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SCREENING OF *Prosopis africana* FOR ANTIMICROBIAL ACTIVITY AGAINST SELECTED ORAL PATHOGENS

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Abstract

Prosopis africana, a chewing stick popularly used in the South Western part of Nigeria was examined for antimicrobial activity against *Lactobacillus casei* ATCC 334, *Staphylococcus aureus* ATCC 35218, *Candida albicans* ATCC 10231, *Escherichia coli* ST2747 and *Streptococcus mutans* using agar well diffusion method. Extraction was done using ethanol and distilled water. The plant was screened for its phytochemical components. Gas chromatography mass spectrometry analysis was done to detect the secondary metabolites. All the extracts showed antimicrobial activity against the organisms with zones of inhibition ranging from 3.33±0.29 - 13.83±1.61mm except *Staphylococcus aureus* ATCC 35218 which was resistant to aqueous extract of the dried sample. Statistical analysis of data showed that there was no significant difference in the antimicrobial effects of ethanolic and aqueous extracts of the plant samples at 95% confidence intervals. The phytochemical screening result showed that phenols, alkaloids, sterols, triterpenes and saponins are components of the chewing stick. Also, fatty acids such oleic, palmitic and stearic acids were chemical constituents detected in the plant extracts by GC-MS. The results obtained in this study indicate that *P. africana* will be useful in the treatment of oral infections.

Keywords: Secondary metabolites, GC-MS, Oral pathogens, *Prosopsis africana*, Chewing stick

INTRODUCTION

Biofilms (plaque) are formed from the extensive growth of microorganisms, resulting from changes in the oral bacterial ecosystem. Once a biofilm is established it may lead to the formation of dental caries (tooth decay) or even more severe periodontal diseases. Dental caries and periodontal diseases in humans have an astonishing impact on the health and welfare of communities. Sick leave due to oral infections, and the consequent cost of dental treatment results in costing billions of dollars each year (Henley-Smith *et al.*, 2013). In 2007, the World Health Organization (WHO) stated that 5-10% of public health expenditure was related to dental care. Tooth decay and to a lesser extent periodontal infections, are perhaps the most expensive infections that most individuals have to contend with, during a lifetime (Henley- Smith *et al.*, 2013). Natural plant products are becoming increasingly popular treatments, even for oral health care. There is a long and venerable history of the use of plants to improve dental health and promote oral hygiene. In vast parts of the world where tooth brushing is uncommon, the practice of tooth cleaning by chewing sticks has been known since antiquity. The use of chewing stick persists today among many African and southern Asian communities as well as in isolated areas of tropical America and Southern United States (Kolapo *et al.*, 2009).

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Ogundiya *et al*. (2006) reported that chewing sticks impact varying taste sensation; a tingling peppery taste, a bitter taste and numbness is provided. In addition, chewing stick provides mechanical stimulation of the gum and also destroys microbes.

The choice of chewing sticks to be used in most cases depends on its cleansing action of the teeth; the therapeutic value, or preferred taste or flavour. The sticks (which may be stem or root with bark removed or retained) are cut to convenient lengths and washed thoroughly with fresh water to get rid of the earth or any dirt. The diameter should afford good grip, say between 0.5-1.30 cm. Some of the chewing sticks being used are obtained from the following plants: *Garcinia manni, Masularia accuminita, Terminalia glaucescens, Anogeissus leiocarpus, Pseudocedrela kotschyi, Xanthoxyllum gilletti* and *Azadiracta indica* (Akande and Hayashi, 1998). In a related development, Agboola, (2005) stated that *Prosopis africana* is used as chewing stick by Yorubas in Southwestern Nigeria.

It must be stressed that the development of several herbal toothpastes is based on the bioactivity of the constituent chewing sticks against a wide range of oral pathogens.

There is inherent belief of individuals in the use of chewing sticks as being effective in curing tooth ache and sore throat. It became imperative to discover whether or, not their claims were true.

MATERIALS AND METHOD

Collection and Identification of Plant

Fresh matured plants were collected from a residential garden in Ogidi, Ilorin, Kwara state. The *P. africana* stems together with the leaves were harvested by cutting the plant at the base with knife and identified at the herbarium, department of Plant biology, University of Ilorin with the voucher number, UILH/ 001/ 472.

Dried and cut chewing sticks (*P. africana*) were bought at Oja Oba market, Ilorin, Kwara state.

Preparation of Plant Material

The leaves were shredded and the stems were then cut into small pieces and dried at room temperature for 2 weeks. They were grinded in an electrical blender to a fine powder to increase the surface area for efficient extraction.

The dried and cut chewing sticks collected from the market were also further dried and grinded to a fine powder.

Preparation of Extracts

One hundred and sixty grams of the fine powder (dried at room temperature and sun dried respectively) was dissolved in 1500ml of sterile distilled water each in separate tight jars, while 160g of the fine powder (dried at room temperature and dried respectively) was dissolved in 1500ml of 70% ethanol each in separate tight jars, for aqueous and ethanol extractions respectively. The mixtures were placed on an electric shaker for 48 hours. The mixtures were filtered using Whatman No. 1 filter paper into beakers, to obtain the filtrates (aqueous and ethanol filtrates of the sun dried and dried at room temperature dried at room temperature samples).

Concentration of extracts

Extracts were concentrated using a rotary evaporator and the resulting concentrates were lyophilized using a freeze drier, model LAB KITS FD- 12- MR. The extracts were put in sterile sample bottles and stored in the refrigerator at 4°C until they were required.

Source of test organisms

The test organisms used in this research work are pure cultures of *Candida albicans* ATCC 10231, *Staphylococcus aureus* ATCC 35218, *Escherichia coli* ST2747 and *Lactobacillus casei* ATCC 334. The organisms were purchased as typed organisms in vials gotten from American Type Culture Collection (ATCC) and stored in the refrigerator at 4°C until needed. *Streptococcus mutans* was obtained from the Microbiology Laboratory at the University of Ilorin Teaching Hospital (UITH).

Antimicrobial Susceptibility Tests

Determination of antimicrobial activity of aqueous and ethanolic extracts of the stems of *P. africana* and herbal toothpaste

The antimicrobial activity of the extracts against the test organisms was determined by agar well diffusion assay as described by Mbajiuka *et al.* (2014). Sterile Mueller Hinton Agar (MHA) was poured into sterile petri dishes and allowed to solidify. Test organisms were standardized to a

turbidity equivalent of 0.5 McFarland standard (corresponds to 1.5 x 10^8 CFU/ml) (Ortez, 2005). Then a sterile cotton swab was dipped into the suspension. The entire plate was covered by streaking back and forth from edge to edge. The MHA plates were labeled A, B, C, and D and a 6mm sterile cork borer was used to bore wells in each quadrant for the 100, 50, 25, and 12.5mg/ml concentrations of the extracts respectively introduced aseptically into the wells using micropipette, and a centre well for the negative control using DMSO. The plates were allowed to stand for some minutes for proper diffusion of the extracts and then incubated uprightly at 35°C for bacteria and at 25°C for *Candida albicans*. The same procedure was repeated for herbal toothpaste (in its concentrated form) as a positive control. Results were determined by measuring the zones of clearance to the nearest millimetre (mm).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration was determined using the method described by Pandey *et al.* (2010) with some modifications.

The concentration of extracts that produced the least zone of inhibition in the antimicrobial susceptibility test was serially diluted further up to three new concentrations. The test organisms were standardized and inoculated on sterile MHA plates. A 4mm sterile cork borer was used to make wells in the MHA plates into which the different concentrations of extracts were aseptically introduced.

The plates were incubated and the least concentration at which antibacterial activity is observed is taken as the minimum inhibitory concentration.

2.12.3 Determination of Minimum Bactericidal Concentration (MBC)

The resulting minimum inhibitory concentrations of both extract for each organism was prepared and used to determine whether the MIC is bactericidal or bacteriostatic as follows:

9.7 ml of sterile nutrient broth in test tubes were prepared onto which 0.2ml of the extracts and 0.1ml of standardized test organism were added. The test tubes were incubated at the appropriate temperatures for 18-24 hours. After incubation, 0.1ml from the broth cultures was cultured in nutrient agar by the pour plate method. The Petri dishes were swirled to mix the inoculum and the media properly. The media was allowed to solidify and the plates were incubated for 18-24 hours. Plates which showed no growth of colonies were considered microbiocidal while plates which showed visible growth of colonies were considered microbiostatic. **Phytochemical screening of extracts**

Phytochemical examinations as described by Tiwari *et al.,* (2011) were carried out on both aqueous and ethanol extracts to screen for the presence of the following: saponins, tannins, flavonoids, phenols, alkaloids, triterpenes, sterol, and carbohydrates.

Gas Chromatography Analysis

GC analysis was performed in an orion micromat 412 double focusing Gas Chromatography system fitted with two capillary column crated with Cp-sil 5 and Cp-sil 19 (fused silica, 25m× 0.25mm ×0.15 film thickness) and flame ionization detector (FID). The volume injected was 1.00 µl and the split ratio was 1:30. Oven temperature was programmed from 50 - 230 at 5 /min, using hydrogen as carrier gas. Injection and detector temperatures were maintained at 200 and 250 respectively. Qualitative data were obtained by electronic integration of FID area percent without the use of correction factor.

Gas Chromatography/Mass Spectrometry (GC-MS) Analysis

A Hewlett-packward ITP5890A GC, interfaced with a VG analytical 70-250S double focusing mass spectrometer was used. Helium was the carrier gas at 1.2ml/min. The MS operating conditions were: ionization voltage 70eV, ion source 230. The GC was fitted with a 25m 0.25mm, fused silica capillary column coated with CP-sil 5. The film thickness was 0.15m. The GC operating conditions were identical with those of GC analysis. The MS data were acquired and processed by on-line desktop computer equipped with disk memory. The percentage compositions of the extracts were computed in each case from GC peak areas. The identification of the components was based on the comparison of retention indices (determined relative to the

retention time of series of n-alkanes) and mass spectral with those of authentic samples and with data from literature (Usman *et al.,* 2010).

Statistical Analysis

SPSS 20 was used for statistical analysis with zone of inhibition as dependent variable. The data were expressed as mean ± SD and analysed using ANOVA, one way analysis of variance, followed by a Turkey's pos-hoc test. Difference between the means was considered significant at 95% Confidence Interval

RESULTS

Percentage Yield of Extracts

The percentage yield of the extracts; aqueous dried at room temperature, aqueous sun dried, ethanol dried at room temperature and ethanol sun dried are as 6.30%, 6.90%, 9.40% and 13.80% respectively as shown in table 1 below.

Phytochemical Screening of Ethanolic and Aqueous Extracts of Dried at room temperature and Sun dried stems of *P. africana*

Table 2 shows the result of the qualitative screening of phytochemicals present in the ethanolic and aqueous extracts of dried at room temperature and dried stems of *P. africana*. The results show the presence of alkaloids, triterpenes (except aqueous dried at room temperature), sterols, phenols and saponins in all the extracts. Tannins was absent in all but, carbohydrate was present in the ethanol extracts only.

Table 1: The percentage yield of extracts

Extracts	Weight of plant samples(g)	Weight of lyophilized extracts(g)	% recovery
Aqueous dried at room temperature	160	10.0	6.30
Aqueous sun dried	160	11.0	6.90
Ethanolic dried at room temperature	160	15.0	9.40
Ethanolic sun dried	160	22.0	13.80

Table 2: Qualitative Phytochemical Screening of stem extracts of *P. africana*.

Phytochemical constituents	ED	EW	AD	AW
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Phenols	+	+	+	+
Tannins	-	-	-	-
Sterols	+	+	+	+
Triterpenes	+	+	+	-
Carbohydrates	+	+	-	-
Flavonoids	-	-	-	-

KEY:
+; Present
-; Absent
ED; Ethanolic (sun dried) extract, EW; Ethanolic (dried at room temperature) extract, AD; Aqueous (sun dried) extract, AW; Aqueous (dried at room temperature) extract.

Antimicrobial activities of Ethanolic and Aqueous Extracts of dried at room temperature and sun dried stems of *P. africana* and herbal toothpaste on test organisms

Table 3 shows the antimicrobial activity of the aqueous and ethanolic extracts of the sun dried

and dried at room temperature stems of *P. africana* and herbal toothpaste against *Lactobacillus casei* ATCC 334 (Lc), *Staphylococcus aureus* ATCC 35218 (Sa), *Escherichia coli* ST2747 (Ec), *Candida albicans* ATCC 10231 (Ca) *and Streptococcus mutans* (Str). All the organisms were susceptible to the extracts except *Staphylococcus aureus* ATCC 35218 which was resistant to the aqueous extract of the sun dried sample and all were susceptible to the herbal toothpaste except *Streptococcus mutans.* Antimicrobial activity generally increased with increasing concentrations for the plant extracts.

Minimum Inhibitory Concentrations

The least concentration at which the extracts had antimicrobial activity against the test organisms is shown in Table 4 below.

Minimum Bactericidal Concentrations result

The minimum bactericidal concentrations for all the extracts are shown in Table 5. Only *Streptococcus* had a minimum bactericidal concentration similar to the MIC for all the extracts except ethanol extract of the sun dried sample.

Table 3: Antimicrobial activities of stem extracts of *P. africana* and herbal toothpaste

Extracts	Conc. (mg/ml)	Zones of Inhibition (mm)±SD				
		Test organisms				
		Sa	Lc	Str	Ca	Ec
AW	100	12.50±0.50	10.17±0.76	9.83±0.76	13.83±1.61	12.50±0.50
	50	8.17±1.26	6.00±1.00	7.83±0.76	10.00±1.00	11.00±1.00
	25	4.67±1.53	4.17±0.29	4.00±0.50	7.00±1.00	7.67±0.58
	12.5	4.00±1.00	0.00±0.00	0.00±0.00	4.50±0.50	5.33±1.04
AD	100	0.00±0.00	13.33±0.79	7.83±0.29	11.17±0.76	11.83±0.29
	50	0.00±0.00	10.50±1.32	5.50±0.50	8.00±0.50	10.33±0.58
	25	0.00±0.00	8.33±0.76	4.67±1.32	5.50±0.50	8.00±0.50
	12.5	0.00±0.00	3.83±0.76	0.00±0.00	0.00±0.00	5.33±0.29
EW	100	8.17±0.76	8.50±0.50	12.33±0.29	7.00±0.50	9.33±1.08
	50	6.83±0.76	7.50±0.50	10.17±0.76	6.50±0.50	8.00±1.00
	25	5.50±0.50	4.83±0.89	7.83±0.76	5.33±0.58	6.50±1.32
	12.5	4.00±0.50	3.83±0.96	4.00±0.50	4.00±0.50	5.00±1.00
ED	100	8.00±0.50	8.83±0.76	8.17±0.76	10.50±0.50	10.17±1.89
	50	6.17±0.76	7.00±0.50	7.00±1.00	7.83±1.26	7.17±2.02
	25	4.83±0.76	5.83±0.76	5.00±0.50	6.83±1.04	5.50±0.50
	12.5	3.33±0.29	0.00±0.00	3.50±0.50	3.83±0.84	3.83±0.50
A		26.50±0.50	16.00±0.00	0.00±0.00	21.00±1.00	16.00±0.00

KEY:

Conc.; Concentrations, A; Herbal toothpaste, ED; Ethanolic (sun dried) extract, EW; Ethanolic (dried at room temperature) extract, AD; Aqueous (sun dried) extract, AW; Aqueous (dried at room temperature) extract.

Sa = *Staphylococcus aureus* ATCC 35218, Ca = *Candida albicans* ATCC 10231

Ec = *Escherichia coli* ST2747, Lc = *Lactobacillus casei* ATCC 334

Str = *Streptococcus mutans*.

Table 4: Minimum Inhibitory Concentration (all extracts) in mg/ml

Test organisms	Extracts			
	EW	AD	AW	ED
Lc	12.50	12.50	15.00	25.00
Sa	7.50	0.00	7.50	2.50

Ec	7.50	12.50	2.50	2.50
Ca	12.50	25.00	12.50	12.50
Str	7.50	25.00	25.00	7.50

KEY:
Sa = *Staphylococcus aureus* ATCC 35218, Ca = *Candida albicans* ATCC 10231
Ec = *Escherichia coli* ST2747, Lc = *Lactobacillus casei* ATCC 334, Str = *Streptococcus mutans*.
ED; Ethanolic (sun dried) extract, EW; Ethanolic (dried at room temperature) extract, AD; Aqueous (sun dried) extract, AW; Aqueous (dried at room temperature) extract.

Table 5: Minimum Bactericidal Concentration for all extracts (mg/ml)

Test organisms	Extracts			
	EW	AD	AW	ED
Lc	NIL	NIL	NIL	NIL
Sa	NIL	NIL	NIL	NIL
Ec	NIL	NIL	NIL	NIL
Ca	NIL	NIL	NIL	NIL
Str	7.50	25.00	25.00	NIL

KEY:
Sa = *Staphylococcus aureus* ATCC 35218
Ca = *Candida albicans* ATCC 10231
Ec = *Escherichia coli* ST2747
Lc = *Lactobacillus casei* ATCC 334
Str = *Streptococcus mutans*.
ED; Ethanolic (sun dried) extract, EW; Ethanolic (dried at room temperature) extract, AD; Aqueous (sun dried) extract, AW; Aqueous (dried at room temperature) extract.

Gas Chromatography- Mass Spectrometry results

Table 6, 7, 8 and 9 show the secondary metabolites of ethanol extracts of sun dried and dried at room temperature samples, aqueous extracts of sun dried and dried at room temperature samples respectively, the retention time in minutes, chemical formulae, molecular weights, area percentage, and mass- spectra data.

Table 6: Secondary Metabolites identified from Ethanolic extract of sun dried *P. Africana* by GC-MS

Peaks	Retention time	Metabolites	Area%	Formula	Weight	MS data
1	3.275	1,1- Diethoxy-3-methylbutane	1.40	$C_9H_{20}O_2$	100	75, 103, 113
2	3.808	Dimethyl sulfoxide	0.74	C_2H_6OS	78	45, 63, 78
3	5.025	Thymine	1.85	$C_5H_6N_2O_2$	126	55, 126
4	5.375	Niobe oil	1.13	$C_8H_8O_2$	136	77, 105, 136
5	6.058	2,3- dihydro- 3,5- dihydroxy- 6- methyl	1.86	$C_6H_8O_4$	144	101, 115, 144
6	10.300	4- Methyl- 1- penten- 3- ol	4.57	$C_6H_{12}O$	100	57, 73
7	11.942	3-O-Methylhexose	14.41	$C_7H_{14}O_6$	194	73, 87, 103
8	12.242	Pentyl chloroacetate	2.90	$C_7H_{13}ClO_2$	164	95, 105, 177
9	13.450	Methyl hexadecanoate	1.84	$C_{17}H_{34}O_2$	270	74, 87, 143
10	13.717	n- Hexadecanoic acid	18.14	$C_{16}H_{32}O_2$	256	60, 73, 129
11	14.558	Methyl cis- 6- octadecenoate	2.43	$C_{19}H_{36}O_2$	296	55, 264
12	14.833	Oleic acid	21.55	$C_{18}H_{34}O_2$	282	55, 69, 83
13	14.958	Stearic acid	10.96	$C_{18}H_{36}O_2$	284	73, 129, 183
14	15.733	Cedran- diol (8S, 14)	4.14	$C_{15}H_{26}O_2$	238	93, 189, 207
15	16.675	Oleamide	12.09	$C_{18}H_{35}NO$	281	55, 59, 72

Table 7: Secondary Metabolites identified from Ethanolic extract of dried at room temperature *P. africana* by GC-MS

Peaks	Retention time	Metabolites	Area%	Formula	Weight	MS data
1	3.267	Methylene chloride	1.44	CH_2Cl_2	84	49, 84
2	5.025	4- Methylpiperidine- 1- carboxylic acid	1.72	$C_{13}H_{17}NO_2$	219	55, 83, 126
3	5.375	Niobe oil	0.12	$C_8H_8O_2$	136	51, 77, 105, 136
4	6.050	2,3- dihydro- 3,5- dihydroxy- 6- methyl	1.71	$C_6H_8O_4$	144	101, 144
5	7.175	Dowanol	0.29	$C_8H_{10}O_2$	138	77, 94, 138
6	10.292	2- Propyl- 1- pentanol	4.70	$C_8H_{18}O$	130	57, 73, 86
7	11.967	3-O-Methylhexose	29.12	$C_7H_{14}O_6$	194	73, 87
8	12.325	1- (Chloromethoxy)octane	1.36	$C_9H_{19}ClO$	178	84
9	13.458	Methyl hexadecanoate	2.86	$C_{17}H_{34}O_2$	270	74, 87, 143
10	13.717	n- Hexadecanoic acid	17.79	$C_{16}H_{32}O_2$	256	60, 73, 129
11	14.275	O,O- diethyl O- (3, 5, 6- trichloro- 2-pyridinyl) ester	0.44	$C_9H_{11}C_{l3}NO_3PS$	349	97, 258, 314
12	14.567	Methyl elaidate	2.62	$C_{19}H_{36}O_2$	296	55, 69, 74
13	14.833	Oleic acid	21.23	$C_{18}H_{34}O_2$	282	55, 69, 83
14	14.958	Stearic acid	9.70	$C_{18}H_{36}O_2$	284	73, 129, 284
15	16.675	Oleamide	4.91	$C_{18}H_{35}NO_2$	297	59, 72, 153

Table 8: Secondary Metabolites identified from Aqueous extract of sun dried *P. africana* by GC-MS

Peaks	Retention time	Metabolites	Area%	Formula	Weight	MS data
1	3.267	N- tert- Butyethanolamine	2.47	$C_6H_{15}NO$	117	86, 102
2	3.800	Dimethyl sulfoxide	0.75	C_2H_6OS	78	45, 63, 78
3	3.908	Glycerol	2.46	$C_3H_8O_3$	92	61
4	5.033	Thymine	4.46	$C_5H_6N_2O_2$	126	55, 126
5	6.050	2,3- dihydro- 3,5- dihydroxy- 6- methyl	7.44	$C_6H_8O_4$	144	73, 101, 144
6	7.175	Dowanol	0.02	$C_8H_{10}O_2$	138	94, 138
7	8.300	Acetylcyclohexane	2.38	$C_8H_{14}O$	126	55, 126
8	8.550	1-(-2- Methyl- 1,3- oxathiolon- 2-yl)ethanol	2.59	$C_6H_{12}O_2S$	148	61, 103
9	9.083	1,8- Nonadien- 3- ol	1.44	$C_9H_{16}O$	140	49. 81, 86
10	10.308	Hexaglycerine	2.33	$C_6H_{14}O_3$	134	57, 86
11	10.667	Methyl 4-methyldodecanoate	0.58	$C_{14}H_{28}O_2$	228	74, 87
12	11.958	3- O- Methylhexose	17.18	$C_7H_{14}O_6$	194	73, 87, 103
13	12.325	Eicosanoic acid	2.38	$C_{20}H_{40}O_2$	312	73, 129, 185
14	13.450	Methyl hexadecanoate	2.78	$C_{17}H_{34}O_2$	270	74, 87, 143
15	13.717	n- hexadecanoic acid	15.30	$C_{16}H_{32}O_2$	256	60, 73, 129
16	13.950	2,3- Dimethylcyclohexanol	1.36	$C_8H_{16}O$	128	57, 110
17	14.283	O,O- diethyl O- (3, 5, 6- trichloro- 2-pyridinyl) ester	0.19	$C_9H_{11}C_{13}NO_3PS$	349	97, 197
18	14.567	Methyl elaidate	2.91	$C_{19}H_{36}O_2$	296	55, 69, 98

19	14.833	Oleic acid	18.72	$C_{18}H_{34}O_2$	282	55, 69, 83, 97
20	14.958	Stearic acid	7.23	$C_{18}H_{36}O_2$	284	73, 129, 284
21	16.675	Oleamide	5.02	$C_{18}H_{35}NO$	281	55, 59, 72

Table 9: Secondary Metabolites identified from Aqueous extract of dried at room temperature *P. africana* by GC-MS

Peaks	Retention time	Metabolites	Area%	Formula	Weight	MS data
1	8.925	Eugenol	3.64	$C_{10}H_{12}O_2$	164	131, 149, 164
2	11.992	3- O- Methylhexose	32.27	$C_7H_{14}O_6$	194	57, 73, 87
3	13.458	Hexadecanoic acid	2.39	$C_{17}H_{34}O_2$	270	74, 87, 143
4	13.717	n- Hexadecanoic acid	16.08	$C_{16}H_{32}O_2$	256	60, 73, 129
5	14.283	O,O- diethyl O- (3, 5, 6- trichloro- 2-pyridinyl) ester	2.99	$C_9H_{11}C_{13}NO_3PS$	349	97, 197, 314
6	14.567	Methyl elaidate	2.53	$C_{19}H_{36}O_2$	296	55, 69, 97
7	14.833	Oleic acid	18.64	$C_{18}H_{34}O_2$	282	55, 69, 83, 97
8	14.933	Stearic acid	7.23	$C_{18}H_{36}O_2$	284	73, 129, 284
9	16.683	Oleamide	14.21	$C_{18}H_{35}NO$	281	55, 59, 72

DISCUSSION

The difference in percentage yield of extracts (Table 1) observed in *P. africana* stems extracts may have resulted from different solvents used in extraction as reported by Kordali *et al* (2003) and Srinivasan *et al* (2001) that different solvents have different extraction capacities and different spectrum of solubility for the phytoconstituents respectively.

P. africana contains quite a number of active compounds as was observed in the phytochemical screening (Table 2). The result showed that alkaloids, an important antimicrobial substance is present in all of the samples, phenols, sterols and triterpenes are also present. Only the ethanolic extracts contained carbohydrates. Various phytocomponents such as alkaloids, tannins, saponins, cyanoglycosides, terpenoids, oleic and stearic acids which are naturally present in plants have been implicated in the conferment of antimicrobial activities on the plant (Abd El Rahman *et al.*, 2003). The presence of some of these plant secondary metabolites in a significant amount in the investigated part of *P. africana* may have conferred antimicrobial activity on extracts of this plant which is in agreement with Kolapo *et al.* (2009).

All the test organisms used in this research were susceptible to extracts of *P. africana* with the exception of *Staphylococcus aureus* ATCC 35218 (table 3) which was not susceptible to the aqueous extract of the sun dried sample. Ajiboye *et al.* (2013) reported that extracts which exhibit zones of inhibition ≥10mm are considered to have good antimicrobial activity. It has been reported that the root decoction of *P. africana* is used to treat toothache in Ghana and the bark and root used to treat and relieve tooth decay in Mali. In the study carried out by Kolapo *et al.* (2009), it was revealed that there is scientific basis for such traditional medicine practices. In addition, the relative efficiency of stem of *P. africana* to inhibit the growth of oral pathogens was presented in the report.

All the test organisms were susceptible to the herbal toothpaste used except *Streptococcus mutans.* (Table 3). This is contrary to the study carried out by Nwankwo & Ihesiulo, (2014). Fatima *et al*., 2000 also reported that the secondary metabolites present in the herbs used for the production of herbal toothpastes are responsible for their antimicrobial activity. The crude extracts of *P. africana* were more effective against *Streptococcus mutans* when compared to the herbal toothpastes (table 3) and as such the use of this chewing stick may provide greater

protection against oral infections caused by *Streptococcus mutans*.

The result of the statistical analysis showed that there is no significant difference between the two solvents of extraction. However, this is contrary to what Kolapo *et al.* (2009) reported in his study which is that the ethanol extracts had a significant higher effect on *C. albicans* as compared to the aqueous extract.

The GC-MS analysis results shows that Eugenol found in aqueous dried at room temperature extract is an antimicrobial substance which may have contributed to the antimicrobial activity of the extract. Eugenol is a component of clove oil and other essential oils; it plays a prominent role in dental and oral hygiene preparations. It has an antioxidant, anti-inflammatory, antibacterial and antiviral effects (Pavithra, 2014).

The detection of methylhexose in the extracts corroborates the presence of carbohydrates as shown in the phytochemical screening (Table 2).

Palmitic (n- hexadecanoic) acid was also detected in all of the extracts (area % ranging between 15.30- 18.14). It is a saturated fatty acid which may also have contributed to the antimicrobial effect of *P. africana* on the test organisms. A study carried out by Agoramoorthy *et al.* (2007) revealed that many fatty acids including palmitic acid have antibacterial and antifungal activities. Fatty acid methyl esters were extracted from *Excoecaria agallocha* and tested against four strains of Gram positive bacteria including *S. aureus*, three strains of Gram negative bacteria including *E. coli* and Yeasts such as *C. albicans.*

Stearic acid is a saturated fatty acid which has been found to have antimicrobial effect. Its presence (area % ranging between 7.23- 10.96) in all extracts of *P. africana* is another factor which may have contributed to its antimicrobial activity. Choi *et al.* (2013) has reported that long- chain saturated fatty acids such as palmitic and stearic acids have antibacterial activity. However, long- chain unsaturated fatty acids have higher antibacterial activity as presented in this study.

Oleic acid is a long- chain unsaturated fatty acid which has also been reported to have antimicrobial effect. It was detected in all the extracts with an area % ranging between 18.64-21.55. Choi *et al*. (2013) reported that the antibacterial activity of long- chain unsaturated fatty acids has been well- known for years. Long chain unsaturated fatty acids such as linoleic and oleic acids are bactericidal to important pathogenic organisms including methicillin-resistant *Staphylococcus aureus*, *Helicobacter pylori* and *C. albicans*.

Oleamide does not seem to have antimicrobial activity, but it has been characterized and identified as a signaling molecule responsible for causing sleep (Driscoll *et al*., 2007).

Other chemical constituents such as dowanol, cedran di ol, etc detected in the ethanol extracts are probably as a result of the chemical interaction of ethanol with the phytoconstituents of *P. africana.*

CONCLUSION

The results from this study clearly demonstrate the presence of antimicrobial properties in *P. africana.* Also, the susceptibility of the organisms observed in this study indicates that *P. africana* will be useful in the treatment of oral infections such as tooth decay and sore throat. Furthermore, the findings from this study suggest that *P. africana* may be a potential candidate in the production of dentrifice for oral hygiene.

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