Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(5): 398-406
Received: 24-07-2016
Accepted: 25-08-2016

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Antimicrobial value of *Olax subscorpioidea* and *Bridelia ferruginea* on micro-organism isolates of dental infection

IC Orabueze, AA Amudalat and AA Usman

Abstract
Dental – oral associated diseases are increasable affecting a considerable portion of the population and is considered one of the major causes of tooth loss, discomfort, mouth odour and loss of confidence. This study focused on the ethnobotanical survey of medicinal plants used in oral hygiene and evaluation of the antimicrobial activities of methanolic extracts of two selected plants from the survey for their efficacy against dental microorganisms. The ethnobotanical survey was carried out in three herbal markets in Lagos State, Nigeria by oral interviewing and information obtained from an old family manually complied herbal medication book. Methanolic extracts of *Olax subscorpioidea* (stem bark) and *Bridelia ferruginea* (stem bark) were assayed for their antimicrobial activities against clinical oral isolates (*Aspergillus fumigatus, Candida albicans*, *Streptococcus spp, Staphylococcus aureus, Lactobacillus acidophilus* and *Pseudomonas aeruginosa*). *In vitro* microbial technique (agar well diffusion method and minimum inhibitory concentration (MIC) assay) were employed for the assay. Chlorhexidine gluconate was used as the reference drug for comparison with the extract results. And the preliminary phytochemical screening of the constituents of the plants were done. The ethnobotanical survey produced plants (39) of diverse family. *O. subscorpioidea* showed considerable antifungal activity with zone of inhibition ranging from 20.00–26.50 mm against *Aspergillus fumigatus* but no such encouraging inhibitory activity was observed in the other assayed organisms. *B. ferruginea* showed antibacterial sensitivity against *Streptococcus spp, Staphylococcus aureus, Lactobacillus acidophilus* and *Pseudomonas aeruginosa* with zone of inhibitions ranging from 25.00 – 34.00; 16.00 – 22.50; 19.50 – 27.00 and 15.25 – 22.25 mm respectively. The minimum inhibitory concentration of *O. subscorpioidea* against *Aspergillus fumigatus* was 51.2 mg ml^{-1} while that of *B. ferruginea* against *Streptococcus spp* was 0.1mg ml^{-1} and for *Staphylococcus aureus, Lactobacillus acidophilus* and *Pseudomonas aeruginosa* were 25.6 mg ml^{-1}. A phytochemical analysis reveals the presence of several secondary metabolites. The barks of both plants exhibited antimicrobial properties against periodontal diseases causing organisms assayed, thus up-holding their folkloric use in oral disorder management.

Keywords: Antimicrobial activities, *Bridelia ferruginea*, dental disinfection, methanolic extract, *Olax subscorpioidea*, ethnobotanical survey

Introduction
The effective life spans of most antibiotic drugs are highly limited due to the ability of the microorganisms in developing resistance to antimicrobial agents [1]. The current observed level of resistance of micro-organisms to most of the available antimicrobial agents, challenges the effective management of infections at affordable cost [1]. It also increases the exposure to drug adverse effect due to increase in dosage needed to achieve desired result [1]. These pitfalls have necessitated a continuous search for new, safe, available and effective agents for the management of common infections [2]. Search for new drug "leads" has led to increased interest in existing informal information system about natural products and therapeutic relieves that can be obtained from them [3]. The wealth of information concerning the use of medicinal plants by indigenous people is invaluable and plays a key role as the starting point in natural products discovery. Study has shown that such information reveal long history of successful usage and effectiveness of medicinal plants in dealing with common occurring primary healthcare needs [4, 5, 6]. One of the easiest place to get the needed information on medicinal plant use of a locality or people is their herbal market place. These special places serve as herbal pharmacies [7], a meeting point of the herbal sellers and buyers, herbal users and prescribers. These herbal market places are also places of exchange of information among the herbal sellers. Plant extracts have been confirmed to have a series of bioactive phytoconstituents which may possibly give a higher level of clinical microbial clearance in multidrug-resistant infection than a single isolated compound [1, 8].

Chewing sticks have been recommended for orodental hygiene by the World Health Organization, and some of them are also used in the ethnomedical treatment of oral infections [9]. In Nigeria, chewing stick is considered an integral part of all its cultural tribes and plays pivotal and indispensable role in daily cleaning of the teeth and dental healthcare [10, 9]. Research findings have proved that some of these chewing sticks contain natural plaque inhibiting substances that can reduce bacterial colonization and plaque formation, thus their use in dental care and infections [11]. A number of chewing stick extract containing herbal dental formulations, such as toothpaste, antimicrobial plaque mouth wash and gels are available in the markets [12]. Antimicrobial susceptibility profiles of some of such products in the market have been done to ascertain their level of effectiveness against a wide range of oral pathogens [13].

Traditionally the chewing sticks or twigs are chewed to create natural disposable bristle tooth brushes for ease of cleaning [11]. The twigs of sticks are flexible and make an easy cleaning of the whole part of the dental or oral structure. The extract or liquid obtained during the course of chewing the stick/twig into brush-like structure contains cleansing and healing agents for the teeth and the gum.[14] Some identified healing effects of chewing sticks include reduction of gum bleeding, gingivitis, inhibition of the growth of aerobic, micro-aerophilic and anaerobic bacteria and analgesic properties [15, 16, 17]. In most cases, the choice of chewing sticks to be used depends on desired action (cleansing action of the teeth, the therapeutic value, taste and flavour) and availability within a locality.

Nigeria is blessed with a good biodiversity of plants having various bioactivities [18], which could not only serve as source of immediate medical products but could be processed into household daily welfare items such as toothpaste, mouth wash, soaps, hair shampoos, hand wash, antiseptic liquids etc.

Bridelia ferruginea (Euphorbiaceae) is a medicinal plant of interest found widespread in West Africa. The plant is widely used in traditional African medicine to treat a range of diseases, some of which have been pharmacologically validated [19]. The efficacy of leaf extract of *B. ferruginea* as a potential oral hypoglycemic [20, 21], antihypertensive [22], analgesic, anti-inflammatory [23] has been reported [24]. In the western part of Nigeria, the stem and its bark are used in the treatment of oral candidiasis and other various oral infections. In northern Nigeria the bark is used for the treatment of infections generated by the wounds of poisoned arrow [25, 26]. Other reported activities of *B. ferruginea* include reduction of free radicals and antimicrobial properties [19].

Olax subscorpioidea is a shrub or tree which belongs to the family of Olacaceae and it is a widely used medicinal plant in West African countries [27]. The plant is a highly valued medicinal plant in the traditional management of asthma, cancer, infectious diseases, pain-killers, mental illnesses [28]. The antimicrobial activity of ethanol extract of the stem of *O. subscorpioidea* has been reported on some selected micro-organisms [27].

The present study aims at evaluating the antimicrobial activity of these two medicinal plants used as chewing stick and in preparation of dento-oral herbal remedies in the Western part of Nigeria in view to check the therapeutic validity of the medicinal plants. It is based on a preliminary research (ethnobotanical survey) conducted in the mentioned locality for the medicinal plants extensively used for oral hygiene and traditional dental health care system.

Materials and Methods

Ethnobotanical survey

The survey was undertaken using three herbal markets in Lagos state, Nigeria, to obtain information on local herbal remedies used for the treatment of dental/oral disorders by means of oral questionnaire majorly in the local language (Yoruba, the common and local language spoken in the study area). Most of the herb sellers are elderly females. A total of seventeen (17) female sellers were interviewed while a lot of the sellers declined the request. The herbal sellers were informed that the ethnobotanical survey was a student study research work. Information on medicinal plants used for orodental care was also further obtained from close relatives (extended family members) with history and knowledge of herbal medicine and from an old family book containing herbal remedies for different aliments passed from one generation to another (three generations now).

The herbal markets used for this research include:

- Gorodomu - Ebutero Market, (Lagos Island).
- Ita Onikoyi Market (Lagos Island).
- Orile herbal Market.

Simplified questions were used during the interviews for better understanding and communication between the interest groups. Questions asked included but not limited to: if there are medicinal or herbs used in treatment of oral and dental care, if yes, the names (local names were given), part (s) of the plant used, possible combinations (poly-herbal) etc. Two (2) plants that were frequently mentioned were selected for further study.

Plant collection and identification

The plants used for the research are *Olax subscorpioidea* and *Bridelia ferruginea.* The stem barks were purchased from Mushin herbal market, Mushin Lagos state, Nigeria. Arrangement was made with the herb seller to source directly from a plant collector, who in turn collected from the wild within Ogun State, Western part of Nigeria. The identification and authentication of *Olax subscorpioidea* was done by Mr Oyebanji O.O, a taxonomist at the herbarium in the Department of Botany, University of Lagos, with herbarium specimen number LUH 6607. Authentication and herbarium preparation of *Bridelia ferruginea* was done by Mr Nwafor of International Centre for Ethnomedicine and Drug Development (Inter CEDD), Nsukka, Enugu state, with voucher specimen number INTERCEDD/606.

Collection of clinical isolates

The microorganism samples used for the research were obtained by culturing mouth isolates with the use of a sterile swab stick. A group of 15 high institution students attending dental clinic (presenting dental caries or periodontal diseases) were randomly selected for the study. Two swab samples were taken from the teeth region of the mouth before brushing for each person.

Preparation of culture agar and biochemical reagents

All media and reagents used were prepared according to the manufacturer's direction, following proper aseptic techniques. The swab sticks were classified into two groups of 15 swabs each. The first group were inserted into Brain Heart Infusion (BHI) broth for enrichment of aerobic organisms while the second group went into Fluid Thioglycolate medium for the anaerobes. They were incubated at 37 ^{o}C for 24 h after which the media went turbid indicative of growth in both media.

Cultivation, isolation and identification

The growing microbial strains in the liquid cultures were subcultured onto diagnostic solid media (Blood Agar, Sabourald Dextrose Agar, Mannitol Salt Agar, Tryptic soy Agar and Nutrient Agar). These plates were used for identification and isolation. Gram staining was used for identification and characterization and sub-culturing was further done to isolate the distinct colonies. The isolates from the Fluid Thioglycolate medium went into anaerobic jar. The organisms isolated for the research were *Aspergillus fumigatus, Candida albicans, Pseudomonas aeruginosa, Streptococcus spp, Staphylococcus aureus and Lactobacillus acidophilus*. The microorganism cultures were calibrated using 0.5 McFarland turbidity standard.

Extract Preparation

The dried and ground stem barks (0.76 kg) of *O. subscorpioidea* and *B. ferruginea* were macerated in absolute methanol for three days in an air tight bottle and shook periodically (agitation) to maximize full extraction of the phytoconstituents. The extract was decanted and filtered using Whatman No1 filter paper. The methanolic extracts were concentrated and dried in a temperature controlled water bath at 40 ºC. They were labelled and kept in refrigerator prior to use.

Phytochemical studies of crude extracts

The dried extracts of the stem of *O. subscorpioidea* and the bark of *B. ferruginea* were analysed for the presence of bioactive compounds by carrying out standard procedures as described by various authors [29, 30].

Antimicrobial sensitivity assay

The antimicrobial assay was performed by using the agar well diffusion method [31, 32]. All procedures were done aseptically. 1 mL of calibrated fungi was mixed with 25 mL of molten Sabourald Dextrose agar (65 gL^{-1}) and was allowed to solidify in the petri dishes. A well was bored using 6 mm cork borer and the different concentrations of the test extracts (50, 100 and 200 mg mL^{-1}) were dispensed into marked wells carefully using sterile 1ml pipette. The plates were incubated at the Laboratory's ambient temperature and observed daily until growth. Same procedure was done for the bacteria but Mueller Hinton Agar (39 gL^{-1}) was used and incubated at 37 ºC for 24 h. Chlorhexidine gluconate, a mouth wash active ingredient against both bacteria and fungi was used as the positive control at concentrations 5%, 2.5%, 1.25% and 0.625% w/v. A negative control of the diluent, sterile distilled water was used. The resulting zones of inhibitions were measured. All were done in duplicate (n = 2). The average of those zones were recorded in millimeters.

Minimum inhibitory concentration (MIC)

The determination of minimum inhibitory concentration was carried out according to methods of [33]. Sabourald Dextrose agar media was used for fungus while Mueller Hinton agar was used for bacteria throughout the study. Test solutions were prepared according to manufacturer's standards and autoclaved at 115 ^{0}C for 1 h. Ten (10) different concentrations were prepared for the extracts which were 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.2 mg mL^{-1}. These different extract concentrations were mixed with agar to make 20 ml, transferred into petri dishes and allowed to solidify. Standardised inoculum were prepared using 0.5 McFarland turbidity standards to calibrate them. The organisms (0.01 mL) were inoculated on the different petri dishes and incubated (fungi at ambient temperature with daily observation and 37 ^{0}C for bacteria with daily observation for 72 h). Observation for growth or inhibition of growth was noted. The lowest concentration inhibiting growth was taken as the minimum inhibitory concentration (MIC).

Results

Data obtained from the Ethnobotanical survey.

A lot of different interpretations and understanding as to what dental disease is all about, were given by various people interviewed, some were describing it as mouth odour, difficulties in chewing, coloured teeth, swollen gums, odour from the stomach, rotten tooth/teeth etc. The common angle to the various understandings of what dental infection means was that all has to do with the mouth cavity. Different morphological parts, like roots, bark, leaves, seed, stem and flowers were mentioned as being useful in dental issues. Some were described as chewing sticks, some to be prepared as mouth wash, some to be used alone and some in combination. An interesting angle was that some medicinal plants were advised not to be used together by some herbal sellers. Table 1 shows a summary of names of some medicinal plants, their families, parts that are used, their local names and the frequency at which each medicinal plant was mentioned. Most of the medicinal plants sold in these markets were sourced randomly from the wild, bushes within Lagos environs and adjacent states. The table shows that 39 plant species belonging to 23 families were mentioned in these markets and other mentioned source as herbal remedies for periodontal-dental hygiene and care. The use of leaves accounted for 32.65%, while that of stem bark, fruit, root and seed are 32.65, 14.29, 12.24 and 8.16%, respectively.

Table 1: Ethnobotanical survey on herbs used for dental diseases.

S/N	Botanical Name	Family	Common Name	Part Of Plant Used	Percentage No of Times Mentioned
1.	*Capsicum annum*	Solanaceae	Ata-ijosi	Fruits	100
2.	*Aframomum melegueta*	Zingiberaceae	Atale	Fruits	66.67
3.	*Xylopia aethiopica*	Annonaceae	Eru-awonka	Fruits, seeds	66.67
4.	*Piper guineense*	Piperaceae	Iyere	Fruits, seeds	100
5.	*Spondias mombin*	Anacardiaceae	Akika	Leaves	100
6.	*Baphia nitida*	Fabaceae	Osun	Leaves	100
7.	*Mentha arvensis*	Lamiaeae		Leaves	66.67
8.	*Terminalia catappa*	Combretaceae	Erin mado	Fruits	66.67
9.	*Ocimum gratissimum*	Lamiaceae	Efinrin	Leaves	100
10.	*Alternanthera nodiflora*	Amaranthaceae	Dagunro	Leaves	100
11.	*Anacardium occidentale*	Anacardiaceae	Kandju	Stem or root barks	100
12.	*Cajanus cajan*	Fabaceae	Otili	Young stems, leaves	33.33

13.	*Citrus aurantifolia*	Rutaceae	Osan wewe	Fruits and Leaves	33.33
14.	*Zanthoxylum zanthoxyloides*	Rutaceae	Orin ata	Root bark	100
15.	*Vernonia amygdalina*	Asteraceae	Ewuro	Young stem	100
16.	*Prosopis africana*	Fabaceae	Pako ayan	Root bark	33.33
17.	*Azadirachta indica*	Meliaceae	Dongoyaro	Stem and root bark	33.33
18.	*Mascularia accuminata*	Rubiaceae	Pako Ijebu	Stem and root bark	33.33
19.	*Agelenopsis chevaleri*	Oliaceae	Igo	Root bark, leaves	33.33
20.	*Kalanchoe crenata*	Crussulacceae	Odundun	Leaves	33.33
21.	*Bryophyllum pinnatum*	Crussulacceae	Abamoda	Leaves	66.67
22.	*Emilia coccinea*	Asteraceae	Odundun owo	Leaves	66.67
23.	*Mannihot esculeta*	Euphorbiaceae	Paki	Leaves, tuber skin	45.33
24.	*Alchornea cordifolia*	Euphorbiaceae	Akowo	Twig, Root	33.33
25.	*Allophylus africanus*	Sapindaceae	Eekan-ehoro	Twig, Root	33.33
26.	*Aulococalyx jasminiflora*	Rubaceae	Ekaju	Twig	33.33
27.	*Baphia nitida*		Iyerosun	Twig	66.67
28.	*Carpolopia lutea*	Pologalaceae	Otupe	Twig, (stem)	33.33
29.	*Cassipourea bartei*		Iroko ekun	Twig (stem)	33.33
30.	*Dennettia tripetala*	annonaceae	Igberi		33.33
31.	*Garcinia kola*	Clusiaceae	Orogbo	Fruits	100
32	*Bridelia ferruginea*	Euphorbiaceae	Epo ira	Bark	70
33	*Pseudocedrela kotschyi*	Meliaceae	Pako emi gbegiri	Leaf, stem bark, twig	42
34	*Jatropha gossypifolia*	Euphorbiaceae	Ewe lapa funfun ati pupa	Leaf	57
35	*Distemonanthus benthmianus*	Fabaceae	Pako ayan	Twig,stem ,bark	57.2
36	*Nicotiana tabacum*	Solanaceae	Ewe taba	Leaf	58
37	*Olax subscorpioidea*	Olacaceae	Egbo ifon	Stem/root	54.32
38	*Alstonia booni*	Apocynaceae	Eso boni	leaf	42.5
39	*Sorindeia warneckei*	Asparagaceae	Egbo meyinro	Stem	29

The phytochemical analysis

Phytochemical screening of crude extracts of *O. subscorpioidea* and *B. ferruginea* indicated that both plants had saponins, alkaloids, phenols and tannins. *O. subscorpioidea* has more of glycosides (cardiac) and saponins than *B. ferruginea*. The components, anthraquinones and flavonoid were absent in both plants (Table 2).

Table 2: Phytochemical components of the crude extracts

S/N	Bioactive Constituent	*Olax subscorpioidea*	*Bridelia*
1	Alkaloid	+++	+++
2	Saponin	+++	++
3	Tannin	+++	+++
4	Steroid		+
5	Cardiac glycoside	+++	+
6	Terpenoids	+++	+++
7	Flavonoids		
8	Phenols	+++	+++
9	Carbohydrate		
10	Anthraquinone		

Key: + present; - Absent

The results from antimicrobial assay showing the zone inhibitions

O. subscorpioidea methanolic extract was not active against any of the bacterial strains that were tested in this work but showed a dose dependent activity against *Aspergillus fumigatus* (a fungus). The details of the zone of inhibition, the bacterial strains used, the concentration of the extract used are listed in Table 3. The control which was sterilized distilled water yielded no zone of inhibition.

Table 3: Zone of inhibitions due to the antimicrobial effect of *Olax subscorpioidea*

Extract conc. Zone inhibition of	400mg/ml (mm)	200mg/ml (mm)	100mg/ml (mm)	50mg/ml (mm)	Sterile distilled water (mm)
Fungi					
A. fumigatus	26.50	24.00	22.50	20.00	-
C. albicans Bacteria					-
P. aeruginosa					-
Streptococcus spp					-
S. aureus					-
L. acidophilus					-

n = 2; Key: -- no growth

Methanolic crude extract of *Bridelia ferruginea* exerted microbial activity against all the bacterial strains that were tested in this work but showed no effect on the tested strains of fungi. A dose depended antimicrobial effect was observed in all the bacterial strains, that is as the concentrations increased, there was a corresponding increase in the zones of inhibitions. The degree of sensitivity was more on *Streptococcus spp,* followed by the *L. acidophilus,* which are key players in dental plaque (Table 4). An immeasurable zone of inhibition was observed around the higher doses for *C. albicans.* This will be regarded as no zone of inhibition in this study.

Table 4: Zone of inhibitions due to the antimicrobial effect of *Bridelia ferruginea*

Extract conc. 400mg/ml 200mg/ml 100mg/ml 50mg/ml Sterile distilled Zone of inhibition water	(mm)	(mm)	(mm)	(mm)	(mm)
Fungi					
A. fumigatus	--	--	--	--	--
C. albicans	--	--	--	--	--
Bacteria					
P. aeruginosa	22.25	21.50	19.00	15.75	--
Streptococcus spp	34.00	28.50	26.00	25.00	--
S. aureus	22.50	20.50	19.00	16.00	--
L. acidophilus	27.00	24.00	22.75	19.50	--

n = 2; Key: -- no growth

Table 5 shows the zones of inhibition of the organisms due to the antimicrobial activities of the chlorhexidine gluconate, used as standard. The zones of inhibition for all the test isolates using this standard drug ranged from 12 mm for C. *A. fumigatus* to 33 mm for *S. aureus* at the concentration of 0.625%.

Table 5: Zone of inhibitions due to the antimicrobial effect of chlorhexidine gluconate used as standard

Standard conc.	5%	2.5%	1.25%	0.625%	Sterile distilled
Zone of Inhibition	**(mm)**	**(mm)**	**(mm)**	**(mm)**	**distilled water**
Fungi					
A. fumigatus	18.50	16.00	13.50	12.00	--
C. albican	26.50	24.50	23.00	16.00	--
Bacteria					
P. aeruginosa	35.00	31.00	31.00	28.00	--
Streptococcus	38.00	37.50	37.00	32.00	-- *sp*
S. aureus	38.00	38.00	36.50	33.00	--
L. acidophilus	39.00	37.00	34.00	31.00	--

n = 2; Key: -- no growth

The determination of the minimum inhibitory concentration (MIC) of the crude drugs *O. subscorpioidea and B. ferruginea* was to provide a minimum concentration (MIC) of the extracts that would inhibit the susceptible etiological agents. The methanolic extract of *O. subscorpioidea* showed highest MIC values at 51.2 mg/ml against *A. fumigatus* while the methanolic extract of *B. ferruginea* revealed its highest MIC at 25.6 mg/ml against *S. aureus*, *P. aeruginosa, L. acidophilus* and 0.1 mg/ml for *Streptococcus spp* (Tables 6 and 7).

Table 6: Minimum Inhibitory Concentration (MIC) result for *Olax subscorpioidea*

S/N	Concentrations Plate (mg/ml)	ON	*Aspergillus fumigatus*
1	0.1		++
2	0.2		++
3	0.4		++
4	0.8		++
5	1.6		++
6	3.2		++
7	6.4		++
8	12.8		++
9	25.6		++
10	51.2		-

Key: ++ growth present, -- no growth present

The minimum inhibitory concentration of *Olax subscorpioidea* is 51.2 mg/ml

Table 7: Minimum Inhibitory Concentration (MIC) result for *Bridelia ferruginea*

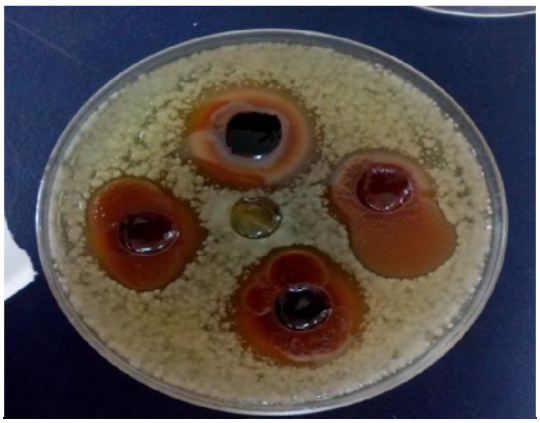
Conc. (mg/ml)	*Streptococcus spp*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Lactobacillus acidophilus*
0.1	-	++	++	++
0.2	-	++	++	++
0.4	-	++	++	++
0.8	-	++	++	++
1.6	-	++	++	++
3.2	-	++	++	++
6.4	-	++	++	++
12.8	-	++	++	++
25.6	-	-	-	-
51.2	-	-	-	-

Key: ++ growth present, -- no growth present

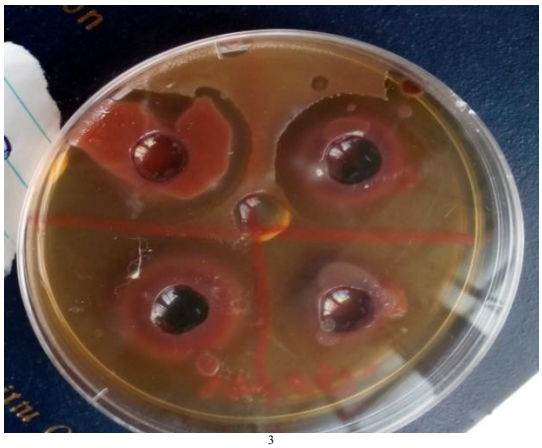
a) *Lactobacillus acidophilus*

b) *Aspergillus fumigatus*

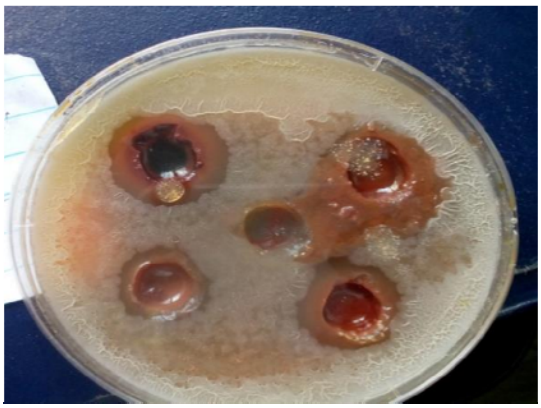
Fig 1: Antimicrobial activities of *Olax subscorpioidea* at various concentrations on



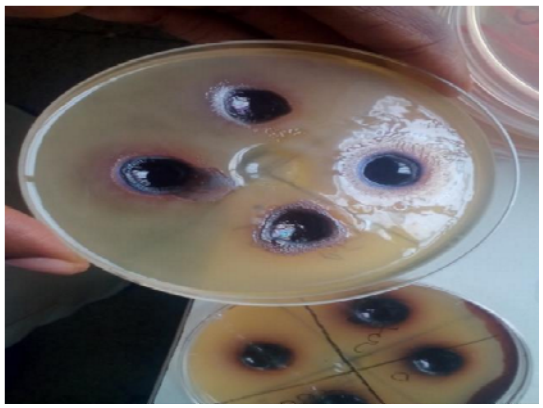
a) *Lactobacillus acidophilus*



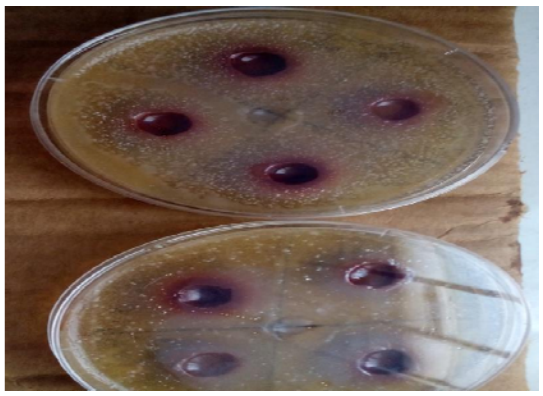
b) *Streptococcus spp*



c) *Staphylococcus aureus*

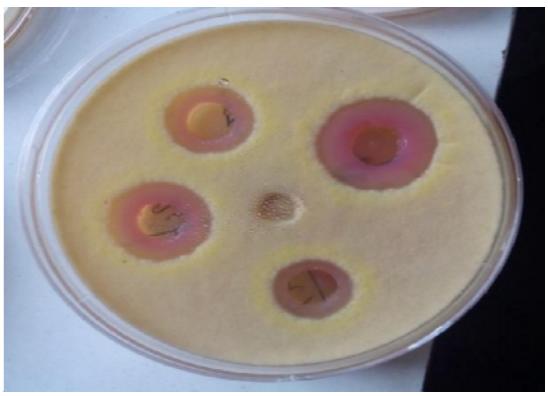


d) *Pseudomonas aeruginosa*

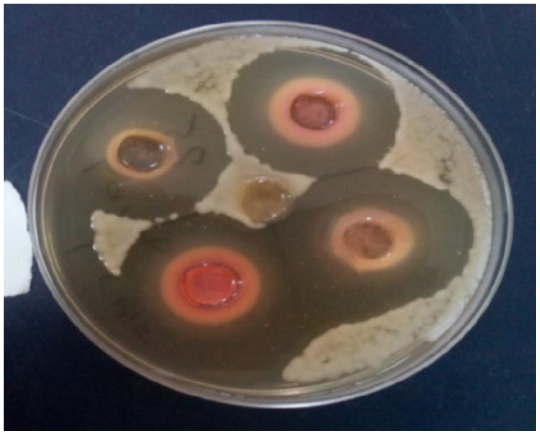


e) *Candida albican*

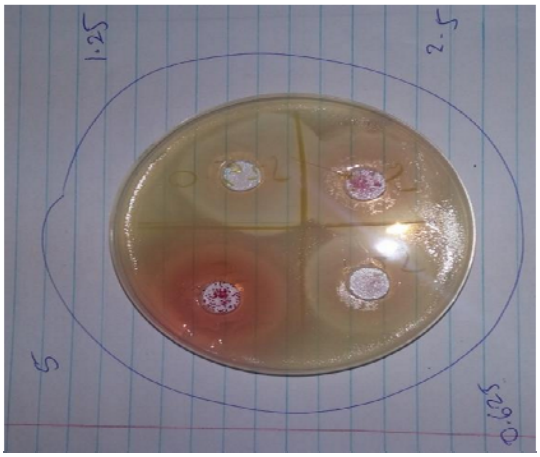
Fig 1: Antimicrobial activities of *Bridelia ferruginea at various concentrations*



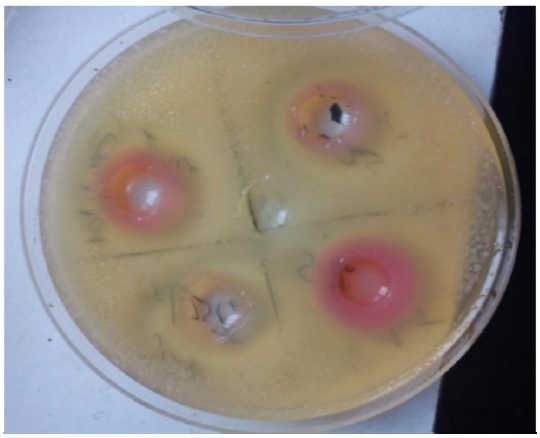
a) *Aspergillus fumigatus*



b) *Lactobacillus acidophilus*



c) *Staphylococcus spp*



d) *Candida albicans*

Fig 1: Antimicrobial activities of Chlorhexidine gluconate
Chlorhexidine gluconate

Discussion

Dental plaque is a major risk factor associated with the development of dental and periodontal diseases [34, 35]. Recent investigation has reported that the cause of dental plaque is as a result of a complex biofilm consisting of a variety of bacteria embedded in a polysaccharide matrix that allows for bacteria to exhibit pathogenic characteristics rather than being an accumulation of bacteria [36]. It is also recognized that mechanical tooth brushing can only remove approximately only 50% of dental plaque. Additional aid in terms of antimicrobial agents may be needed to further reduce the bacterial load with the view of preventing or managing dental health. Thus an effective dental health management may require the use of antimicrobial agents to reduce plaque forming micro-organisms before the biofilm is formed [35]. Many studies have reported on the effectiveness of various chewing sticks and plants in prevention and management of orodental challenges [37]. Herbal toothpastes and other mouth care products containing extracts of these medicinal plants and chewing sticks are available in the market [38, 39, 40].

This study established that methanolic extracts of *B. ferruginea* and *O. subscorpioidea* possess antibacterial and anti-fungal activity respectively against the tested clinical isolated microorganisms which were all sensitive to the reference drug chlorhexidine gluconate at minimum concentrations *in vitro*. When compared to the reference drug, the anti-infective ability of the tested extracts were relatively lower, however, purification processes such as fractionation and isolation of active components of the active extracts may improve their observed activity.

Antimicrobial effect of etthanolic and methanolic extracts of Bridelia ferruginea bark extracts have been reported to exhibit varying responses to some pathogenic microorganisms such as *B. substilis*, *S. aureus*, *E. coli*, *A. niger* and *F. solani*. Different studies revealed that the medium of extraction, strain of the isolate, differences in the concentration of active ingredient in the chewing stick extracts, microbial load used for the anti-microbial assay and source of collection of the plants are capable of bringing about differences in the susceptibility profile [41, 42, 43]. Methanol extract of *B. ferruginea* leaf was reported to be very effective against *Aspergillus* species where the most sensitive was *A. fumigatus* [44] while Owoseni (2010) reported zero activity against the fungi tested for both leaf and stem bark extracts [45]. The anti-fungi growth inhibition of aqueous extract of *B. ferruginea* stem bark was reported to be low by Jose1 and Kayode 2009 which is comparable to observed activity with *C. albican* in this study using methanolic extract. The inability of the methanolic extract of *B. ferruginea* at the doses tested to inhibit significant growth of the test fungi may be as a result of strains developing resistance to the antimicrobial agent(s) in the plant extract or a higher dose needed or absence of active agents.

The no activity of *O. subscorpioidea* methanolic extract on all the tested isolates except *A. fumigatus* may be suggesting that the plant extract contained no active phytochemical against the isolates or that the method of extraction did not yield any active compound(s) with pharmaceutical effect against the tested isolates [46, 47]. This observation is supported by Valenciennes *et al.,* (2001) who reported that antifungal agents might not necessarily exhibit antibacterial effects [48]. Ayandele and Adebiyi 2007 reported the ethanolic extract of *O. subscorpioidea* as abroad spectrum agent against both gram positive and gram negative bacteria and also fungi while the aqueous extract of the same plant on the same isolates has much less active agent [27]. The contradiction in the Ayandele and Adebiyi (2007) results and the present study may lie on the medium of extraction, different microorganism strains and development of resistance to the anti-infective agent [27].

An antimicrobial activity of plant extract with highly active antimicrobial agent gives a low MIC while a low activity against a microorganism has a high MIC (Olasehinde *et al.*,

2016) [49]. Thus the low 0.1 mg ml^{-1} MIC of *B. ferruginea* for *Streptococcus spp* is an indicative of potent antibacterial activity. The minimum inhibitory concentration (MIC) for *A. fumigatus* is 51.2 mg ml^{-1} meaning that higher doses of *O. subscorpioidea*, will be required in infections where *A. fumigatus* is the aetiologic agent. The high MICs (Table 6 and 7) of the extracts could be due to resistance level of the test isolates.

Phytochemical analysis carried out on the study crude extracts *B. ferruginea* and *O. subscorpioidea* stem barks detected the presence of alkaloids, tannin, cardiac glycosides, polyphenols and saponins and was negative for flavonoids (Owoseni *et al.,* 2010; Ayendele and Adebiyi 2007). The inhibitory effects of these medicinal plants on the microorganisms may be due to the presence of the above phytochemical components.

Conclusions and Recommendations

Based on the pharmacological results of the present study, it could be said that the plant extracts contain chemical constituents for good oral health care. This means that regular use of these chewing sticks may decrease the incidence of dental disease cause by microbes. Further research in synergistic action between the two plant crude extracts in a combined formulation is recommended bearing in mind that *B. ferruginea* has good antibacterial activity while *O. subscorpioidea* exhibited susceptibility to fungi infection. Such synergies can be utilized to prevent or reduce the ease of development of resistance, growth of oral opportunist pathogen and targeting of a broad spectrum of microorganisms.

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