanolic stem bark extracts respectively (lowest MIC=0.391mg/ml & lowestMBC=0.781mg/ml), while *S. aureus* demonstrated equal susceptibility to all, but the aqueous leaf extracts. This result indicates the potential of *Prosopis africana* in the search for prototype antimicrobial principal in the chemotherapeutic management of oral diseases.
Keywords: *In-vitro*, Phytochemical, Antibacterial, Orodental, Periodontitis, Agar-well diffusion, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, Zone of Inhibition

INTRODUCTION
One of the prominent African herbal medical practices that have remained strong throughout antiquity, is the use of chewing stick plants as a means of orodental hygiene. This practice is also common in many cultures throughout the world right from pre-Christian times.
The earliest reported use of chewing stick plants dates back to the ancient Babylonians in the period 7000BC (al-Otaibi, 2004; Ndukwe *et al*., 2005 and Niazi *et al*., 2016). This ultimately spread throughout the Greek, Roman, Egyptian and Islamic Civilizations (Muhammad & Shinkafi, 2007). In Nigeria, 70 – 80% of the rural populace rely mainly on chewing stick plants as their primary means of oral hygiene (Ndukwe *et al*., 2005).
These chewing sticks are used either as brush-ended twigs used to clean the teeth or are made into decoctions and/or infusions as prescribed by traditional healers in the control of more severe dental

problems. The type of plants used as chewing sticks, in any given community, however, depends largely on the local floral diversity. For many people this choice also has more to do with the fibrous nature of the plant twig and the taste-sensation or frothing it produces in the mouth, than on any antimicrobial activity that the plant may have.

Sofowora (2006) reported that the tastes sought for in a chewing stick include ". . . the tingling, peppery taste, and numbness, as provided by *Fagara zanthoxyloides* root; a strong bitter and frothing taste as in *Masularia acuminata* stem … and from *Vernonia amygdalina* root". The type of plants used as chewing sticks in Northern Nigeria include the stem and root cuttings of several indigenous and exotic tree plants, including *Prosopis africana* (Kirya), *Commiphora africana* (Dashi), *Securidaca longipedunculata* (Sanya), *Khaya senegalensis* (Madachi), *Fagara zanthoxyloides* (Fasa Kwauri), *Guiera senegalensis* (Sabara), *Azadrichta indica* (Bedi), *Mangifera indica* (Mangwaro), *Salvadora persica* (Arak), *Diospyros mespilliforumia* (Kanya), *Vernonia amygdalina* (Shuwaka), *Anogeissus leiocarpus* (Marke), *Garcinia cola* (Goro), *Psidium guajava* (Gwaba), *Leptadania hastata* (Yadiya) etc.

The antimicrobial investigation of some of these chewing stick plants has shown that some possess antimicrobial activity against pathogenic bacteria and fungi. Thus, in addition to stimulation of the gum, and removing food particles and debris from root crevices like conventional tooth-brushing does, some of these chewing sticks, unlike many conventional toothpastes, also destroy oral microbes (Sofowora, 2006). The anti-microbial activity of chewing stick plants is believed to be a property of their constituent phytochemicals such as alkaloids, saponins, tannins, glycosides, anthraquinones, terpenes and terpenoids, phenols and other phenolic compounds etc (Hooda *et al.*, 2012; Ndukwe *et al.*, 2005). Previous studies have also demonstrated the antibacterial and anti-fungal activity of some of these chewing stick plants, against some wild strains of oral bacteria and fungi such as *Streptococcus mutans, Bacteriodes gingivalis,* and some oral anaerobes commonly implicated in dental caries and other orodental diseases (Ngonda *et al*., 2012 and Alhussaini *et al*., 2015).

Studies of the antimicrobial activity of chewing stick plants have been justified by the prevailing need to find alternative solutions to the phenomena of multi-drug resistance currently bedeviling the medical field (Alimata *et al*., 2020, Kouidhi *et al.*, 2015). It has been hypothesized that plant antimicrobials may inhibit bacteria by a different mechanism than those used by conventional antibiotics, and thus may have clinical value in the treatment of resistant microbial strains (Savoia, 2012), or serve as alternative therapeutic options in clinical multi-drug resistance phenomena (Simões *et al*., 2009) and (Khameneh *et al*., 2019). Beteck *et al*. (2014) infact reported that "in the treatment of chloroquine-resistant strains of *Plasmodium*, the natural quinone obtained from *Cinchona* spp, is a more effective therapeutic option than many of the synthetic anti-malarial agents in the market".

Chewing sticks have been reported to possess anti-plaque activity comparable to conventional toothbrushing (Hooda *et al*., 2012 and Chandra *et al*., 2018), and in some cases, give an even greater mechanical and chemical cleansing of oral tissues and plaque removal (Malik *et al*., 2014).

There is thus a renewed ethno-pharmacological investigation of the medicinal properties of several types of African and Asian chewing sticks (Ayanwuyi *et al*., 2010; David *et al*., 2010; Alimata *et al*., 2020 and Shemishere *et al*., 2020).

Prosopis africana (Guill., Perrott. & Rich.) Taub, also known as the African mesquite and 'Kirya' in Hausa is a small to large tree (4 – 20m in height) plant commonly used as a chewing stick in Northern and many parts of Nigeria. The plant has a drooping foliage with an open crown and slightly rounded buttresses; bark is very dark and scaly. Leaves are alternate and bipinnate with a rachis of about 10-15 cm long with 3-6 pairs of opposite pinnae (5-8 cm long). The plant is native to inter-tropical Africa and widespread throughout the Sudanean and Guinean ecozones, occurring from Senegal to Ethiopia reaching the border of the Sahelian ecozones to the north (Orwa *et al*., 2009).

P. africana has also been reported to possess several medicinal uses, although its economic exploitation for its wood value greatly overshadows its exploitation as a medicinal plant (N'Danikou *et al*., 2011). It has been extensively exploited in the savannah region of West Africa, and has for this reason been described as a threatened-specie that is rapidly disappearing from the savannah and Sahelian ecozones (N'Danikou *et al*., 2011 and Abubakar *et al*., 2018).

Almost all parts of *P. africana* are reported to be used in the traditional treatment of various diseases. The stem bark is used as remedy for dysentery, gonorrhea, bronchitis, and skin diseases. In many parts of Nigeria, the twigs, leaves, bark, and secondary roots are used for the treatment of typhoid fever, dental decay, malaria as well as stomach cramps (Raji *et al*., 2019) and the root poultice is used in the management of bronchitis, dermatitis, tooth decay, dysentery, malaria, stomach cramps, dental caries, sore throat, toothache, tooth decay, and as a dressing for wounds (Muhammad & Shinkafi, 2007).

The major bioactive phytochemicals isolated from *P. africana* have been reported to include alkaloids, saponins and tannins. Sandelier (1999) reported the isolation of the following alkaloids - *prosopine, prosopinone, prosopinine, (+)-prosophylline, isoprosopinine A, isoprosopinine B, prosafrine* and *prosafrinine,* from *P. africana,* while Karla (2008) reported the isolation of *5-hydroxytryptanine, tryptamine, tyramine, prosopine, L-arabinose*, and *D-glucuronic acid* from *P. africana*.

Aworinde *et al*. (2016) reported that *P. africana* root extract exhibited a strong antimicrobial activity against *Staphylococcus aureus,* (with MIC at 20mg/ml), *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (with MIC at 50mg/ml). Atawodi & Ogunbusola (2010) also reported that extracts of *P. africana* leaf and root showed strong *in vitro* anti-trypanosomal activity against *Trypanosoma brucei brucei*, at concentrations of 2mg/ml.

The extracts of *P. africana* have also been reported to exhibit significant hepato-protective activity against paracetamol-induced hepatotoxicity in experimental albino rats (Ojo *et al*., 2006), inhibited radial mycelial growth and sclerotial formation in *Macrophomina phaseolina* (Elaigwu *et al*., 2018), showed a significant inhibitory activity on the growth of *Salmonella typii, Streptococcus pyogenes*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *K. pneumonia, P aeruginosa* and *Candida albicans*, at MICs ranging from 12.5mg/l to 50mg/ml (Aworinde *et al*., 2016; Raji *et al*., 2019).

METHODOLOGY

Collection and Preparation of Plant Materials

Plant materials were collected from Dutsinma environs and identified at the Herbarium of the Department of Botany, Ahmadu Bello University Zaria, and issued with voucher number VN6908.

Extraction of plant materials was carried out as described by Adeiza *et al*. (2009) and Okwute & Ochi, (2017) using sterile de-ionized distilled water and methanol as solvents. Each of the plant material (leaves, stem and root barks) was separately cut into small pieces, shade dried at room temperature (32 – 35^{O}C) until constant weight was obtained, and then separately pulverized to a fine powder with a laboratory mortar and pestle and sieved with 20mm sieve British standard sieve, and thereafter placed into appropriately labeled, air – tight bottles, prior to extraction procedures.

Phytochemical Screening

Phytochemical tests for alkaloids, tannins, flavonoids, saponins, glycosides, anthraquinones and terpenoids were conducted using procedures described by Harborne (1998), Harris (2003) and Raji *et al*. (2019).

Serial Doubling – Dilution

A stock solution of 50mg/ml of the extract was prepared by dissolving 5gm of the powdered extract in 100ml of sterile deionized distilled water and shaken until a homogenate solution was obtained. A 2 – fold serial doubling dilution was thereafter prepared as follows. Four test tubes labeled 'A' – 'D' were arranged on a rack with tube A containing 10ml of the 50mg/ml stock concentrate, and tube 'B' to 'D' containing 5ml of its extraction solvent (deionized distilled water or methyl alcohol) each. Starting with tube 'A', 5mls each of the content of the tube was transferred from tube 'A' to 'B' and shaken, then 'B' to 'C' and 'C' to 'D', while 5ml from tube 'D' was taken and discarded. Each tube was however shaken before its content was withdrawn. The procedure was repeated separately for the 9 aqueous and 9 methanolic extracts to obtain 5mls each of the four test concentrations (50mg/ml, 25mg/ml, 12.5mg/ml, and 6.25mg/ml) of the aqueous and methanolic extract concentrates of the three test plants.

Collection of Bacterial Samples

The orodental pathogens used in this study (S*treptococcus mutans, Staphylococcus aureus* and *Escherichia coli*) were clinical isolates collected from patients that presented periodontitis and/or dental

caries at the dental unit of Katsina General Hospital Katsina and Turai Yar'Adua Maternity and Children's Hospital Katsina. A total of 68 samples were collected over a period of five weeks, using sterile commercial swab sticks, which were thereafter dipped and shaken in sterile bijou bottles containing peptone water, before to being transported to the laboratory as described by Cheesbrough (2005) and Church (2016).

Culturing, Isolation, and Identification of Test Bacteria

A loopful of the microbial suspension from different samples was transferred onto separate sterile nutrient agar plates and incubated at 37^{O}C for 24hrs after which different morphological colonies were severally sub-cultured onto fresh agar plates to get pure cultures, then observed for growth, gram staining and identification according procedures described by Cheesbrough (2005) and Church (2016).

Identification of *Escherichia coli*

Colonies resembling those of *E. coli* (deep red colonies on McConkey agar that tested Gram negative) were sub-cultured onto slanted nutrient agar plates for biochemical tests i.e. Indole, Methyl Red – Voges Proskauer, Simmons's citrate utilization, Urease tests and motility tests as described by Cheesbrough (2005) and Church (2016).

Identification of *Streptococcus mutans*

Test bacteria were first cultured on Mitis – Salivarius agar. Colonies resembling those of *S. mutans* (raised, convex, undulate, opaque, granular, and pale blue or whitish colonies), that had previously tested gram – positive were sub-cultured on blood agar and observed for greenish color along streak line (α – hemolysis), since *S. mutans* are α – hemolytic. The suspected colonies were then tested for catalase activity as described by (Cheesbrough, 2005) and (Church, 2016). Identification for *Streptococcus mutans* was confirmed by biochemical tests – mannitol and sorbitol fermentation and catalase test as described by Cheesbrough (2005), Al-Jumaily *et al*. (2014) and Church (2016).

Identification of *Staphylococcus aureus*

Incubated culture plates were observed for colonies which tested gram positive and appeared yellow, round, smooth, raised and glistening on Mannitol Salt Agar. Suspected colonies were sub-cultured onto fresh nutrient agar plates and confirmatory biochemical tests for the presence of *S. aureus* was carried out using catalase and coagulase tests as described by Cheesbrough (2005) and Church (2016).

Antibacterial Sensitivity Test

Antibacterial susceptibility testing was carried out by the ditch – plate method as described by Oseni (2012) and CLSI (2015).

Twenty millilitres (20ml) of previously warmed Mueller Hinton media (prepared according to NCCLS standards) was aseptically poured into 36 freshly autoclaved disposable polystyrene sterile Pyrex® petri dishes (150 x 20mm) to give a mean depth of 4.00mm ± 0.5mm and left to gel at room temperature. Twelve plates each, were inoculated with *E. coli*, *S. mutans* and *S. aureus* cultures (previously standardized to 0.5 McFarland turbidity) using a sterile inoculation needle. For each inoculation, the needle was swabbed over the entire surface of the Mueller Hinton Agar plate in a "zig – zag" pattern. The procedure was repeated by swabbing two more times while rotating the plate approximately 60° to ensure an even distribution of the inoculum.

Nine wells (1mm depth and 5mm diameter) were aseptically bored in each media plate, using a sterile cork-borer (No 5) and labelled from the underside of the plate. Using Pasteur pipettes, 0.5ml of the four test extract concentrations (50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) were aseptically dispensed separately into eight peripheral wells. Half millilitre of control solutions (distilled water as negative control, 10mg/ml of standard Chlorhexidine Gluconate - containing commercial toothpaste and 100µg/ml Ciprofloxacin® antibiotic as positive controls) were dispensed into the central wells of three petri dishes each as triplicates. The whole process was repeated for each set of test bacteria plates.

The plates were then allowed to pre-diffuse and thereafter incubated at 37^{O}C for 24 hours. Each plate was then examined for anti-bacterial activity as shown by the presence of a zone of inhibition around the wells. The zone diameter of inhibition (ZI) for each well was measured to the nearest millimetre and recorded. The activity index (AI) of all the extract types was measured as reported by Yolwin & Leysa (2012) and Dasgupta *et al*. (2012) using the following formula:

$$Activity\ index\ (AI) = \frac{Maximum\ Zone\ Diameter\ of\ Inhibition\ of\ Extract}{Maximum\ Zone\ Diameter\ of\ Inhibition\ of\ Positive\ Control}$$

Where 'maximum zone diameter of inhibition of positive control' is taken as the maximum zone diameter of inhibition of the Standard Chlorhexidine Gluconate – Containing Commercial Toothpaste.

By convention any zone of antibacterial inhibition for plant's antimicrobial agents above a threshold of 12.00mm is accepted as significant (Bauer *et al*., 1966 and Chattopadhyay *et al*., 2009). Any Activity index below 1.00 is also considered as being lower in antimicrobial activity than the reference positive control, which in this study is standard Chlorhexidine Gluconate – containing commercial toothpaste.

Determination of Minimum Inhibitory Concentrations (MIC)

Minimum inhibitory concentrations (MICs) of the test extracts were determined using procedures described by Anyanwu & Okoye (2017) and Setargie *et al*. (2019). MIC was determined for extracts that showed significant activity (max ZI ≥12mm) against any of the test bacteria.

The 50mg/ml concentrate of each plant extract was diluted by a serial doubling dilution to obtain 10ml each of seven test extract concentrations i.e. 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, 1.563mg/ml, 0.781mg/ml and 0.391mg/ml, using nutrient broth as diluent. Seven tubes each containing 5ml of nutrient broth were placed on a rack and labeled 'A' – 'G'. Starting with the 25mg/ml extract concentration, 1ml of each extract concentration (25mg/ml to 0.391mg/ml) was added to a tube each.

One milliliter of broth culture previously standardized to 0.5 McFarland standard was thereafter inoculated into each of the seven test tubes and shaken then incubated at 37^{O}C for 24 - 48 hours. A control tube was also inoculated with 1.0ml each of the extract and sterile nutrient broth only. The tube with the lowest concentration showing no detectable turbidity (growth) when compared with the control, was considered as the tube with the minimum inhibitory concentration (MIC) (Setargie *et al*., 2019).

Determination of Minimum Bactericidal Concentrations (MBC)

The minimum bactericidal concentration (MBC) of the test extracts were determined using procedures described by (Setargie *et al*., 2019). Test tubes resulting from MIC test were first assayed, and a loopful of each MIC tube was thereafter inoculated by streaking onto fresh sterile agar plate. The plates were incubated at 37^{O}C for 24 hours and observed for possible bacterial growth. The lowest concentration of the sub-culture that shows no bacterial growth was considered as the minimum bactericidal concentration (MBC) (Setargie *et al*., 2019).

RESULTS AND DISCUSSION

The phytochemical analysis of the aqueous and methanolic extracts of *P. africana* leaves, stem and root bark, shows that both extraction types demonstrated the presence of all phytochemicals tested, except anthraquinones, and alkaloids which were differentially demonstrated in the methanolic extracts (**Table 1**). This result agrees with the report of previous workers of the presence of phytochemicals in *P. africana* extracts (Abdulmumin *et al*., 2019 and Raji *et al*., 2019).

Table 1:

Phytochemical screening of aqueous and methanolic extracts of the leaves, stem, and root bark of *Prosopis africana*.

Compound	Aqueous			Methanolic		
	Leaves	Stem	Root	Leaves	Stem	Root
Carbohydrates	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+
Cardiac Glycosides	+	+	+	+	+	+
Free Anthraquinones	-	-	-	+	+	+
Combined Anthraquinones	-	-	-	+	+	+
Saponins	+	+	+	+	+	+
Steroids	-	-	-	+	+	-
Triterpenes	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Alkaloids	-	+	+	+	+	+

Key: L = leaves, S = Stem bark, R = Root bark, + = present, - = absent;

The results of the antibacterial susceptibility test shows that the crude aqueous and methanolic extracts of *P. africana* demonstrated a concentration gradient antibacterial activity against orodental clinical isolates of *E. coli, S. mutans* and *S. aureus* at concentrations between 6.25mg/ml to 50mg/ml. Although the methanolic extracts (meanZI=15.57mm, maxZI=23mm, meanAI=1.15) showed slightly higher zone diameters of inhibition, than the aqueous extract (meanZI=15.17mm, maxZI=22mm, meanAI=1.12), statistical analysis showed no significant difference ($p \leq 0.01$) between the two extract types in their activity against the test pathogens (see Table 2 and Table 3).

Both stem bark (meanZI=15.50mm, maxZI=22.00mm, meanAI=1.15) and root bark (meanZI=17.31mm, maxZI=23.00mm, meanAI=1.24) extracts demonstrated significantly ($p \leq 0.01$) higher zone diameters of inhibition and activity index, than the leaves extract (meanZI=13.88mm, maxZI= 22.00mm, meanAI=1.03) against all three test bacteria (see Table 2 and Table 3).

The zone diameters of inhibition of the stem and root barks extracts, especially at 50mg/ml, 25mg/ml and 12.5mg/ml, as well as their activity index (AI) were also significantly ($p \leq 0.01$) higher than that of the standard chlorhexidine-gluconate containing commercial toothpaste control (meanZI=13.50mm, maxZI=14mm, meanAI=1.00) (see Table 2 and Table 3).

S. aureus (meanZI = 16.76mm; maxZI = 23.00mm), and *E. coli* (meanZI = 15.28mm; maxZI = 21.00mm) showed significantly ($p \leq 0.01$) higher susceptibility than *S. mutans* (meanZI = 14.65mm; maxZI = 22.00mm) was the least susceptible to *P. africana* extracts (see Table 2 and Table 3).

These zone diameters of inhibition of *P. africana* extracts against *E. coli, S. mutans,* and *S. aureus* are well within the range of zone diameters of inhibition reported by other workers (David *et al*., 2010; Ezike *et al*., 2010; Akande & Ajao, 2011; Abdulmumin *et al*., 2019 and Raji *et al*., 2019).

Table 2: Zone diameters of inhibition (ZI) and Antibacterial Activity Index (AI) of Aqueous and Methanolic Extracts of Leaves, Stem and Root barks of *P. africana* against clinical strains of *E. coli, S. mutans* and *S. aureus* isolated from sub-gingival crevices of periodontitis and dental caries patients in Katsina Hospitals

Variable		Zone Diameter of Inhibition (in mm)			Antibacterial Activity Index (AI)	
		Mean ZI	Max ZI	S.E.	Activity Index	S.E.
Aqueous	*E. coli*	14.67*	21.00	0.896	1.09	0.326
	S. mutans	14.29*	21.00	0.763	1.06	0.278
	S. aureus	16.54**	22.00	0.631	1.23	0.229
	Mean	15.17**	21.33	0.763	1.12	0.286
Methanolic	*E. coli*	15.38**	21.00	0.849	1.14	0.309
	S. mutans	14.42*	22.00	0.878	1.07	0.319
	S. aureus	16.92**	23.00	0.568	1.25	0.206
	Mean	15.57***	22.00	0.765	1.15	0.289
Leaves	*E. coli*	12.88ns	19.00	1.056	0.95	0.314
	S. mutans	12.50ns	18.00	0.944	0.93	0.280
	S. aureus	16.25**	22.00	0.674	1.20	0.200
	Mean	13.88ns	19.67	0.891	1.03	0.292
Stem Bark	*E. coli*	15.31**	21.00	1.106	1.13	0.329
	S. mutans	14.56*	21.00	1.000	1.08	0.297
	S. aureus	16.63**	22.00	0.735	1.23	0.217
	Mean	15.50**	21.33	0.947	1.15	0.286
Root Bark	*E. coli*	16.88**	21.00	0.806	1.25	0.239
	S. mutans	16.00**	22.00	0.899	1.18	0.268
	S. aureus	17.31**	23.00	0.794	1.28	0.235
	Mean	16.73**	22.00	0.833	1.24	0.246
50mg/ml	*E. coli*	18.75**	21.00	0.719	1.39	0.186
	S. mutans	18.25**	22.00	0.740	1.35	0.191
	S. aureus	20.08**	23.00	0.514	1.49	0.132
	Mean	19.03**	22.00	0.658	1.41	0.177
25mg/ml	*E. coli*	16.50**	20.00	0.925	1.22	0.238
	S. mutans	15.58**	20.00	0.830	1.15	0.214
	S. aureus	17.83**	20.00	0.345	1.32	0.087
	Mean	16.64**	20.00	0.700	1.23	0.199
12.5mg/ml	*E. coli*	13.92ns	18.00	1.018	1.03	0.262
	S. mutans	13.50ns	18.00	0.883	1.00	0.226
	S. aureus	16.00**	18.00	0.246	1.19	0.063
	Mean	14.47*	18.00	0.716	1.07	0.214
6.25mg/ml	*E. coli*	10.92ns	15.00	0.949	0.81	0.244
	S. mutans	10.08ns	13.00	0.633	0.74	0.161
	S. aureus	13.00ns	13.00	0.369	0.96	0.095
	Mean	11.33ns	13.67	0.651	0.84	0.196
Distilled Water	*E. coli*	5.00ns	5.00	0.000	0.37	0.000
	S. mutans	5.00ns	5.00	0.000	0.37	0.000
	S. aureus	5.00ns	5.00	0.000	0.37	0.000
	Mean	5.00ns	5.00	0.000	0.37	0.000
Standard Toothpaste	*E. coli*	13.50ns	14.00	0.500	1.00	0.057
	S. mutans	13.50ns	14.00	0.500	1.00	0.057
	S. aureus	13.50ns	14.00	0.500	1.00	0.057
	Mean	13.50ns	14.00	0.500	1.00	0.044
Standard Antibiotic	*E. coli*	33.50**	34.00	0.500	2.48	0.057
	S. mutans	32.50**	33.00	0.500	2.41	0.049
	S. aureus	32.50**	33.00	0.500	2.41	0.049
	Mean	32.83**	33.33	0.500	2.43	0.056
Pathogen	*E. coli*	15.28	21.00	0.500	1.13	0.424
	S. mutans	14.65	22.00	0.500	1.08	0.404
	S. aureus	16.76	23.00	0.500	1.24	0.351
	Mean	15.56	22.00	0.500	1.15	0.397

KEY: S.E = Standard error of the mean; AI = Antibacterial Activity Index (measured relative to the activity of the standard toothpaste control); ** = mean ZI is significantly higher than that of toothpaste control at $p \leq 0.01$; * = mean ZI is significantly higher than the toothpaste control at $p \leq 0.05$; NS = mean ZI is not significantly higher than the toothpaste control at $p \leq 0.05$.

In general, the mean antibacterial activity index of the methanolic extracts were higher than that of the aqueous extracts for *S. mutans* (meanAI aqueous =1.06, meanAI methanolic =1.07), *E. coli* (meanAI aqueous =1.09, meanAI methanolic =1.14) and for *S. aureus* (meanAI aqueous =1.23, meanAI methanolic =1.25). This result supports the findings of Nden *et al.* (2019) and Alimata *et al.* (2020) that reported higher zones of inhibition and lower MICs for methanolic extract of *P. africana* than its aqueous extract.

In comparison, the standard antibiotic control (Ciprofloxacin®) at 10mg/ml showed the highest antimicrobial activity index (AI = 2.46), about 146% times more than the activity index of the standard toothpaste (AI = 1.00).

Similarly, at 50mg/ml and 25mg/ml the zone diameter of inhibition shown by all extracts of *P. africana* were significantly ($P \leq 0.05$) higher than that shown by the standard chlorohexidine gluconate-containing toothpaste control, but the zone diameter of inhibition shown by the standard antibiotic control against all the three test bacteria is significantly ($P \leq 0.01$) higher than those shown by all extracts of *P. africana* (Table 3).

Table 3: SPSS® Univariate ANOVA for mean zones of inhibition of Aqueous and Methanolic extracts of *P. africana* leaves, stem and root bark, against clinical isolates of *E. coli, S. mutans* and *S. aureus* isolated from sub-gingival crevices of periodontitis and dental caries patients in Katsina Hospitals.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Part	196.764	2	98.382	14.069	.000
Extract	5.840	1	5.840	.835	.364
Pathogen	144.056	2	72.028	10.300	.000
Concentration	1155.243	3	385.081	55.066	.000
Error	503.500	72	6.993		
Total	36147.000	144			
Corrected Total	2137.493	143			

a. R Squared = .764 (Adjusted R Squared = .532)

MIC and MBC tests showed that all the leaves, root bark (lowest MIC = 0.391mg/ml, lowest MBC = 0.781mg/ml), and aqueous stem extracts (lowest MIC = 0.3911mg/ml, lowest MBC = 1.563mg/ml) demonstrated their lowest MIC and MBC on *S. aureus*, while the methanolic stem extract (lowest MIC = 0.391mg/ml, lowest MBC = 0.781mg/ml) demonstrated its lowest MIC and MBC on *E. coli.* The aqueous root extract (lowest MIC = 0.391mg/ml, lowest MBC = 0.781mg/ml) however also shows lower MIC and MBC on both *S. mutans* (**Table 4**).

These results are also within the range of zone diameters of inhibition, MICs and MBCs of *P. africana* extracts reported by other workers (David *et al*., 2010; Ezike *et al*., 2010; Akande & Ajao, 2011; Ajiboye *et al*., 2013; Aworinde *et al*., 2016; Abdulmumin *et al*., 2019 and Alimata *et al*., 2020).

Table 4: Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) of *P. africana,* against clinical strains of *E. coli, S. mutans* and *S. aureus* isolated from patients that presented periodontal diseases at Katsina General Hospital and Umaru Musa Yar'adua Children and Maternity Hospital Katsina.

Extract	MIC/MBC	*E. coli*	*S. mutans*	*S. aureus*
Aqueous Leaf	MIC	1.563mg/ml	3.125mg/ml	0.781mg/ml
	MBC	3.125mg/ml	6.250mg/ml	1.563mg/ml
Methanolic Leaf	MIC	0.781mg/ml	0.781mg/ml	0.391mg/ml
	MBC	3.125mg/ml	1.563mg/ml	0.781mg/ml
Aqueous Stem	MIC	0.781mg/ml	0.781mg/ml	0.391mg/ml
	MBC	1.563mg/ml	1.563mg/ml	1.563mg/ml
Methanolic Stem	MIC	0.391mg/ml	1.563mg/ml	0.391mg/ml
	MBC	0.781mg/ml	3.125mg/ml	1.563mg/ml
Aqueous Root	MIC	0.781mg/ml	0.391mg/ml	0.391mg/ml
	MBC	3.125mg/ml	0.781mg/ml	0.781mg/ml
Methanolic Root	MIC	0.781mg/ml	0.781mg/ml	0.391mg/ml
	MBC	1.563mg/ml	1.563mg/ml	0.781mg/ml

KEY: MIC = Minimum Inhibitory Concentration; MBC = Minimum Bactericidal Concentration

CONCLUSION

The findings from this study have shown that the aqueous and methanolic extracts of the leaves, stem bark and root of *P. africana* commonly used in Northern Nigeria as chewing stick, has shown an antimicrobial activity against clinical strains of *E. coli*, *S. mutans* and *S. aureus* isolated from sub – gingival crevices of patients that presented periodontitis and dental caries at two hospitals in Katsina, Katsina State Nigeria. The chewing stick plant has demonstrated MICs and MBCs as low as 0.391mg/ml and 0.781mg/ml respectively, against the three clinical strains of *E. coli*, *S. mutans* and *S. aureus*.

RECOMMENDATIONS

This result indicates the potentials of *P. africana* for use in chemotherapeutic management and treatment of orodental infections and as antimicrobial additive to commercial toothpaste formulations.

Given also, the large repository of ethnobotanical information on medicinal plants available with herbal medical practitioners, there is the need for a coherent well – planned policy on traditional medical practice, so as to make available this information, to enable its harnessing in the hunt for antimicrobial prototype principals. This is especially because of the myriad of problems associated herbal medical practice, which include, but are not limited to toxic overdose, mis – diagnosis, fetishes etc.

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