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Antibacterial and phytochemical properties of five African medicinal plants used as chewing sticks south-western part of Nigeria

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Abstract

The antimicrobial activities of the ethanolic, Aqueous and Ethyl acetate extracts of five Nigerian medicinal plant used as chewing sticks were tested against five microorganism, three bacteria and two fungi. The five medicinal plant tested are *Vitellaria paradoxa*, *Vitex doniana*, *Sarcocorophalus latiolia*, *Vernonia amygdalina*, *Olox subcorpudica* and the microorganisms are *Staphylococcus aureus*, *Neisseria sicca*, *Streptococcus mutans* and *Lactobacillus salivarius* and *Actinomyces viscosus*. Out of the three extracting medium, Ethanolic extract shows significant activity than Ethyl acetate and Aqueous extract, follow in that decreasing order, and *Vitellaria paradoxa* were more active in Ethanolic extract, *Vitex doniana* and *Olox subcorpudica* were more active in Aqueous extract and *Vitex doniana* and *Sarcocorophalus latiolia* were more active in Ethyl acetate extract. all the five microorganism react vigorously to the tested medicinal plant The results of in this investigation clearly indicate that the antibacterial and antifungal activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of medicinal plants used in the Southwestern part of Nigeria and Africa as a whole, which could be of considerable interest to the development of new drugs, production of new antibiotic, development of locally made chewing sticks which would be commercially available to the Nigeria populace and transport to entire African region

Keywords: Extraction method, Antibacterial assay, agar well diffusion method, Qualitative Method of Analyses, Quantitative Method of Analyses, Phytochemical screening, Percentage of Crude Constituent, Anti-nutrients composition, Percentage Proximate Composition

Introduction

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyback, these plants are still widely used in ethno medicine around the world, Thomas (1978)^[1]

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has root, been investigated phytochemically and the submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs Srivastava, 1996^[3].

A wide range of medicinal plant parts is used for extract as raw medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs.

In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal. In view of this, the searches for new antimicrobial agents from medicinal plants are even more urgent in the countries like Nigeria where infectious diseases of bacterial origin are not only rampant, but the causative agents are also developing an increasing resistance against commonly used antibiotic (Abebe 2003)^[4] Considering the high costs of the synthetic drugs and their various side effects, the search for alternative products palnt used for antibiotic are justified.

Medicinal plant are rich source of wide variety of secondary metabolites belonging to chemical classes such as sterols, alkaloids, glycosides, saponins, flavonoids, tannins, and carbohydrates are generally superior in their anti-microbial activities Cowan 1999^[5].

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Leaves and flower of experimental plants have be in the treatment of different variety of infection and diseases.

Chewing sticks on the other hand is a very important plant/tree in Africa most especially Nigeria. The chewing sticks are used for three reasons, firstly, for cleaning of teeth and gums, Secondly to prevent tooth decay traditionally and thirdly, as a good source of medicinal plant. 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. Akande and Hayashi (1998) ^[6] reported that some of the chewing sticks being used are obtained from the following plants: *Garcinia manni*, *Masularia acuminita*, *Terminalia glaucescens*, *Anogeissusleiocarpus*, *Pseudocedrela kotschyi*, *Xanthoxylum gilletti* and *Azadiracta indica*. Investigations carried out on some of these chewing sticks showed that they posses antimicrobial activity against oral microbial flora such as *Staphylococcus aureus* and *S. auricularis* (Akande and Hayashi,1998) ^[6], *Candida albicans*, *Aspergillus flavus*, *Microsporium gypseum* and *Trichophyton metagrophytes* (Adekunle and Odukoya, 2006) ^[7].

The whole of Nigerian especially the southwestern part and Africa landscape is blessed with an abundant supply of the plants from which chewing sticks are obtained. These

indigenous raw materials need to be processed and packaged for local consumption and perhaps for export. This become more imperative as Indian product-Darbur herbal toothpaste has become a household name in Nigeria, the use of chewing sticks should be encourage and the addition of some extract of the chewing stich can also be added to the conventional toothpaste like Darbur and close up toothpaste,

it must be stressed that the development of virile herbal toothpaste is consequent upon the bioactivity of the constituent chewing sticks against a wide range of oral pathogens. Hence the aim of this paper is to report the antimicrobial activity of the ethanolic extracts of three Nigerian chewing sticks, *Olax subcorpida*, *Vitellaria paradoxa*, *Veronia amydalina*, *Vitex doniana* and *S latiofolia* on oral pathogens such as *Staphylococcus mutan*, *Neisseria sieca*, *Lactobacillus salivarius*, *Actinomyces viscosus* and *Streptococcus aureus*.

The purpose of this present study was to carry out preclinical evaluation of some popular medicinal plant species, i.e., biological and phytochemical screening with particular emphasis on those that seems to have very little scientific information in the areas intended for the investigation. This study facilitated the selection of plants with relatively high level of potency and wide range of biological activities suggesting that the strength of biological activities of a natural product is dependent on the diversity and quantity of such constituents.

Table 1: Family, Common, and Botanical Name and part used of Some Chewing Sticks

Common Name	Botanical Name	Authority	Part Used
Ifon	<i>Olax subcorpida</i>	Nguelefack et al (2005)	Root
Egbesi/Gberesi	<i>Sarcoccephalus latiolia</i>	Oluma et.al (2004)	Leave/bark
Emi	<i>Vitellaria paradoxa</i>	Adekunle and Odukoya, 2006;	Leave/bark
Ewuro	<i>Vernonia amygdalina</i>	(Schum. & Thonn.) Mull.Arg.	Root
Madumaro	<i>Vitex doniana</i>	(Schum. & Thonn.) Mull.Arg.Mull.Arg.	Leave/bark

Materials and Method

The experiment involved five types of chewing sticks, three different concentration of extracts, four bacteria and one fungus, and three trials.

Source of plant materials

The chewing sticks selected were obtained from the roots of *Olax subcorpida*, *Vitellaria paradoxa*, *Veronia amydalina*, *Vitex doniana* and *S latiofolia* They were all collected from Ado Ekiti, Ekiti State(a woody savanna vegetation) of Ekiti state, Nigeria. The plant parts were authenticated by a Botanist at the Department of Botany, Ekiti State University Ado Ekiti, Nigeria.

Source of microorganisms

Pure cultures of *Staphylococcus aureus*, *Neisseria sieca*, *Streptococcus mutans* and *Lactobacillus salivarius* and *Actinomyces viscosus* isolated from patient with dental diseases were obtained from the Federal Medical Center (FMC), Owo, Ondo State, Nigeria Bacterial cultures were maintained on Blood agar slant and the fungus on Potato dextrose agar slant.

Extract preparation

The root and branch of the test plant were well dried and then grinded to powder. About 200g of the powder were separately soaked in 400ml of (1)95% Ethanol, (2) Distilled

water and (3) Ethyl acetate in a 500ml reagent bottle and stoppered. This was allowed to stand for 14 days to permit full extraction of the active ingredients. The fluids were then filtered using Whatman No1 filter paper. The extracts were rotary dried to obtain the concentrate. It was then kept in fridge prior to use. A 2.0g/l solution of each extract was prepared and fractionated into 0.4g/l, 0.2g/l and 0.1g/l concentrations needed for the bioassay.

Bioassay

The method reported by Akande and Hayashi (1998) ^[6] was employed for the antimicrobial testing. The bacterial strains were subcultured three times in the fresh media (Bacto *Staphylococcus* base medium) stocks to obtain a more vigorous population. However, potato dextrose agar was used for the assay on *Actinomyces viscosus*. Fresh stocks were also used to seed the 20ml no-agar medium and incubated at optimal temperature of 35°C for 60 h. A 0.2-0.5ml portion of the new culture was aseptically transferred into Pyrex plates containing melted soft medium, gently agitated and poured as overlay on assay-plate containing the base medium. The preparation was left to gel and dry under a hood. Spots where extracts were to be introduced into the plates were carefully marked using sterile cork borer (6mm diameter) and small drops of extract of different concentrations were added. The plates containing bacteria were incubated at 35°C and fungal plates at 28°C, and the

zone of inhibition measured in mm after 24h, 48h and 72 h growth. A control experiment was set up by using drops of sterile distilled water in place of different extract concentrations.

Preliminary Phytochemical Studies

Basic phytochemical screening were performed on the chewing sticks extract as described by Harbone (1998) ^[9]

Phytochemical Screening

The extract were analysed for the presence of Alkaloid, Glycosides, Tannins, Saponins, Anthraquinones, Anthocyanosides, Flavonoids, Reducing sugars, Cyanogenic. The plant were collected in Ikare Akoko, a tropical rainforest, Ondo State, Nigeria with latitude (7.21692 North) and longitude (5.21561 East) Osuntokun & Ajayi (2014).

Qualitative Method of Analyses

Plant filtrate were prepared by boiling 20 g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrate were used for the phytochemical screening for flavonoids, tannins, saponins, alkaloids, reducing sugars, anthraquinones and anthocyanosides.

(i). Test for Alkaloids

About 0.2 gram were warmed with 2% of H₂SO₄ for two minutes, it was filtered and few drops of Dragendoff's reagent were added. Orange red precipitate indicate the present of Alkaloids (Trease and Evans 1989).

(ii) Test for Tannins

One milliliter of the filtrate was mixed with 2ml of FeCl₃. A dark green colour indicated a positive test for the tannins.

(iii) Test for Saponins

One milliliter of the plant filtrate were diluted with 2 ml of distilled water; the mixture were vigorously shaken and left to stand for 10min during which time, the development of foam on the surface of the mixture lasting for more than 10mm, indicates the presence of saponins.

(iv) Test for Anthraquinones

One milliliter of the plant filtrate were shaken with 10ml of benzene; the mixture was filtered and 5 ml of 10 % (v/v) ammonia were added, then shaken and observed. A pinkish solution indicates a positive test

(v). Test for Anthocyanosides

One milliliter of the plant filtrate were mixed with 5 ml of dilute HCl; a pale pink colour indicates the positive test.

(vi) Test for Flavonoids

One milliliter of plant filtrate were mixed with 2 ml of 10% lead acetate; a brownish precipitate indicated a positive test for the phenolic flavonoids. While for flavonoids, 1 ml of the plant filtrate were mixed with 2ml of dilute NaOH; a golden yellow colour indicated the presence of flavonoids

(vii) Test for Reducing Sugars

One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown colour with Fehling B and a green colour with Fehling A indicate the presence of reducing sugars.

(viii) Test for Cyanogenic glucosides

This was carried out by subjecting 0.5g of the extract 10ml sterile water filtering and adding sodium picrate to the filtrate and heated to boil (Trease and Evans 1989).

(ix) Test for Cardiac glucosides

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydride plus H₂SO₄ (Trease and Evans 1989)

Quantitative Method of Analyses

(i) Saponins

About 20grams each of dried plant samples were ground and, put into a conical flask after which 100 ml of 20 % aqueous ethanol were added. The mixture were heated using a hot water bath. At about 55°C, for 4 hour with continuous stirring, after which the mixture were filtered and the residue re-extracted with a further 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether were added and then shaken vigorously. The aqueous layer were recovered while the ether layer was discarded. The purification process was repeated three times. 60 ml of n-butanol were added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution were heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material, (Osuntokun 2015).

(ii) Flavonoids

About 10 g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol, at room temperature. The whole solution were filtered through Whatman filter paper No 42. The filtrate were later transferred into a crucible and evaporated into dryness over a water bath; the dry content were weighed to a constant weight (Trease and Evans 1989).

(iii) Cardiac glucosides

Legal test and the killer-kiliani was adopted, 0.5g of the extract were added to 2ml of acetic anhydride plus H₂SO₄ (Trease and Evans 1989)

(iv) Tannins

About 500 mg of the plant sample were weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M FeCl₃ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance were measured at 120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract. (Osuntokun *et al.* 2014).

(v) Alkaloids

Five grams of the plant sample were weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then added, the reaction mixture were covered and allowed to stand for 4 hour. This were filtered and the extract will be concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added

drop-wise to the extract until the precipitation is complete. The whole solution were allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid, which was dried and weighed to a constant mass (Trease and Evans 1989).

(vi) Phlobatannins

About 0.5grams of each plant extracts were dissolved in distilled water and filtered. The filtrate were boiled in 2% HCl, red precipitate show the present of phlobatannins.

Results and Discussion

Table 2: Antibacterial Activities of Crude Ethanol Extract at 10 and 20mg/ml Concentrations

Extract	<i>Staphylococcus mutan</i> 10mg/ml	20mg/ml	<i>Neisseria sieca</i> 10mg/ml	20mg/ml	<i>Lactobacillus salivarius</i> 10mg/ml	20mg/ml	<i>Actinomyces viscosus</i> 10mg/ml	20mg/ml	<i>Streptococcus aureus</i> 10mg/ml	20mg/ml
A	0.0	0.0	7.0	10.0	0.0	0.0	8.0	10.0	3.0	7.0
B	7.0	9.0	4.0	7.0	6.0	3.0	6.0	9.0	4.0	6.0
C	0.0	0.0	0.0	0.0	0.0	7.0	6.0	8.0	4.0	8.0
D	4.0	1.0	5.0	8.0	4.0	6.0	9.0	0.0	4.0	8.0
E	6.0	8.0	0.0	3.0	6.0	8.0	6.0	10.0	8.0	10.0

Unit of Zone of Inhibition –mm

KEY- A- Vitellaria paradoxa, B- Vitex doniana, C- Sarcorcephalus latiolia, D- Vernonia amygdalina, E- *Olax subcorpidica*

Table 3: Antibacterial activities of Crude Aqueous Extract at 10 and 20mg/ml Concentrations

Extract	<i>Staphylococcus mutan</i> 10mg/ml	20mg/ml	<i>Neisseria sieca</i> 10mg/ml	20mg/ml	<i>Lactobacillus salivarius</i> 10mg/ml	20mg/ml	<i>Actinomyces viscosus</i> 10mg/ml	20mg/ml	<i>Streptococcus aureus</i> 10mg/ml	20mg/ml
A	4.0	8.0	2.0	6.0	5.0	8.0	4.0	7.0	3.0	5.0
B	0.0	0.0	0.0	2.0	4.0	7.0	6.0	10.0	2.0	4.0
C	3.0	5.0	6.0	2.0	4.0	8.0	4.0	8.0	0.0	0.0
D	3.0	6.0	0.0	6.0	4.0	8.0	4.0	7.0	2.0	5.0
E	6.0	10.0	5.0	8.0	6.0	10.0	5.0	8.0	4.0	7.0

Unit of Zone of Inhibition –mm

KEY- A- Vitellaria paradoxa, B- Vitex doniana, C- Sarcorcephalus latiolia, D- Vernonia amygdalina, E- *Olax subcorpidica*

Table 4.Antibacterial Activities of Crude Ethyl Acetate Extract at 10 and 20mg/ml Concentrations

Extract	<i>Staphylococcus Mutan</i> 10mg/MI	20mg/MI	<i>Neisseria Sieca</i> 10mg/MI	20mg/MI	<i>Lactobacillus Salivarius</i> 10mg/MI	20mg/MI	<i>Actinomyces viscosus</i> 10mg/MI	20mg/MI	<i>Streptococcus Aureus</i> 10mg/MI	20mg/MI
A	0.0	2.0	0.0	1.0	2.0	4.0	0.0	0.0	0.0	2.0
B	0.0	9.0	2.0	5.00	5.0	10.0	0.0	3.0	0.0	0.2/
C	4.0	8.0	2.0	5.0	0.0	0.0	0.00	0.0	0.20	0.5.
D	0.0	0.0	1.0	5.0	6.0	8.0	6.0	7.0	0.0	0.40
E	4.0	6.0	4.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0

Unit of Zone of Inhibition -mm

Key- A- Vitellaria paradoxa, B- Vitex doniana, C- Sarcorcephalus latiolia, D- Vernonia amygdalina, E- *Olax subcorpidica*

Table 5: Phytochemical Screening of Chewing Sticks (Medicinal Plant)

Extract	Alkaloids	Tannins	Saponin	Steroids	Plobatanin	Terpenoids	Flavonoids	Cardiac glycosides
A	+	+	+	+	+	+	+	+
B	+	+	+	-	-	+	+	+
C	+	+	+	+	+	+	-	+
D	+	+	-	-	+	+	-	+
E	+	+	+	+	-	+	-	-

Key = (+) = Positive & Present (-) = Negative & Absent

Key- A- Vitellaria paradoxa, B- Vitex doniana, C- Sarcorcephalus latiolia, D- Vernonia amygdalina, E- *Olax subcorpidica*

Table 6: Percentage of Crude Constituent of Chewing Sticks (Medicinal Plant) Investigated

Extract	Alkaloids %	Phenols %	Tannins %	Flavonoids %	Saponin %	Phytate %	Oxalate %
A	8.38	1.34	3.41	8.96	6.48	1.97	0.85
B	3.62	0.24	0.71	3.42	12.11	1.03	0.50
C	4.28	0.23	0.65	1.90	10.74	1.10	0.72
D	14.70	1.15	3.45	1.90	N.D	1.15	0.68
E	3.17	1.15	2.02	2.17	6.05	1.21	0.65

Key- A- Vitellaria paradoxa, B- Vitex doniana, C- Sarcorcephalus latiolia, D- Vernonia amygdalina, E- *Olax subcorpidica*

Table 7: Anti –nutrients present in plant extracts result in percentage (%)

Parameters	Plant Extract used									
	A		B		C		D		E	
	A	B	A	B	A	B	A	B	A	B
Tannin	2.20	2.10	2.32	2.37	2.30	2.25	ND	ND	2.10	3.00
Phenol	3.50	3.55	2.50	2.47	3.42	3.47	ND	ND	3.41	2.25
Phylate	17.30	17.27	15.65	15.71	12.36	12.42	1.25	1.30	1.87	3.89
Oxalate	3.69	3.70	6.57	6.55	8.55	8.59	1.50	2.00	2.09	3.90
Saponin	13.89	14.01	9.71	9.75	7.51	7.60	ND	ND	4.0	ND
Flavonoid	8.53	8.55	6.49	6.55	10.32	10.40	ND	ND	3.89	4.0
Alkaloids	1.23	1.25	4.25	4.31	4.36	4.37	ND	ND	3.0	2.90

Key: ND =Not Detected

Key- A- Vitellaria paradoxa, B- Vitex doniana, C- Sarcoccephalus latiolia, D- Vernonia amygdalina, E- *Olox subcorpida*

Table 8: Proximate Composition percentage of the plant extracts (%)

Plant used	Ash	MC	CP	Fat	Fibre	CHO
A	11.26	5.70	16.25	5.36	8.47	52.96
B	11.24	5.68	16.30	5.37	8.50	52.91
C	10.30	5.72	15.46	3.68	7.33	57.51
D	9.75	6.10	15.49	4.25	6.75	57.66
E	7.25	5.36	14.75	6.38	5.32	60.94

Keys: CP= Crude Protein, MC= Moisture content, CHO= Carbohydrate

Key- A- Vitellaria paradoxa, B- Vitex doniana, C- Sarcoccephalus latiolia, D- Vernonia amygdalina, E- *Olox subcorpida*

Discussion

Five plants were reported as sources of chewing-sticks in this study community. Five of these plants namely Vitellaria paradoxa, Vitex doniana, Sarcoccephalus latiolia, Vernonia amygdalina, *Olox subcorpida* have previously been documented as sources of medicinal plant and chewing-sticks in Southwestern part of Nigeria communities shown in table 1. (Eggeling, 1952; Kokwaro, 1976; Katende et al., 1998)

Medicinal herbs possess curative properties due to the presence of various complex chemical substance of different composition, which are found as secondary plant metabolite found in one or more part of the plant. Patil 2009. There is continuous and urgent need for discovery of new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because of alarming increase in the incidence of new and re-emerging infectious diseases [Parekh 2008]. Natural products are known to play an important role in both drug discovery and chemical biology. In fact, many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs. Parekh 2008.

In table 2, it was observed that in ethanol extraction, *Staphylococcus mutan* were significantly active against all the plant extract except for Vitellaria paradoxa, and Sarcoccephalus latiolia at the varying concentration of 10mg/ml and 20mg/ml. then *Neisseria sieca* were active against all the plant extract except Sarcoccephalus latiolia, *Lactobacillus salivarius* is significantly active against Vitex doniana,, Vernonia amygdalina, *Olox subcorpida* but moderately active against Sarcoccephalus latiolia and less active against Vitellaria paradoxa.

Actinomyces viscosus and *Streptococcus aureus* is highly active against all the plant extract at a geometrical ratio.(Bone et.,al 2005).it was observed that the part of the plant used for antibacterial assay depends on their activity, if the leaves/ bark were used, there may be an increase in activity of the plant extract compared to the root,this is not to say that there are no antibacterial activity in the root, but in the leaves ,there is a storage of nutrient, food and importance phytochemical, which are useful to the plant, if

this can be useful to the plant , it is also useful to human being because of the storage of the nutrient . Behera, 2005.

In table 3,it was observed that in aqueous crude extraction, all the plant extract were significantly active except Vitex doniana against *Staphylococcus mutan* and Sarcoccephalus latiolia against *Streptococcus aureus* ,this is due to the fact that water is a good solvent for extraction, reason for significant high result obtained. Kumaraswamy 2000.

In table 4, all plant were active against the test microorganism except *Staphylococcus mutan* against Vernonia amygdalina and Vitellaria paradoxa. *Actinomyces viscosus* against *Olox subcorpida*. the reason for the high rate of antibacterial activity is as a result of the part used for extraction, leaf and bark extract showed significant antibacterial activity against the test pathogens. Leaf extract showed significant activity when compared with the bark/root extract of all the test plant extract. Samy 2000.

In table 5,6 and 7, Quantitative analysis of the phytochemical screening of chewing sticks(Medicinal plant) were observed, it was shown that Vitellaria paradoxa has all phytochemical constituents, Vitex doniana has all phytochemical constituents except Steroids and Plobatanin. In the case of Sarcoccephalus latiolia,all phytochemical constituent are present, only Flovonoids were absent. In the case of Vernonia amygdalina, Flavonoid, Saponin and Steroid were absent, other phytochemical constituents were present at a significant percentage. *Olox subcorpida*, Plobatanin, Flavonoids, and Cardiac glycosides were absent while others are present.

In table 6, shows the significant present of crude constituent at various percentage and ratio which are significant to the medicinal plant used as Chewing sticks. the crude constituent are Alkaloids, Phenol, Tannins, Flavonoids, Saponin, Phytate and Oxalate. Alkaloids are useful as Analgesic, antispasmodiac, bactericidal activity, (Harborne, 1973 and Okwu, 2004), Phenols are good antiseptic, anti-inflammatory, antimicrobial, anti-tumor in the activity(Leighton, 2005.Okwu, 2005),Tannins has Soothing relief, Regenerates skin, anti-inflammatory, Diuretics in its action (Okwu and Okwu, 2004), Saponin has expectorant action, cough suppressant, hemolytic activity(Sofowora,

1993 and Okwu, 2005). This is the reason the medicinal plant extract are useful to our dally life and better antimicrobial activity than the conventional toothpaste, its uses should be encouraged.

In table 7 shows the present of anti-nutrient and table 8 shows the proximate analysis of the nutrient present in the medicinal plant used as chewing sticks in the southwestern part of Nigeria.

Conclusion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [Tona 1998]. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [Samy 2000, Palombo 2001]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations for applications in crop protection.

The results of in this investigation clearly indicate that the antibacterial and antifungal activity vary with the species of the plants and plant material used. Thus, the study ascertains the value of medicinal plants used in the Southwestern part of Nigeria and Africa as a whole, which could be of considerable interest to the development of new drugs, production of new antibiotic, development of locally made chewing sticks which would be commercially available to the Nigeria populace and transport to entire African region, this we can be proud of ,compared to the conventional toothpaste which is made for European tooth, Nigeria and Africa is blessed with abundant resources like the Medicinal plant which should be converted to our dally use. God bless Nigeria and Africa.

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