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Research Article

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Anti-microbial activities and phytochemical screening of some commonly used chewing sticks in Kano, Nigeria

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ABSTRACT

This research work was aimed to determine the antibacterial activity of aqueous and ethanolic extract of plants commonly used in Kano on a clinical isolate of *Staphylococcus* and *Streptococcus* species obtained from the dental problem with a view to find the most efficacious one among them. The sensitivity disc method was used to test the antibacterial activity of chewing sticks, *Eucalyptus globulus*, *Salvadora persica*, *Gledistsia triacanthos*, *Azadirachta indica* and *Jatropha curcas* were the plants. It was found that none of the plants 'aqueous extract had activity on the two species of the bacterial isolate at various concentrations. But ethanolic extract was active against all the test bacterial isolate obtained from the dental problem with a greater zone of inhibition in *A. Indica*, followed by *E. globulus* and a smaller zone of inhibition in *J. curcas*. Some of the secondary metabolites were all present with high content in ethanolic extract. The extracts of these plants may serve as sources for chemotherapeutic agents of the management of orofacial infection.

KEYWORDS: *Staphylococcus*, *Streptococcus*, chewing stick, infection, plant-extract

INTRODUCTION

Our mouth contains a variety of microorganisms but few specifically engage in dental caries. Bacterial invasion causes demineralization and destruction of hard tissues of teeth. The acid production by bacteria causes accumulation of tooth surface, finally producing dental caries. Several bacteria are responsible for dental caries and periodontal infections i.e. *S. sobrinus* , *Lactobacillus acidophilus* , *Actonomyces* spp., *N. ocardia* spp., *Camphylobacter*, *Fusobacterium*, *Haemophilus*, *Prevotella*, *Porphylomonas*, *Veillonella* [1,2]. Some of these organisms produce high level of lactic acid causing fermentation of dietary sugars and are resistant to the adverse effect of low pH [3].

In Nigeria, as in other developing countries, a very significant proportion of orofacial diseases are due to microbial infections [4]. This being the case, there is widespread use of antibiotics in dental practice in these regions and this gives microorganisms' enhanced opportunities for the development of resistance to a broad spectrum of antibiotics. Antibiotics are also widely used and misused in the management of other infections within the regions [5].The need to conserve antibiotics in order to prevent the selection of antibiotics resistance organisms has now been recognized and there is, therefore, the need to work for non-antibiotics substances with proven antimicrobial activity, which can be used in the treatment of microbial infections, including those that are encountered in dental practice [6].

Chewing sticks are important Non Timber Forest Product (NTFP) widely used for dental cleaning in the tropical West Africa [7]. Plants from which chew sticks are derived are abundant and diverse in Nigerian rural communities. Almost the entire rural population of Nigeria uses chewing sticks for orodental hygiene. Chewing sticks are recommended for oral hygiene by the World Health Organization, and some of them, or their extracts, are also used in the ethnomedical treatment of oral infections [8].

Previous studies have demonstrated the antiplaque and antibacterial actions of extracts of these Nigerian chewing sticks (NCS) against oral bacteria, such as *Streptococcus mutans*[9], *Streptococcus mitis* and oral anaerobes [10], which are the organisms commonly indicated in dental caries and orodental infections. All surfaces in the mouth are colonised by a resident microflora that is highly diverse in composition [11]. The largest numbers of microorganisms are found on the tooth

surfaces, especially at stagnant sites and are termed dental plaque, the composition of which varies at distinct surfaces (e.g. approximal surfaces, and the gingival crevice) due to the prevailing biological properties of the site.

MATERIALS AND METHODS

Plant Materials Chewing sticks from five plant species, commonly used in Kano State region, in Nigeria, were collected in October, 2019. Chewing sticks were identified by asking people to show which plants and plant parts they used for toothbrushing. The plants were taxonomically identified in Biological sciences Department, Yusuf Maitama Sule University, Kano State. Voucher specimens are deposited in the Herbarium of the Department.

EXTRACT PREPARATION

The root and branch of the test plants were well dried at the room temperature and then ground to powder using mortar and pestle. The powders were stored in a cool dry place. About 10g of the each plants were separately soak in 100ml of distilled water and 95% ethanol in a bottle. This was allowed to stand in shaker for 7 days (a week) and 14 days (2 weeks) respectively to allow full extraction of the active ingredients. The fluids were then filtered using whatman No 1 filter paper. The extracts were dried using water bath to obtain the concentration. It was then kept in refrigerator prior to use. The residues of both aqueous and ethanolic extract for each plant were in (4000, 2000, 1000 and 500) ug concentrations needed for the bioassay [12].

TEST ORGANISMS

Clinical isolates were recovered from patient with dental disease and cultured in the medical microbiology laboratory, Pathology Department Aminu Kano Teaching hospital (AKTH), Kano, Nigeria. The clinical isolates were identified and confirmed by conventional biochemical techniques as described by Checesbrougth, 2004 [13]. The staphylococcus was maintained on nutrient agar slant and streptococcus was maintained on blood agar slant.

Phytochemical Analysis of Chewing Stick Extracts

Qualitative screening of the phytochemical components of the chewing sticks were carried out using the method outlined by [14] to detect the presence of glycosides, alkaloids, saponin, tannins, flavonoids and reducing sugar.

Phytochemical Screening

The phytochemical analysis of plant extracts were carried out by standard qualitative methods [15,16].

Test for alkaloids

The test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloid.

Test for flavonoids

On addition of conc. HCl in methanolic extract of the material, a red colour appeared which indicated the presence of flavonoids.

Test for tannins

Extract was added in 1% ferric chloride and the colour was observed. Bluish black colour appeared which disappeared on addition of dilute H_2SO_4 followed a yellow brown precipitate showed the presence of tannins.

Test for saponins

Extracts were diluted with water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

Nutrient agar was prepared using standard microbiological procedure and carefully poured in to sterile Petridishes to solidify, they were then streak with clinical isolates as described previously. One disc with appropriate potency (500ug/disc, 1000ug/disc 2000ug/disc and 4000ug/disc) was picked for each concentration and aseptically placed on the plates, both positive and negative controls were prepared. The former was set up using tarivid acid on the streaked plates while the later was set up with disc containing DMSO. The plates were incubated aerobically at 35^0C for 16-18 hours

RESULTS AND DISCUSSION

The physical characteristics of aqueous extract presented in tables (Tables 1 and 5-7) above shows that, among five different chewing sticks *Salvadora persica* yield more extract than *Eucalyptus globulus*, *Azadirachta indica* and *Gledistsia triacanthos* while *Jatropha curcas* was the least. In ethanol extracts also *Salvadora persica* showed higher yield of extract than the rest (Table 2).

Table 1: Physical characteristics of the aqueous extracts

Extract	Original weight perculated(g)	Weight recovered(g)	Coloured	Appearance	Texture
Salvadora persica	10	1.30	Brown	Solid	Bristle
Azadirachta indica	10	1.00	Brown	Solid	Bristle
Jatropha curcas	10	0.90	Milk	Creamy	Soft
Gledistsia triacanthos	10	1.00	Yellow	Solid	Bristle
Eucalyptus globulus	10	1.01	Milk	Solid	Bristle

Table 2: Physical characteristics of ethanolic extracts

Extract	Original weight perculated(g)	Weight recovered (g)	Coloured	Appearance	Texture
Salvadora persica	10	1.32	Brown	Solid	Bristle
Azadirachta indica	10	1.10	Brown	Solid	Bristle
Jatropha curcas	10	0.78	Milk	Creamy	Soft
Gledistsia triacanthos	10	1.20	Yellow	Solid	Bristle
Eucalyptusglobulus	10	0.90	Milk	Solid	Bristle

Table 3: Sensitivity (mm) of streptococcus isolates of dental disease to aqueous plants extracts

Extract	Disc potentency (ug/disc)			
	500	1000	2000	4000
Salvadora persica	0	0	0	0
Azadirachta indica	0	0	0	0
Jatropha curcas	0	0	0	0
Gledistsia triacanthos	0	0	0	0
Eucalyptus globulus	0	0	0	0

Table 4: Sensitivity (mm) of streptococcus isolates of dental disease to aqueous plants extracts

Extract	Disc potentency (ug/disc)			
	500	1000	2000	4000
Salvadora persica	0	0	0	0
Azadirachta indica	0	0	0	0
Jatropha curcas	0	0	0	0
Gledistsia triacanthos	0	0	0	0
Eucalyptus globulus	0	0	0	0

Table 5: Sensitivity (mm) of staphylococcal isolates of dental dieses to ethanolic plants extracts

Extract	Disc potentency (ug/disc)			
	500	1000	2000	4000
Salvadora persica	0	07	09	12
Azadirachta indica	0	08	10	15
Jatropha curcas	0	00	08	10
Gledistsia triacanthos	0	00	10	11
Eucalyptus globulus	0	07	08	14

The sensitivity of staphylococcus and streptococcus specie to the aqueous extract of the tested chewing sticks had no activity as shown in Table 3 and 4. Even though they contained most of the phytochemicals present in ethanolic extract the fact that the plant are being used as local chewing sticks may be suggesting that the level of these photochemical might be low. While these tested chewing sticks in ethanol extract revealed that the antibacterial compounds. In addition, the result revealed that the antibacterial activities of the five different tested chewing sticks vary and are target- microbe specific of the five extract, that of *Azadirachta indica* was the most effective against staphylococcus and streptococcus specie, followed by the *Eucalyptus globulus*, *Salvadora persica* and then *Gledistsia triacanthos* whereas *Gledistsia triacanthos* has least activity to all tested bacterial isolates. Bioactivities of these chewing sticks would be consequent upon the phytochemicals they contained in Table 8.

Table 6: Sensitivity (mm) of streptococcus isolates of dental dieses to ethanolic plants extracts

Extract	Disc potentency (ug/disc)			
	500	1000	2000	4000
Salvadora persica	00	00	07	10
Azadirachta indica	00	00	08	12
Jatropha curcas	00	00	00	00
Gledistsia triacanthos	00	00	07	10
Eucalyptus globulus	00	00	07	11

Table 7: Phytochemical constituent of aqueous extract

Sample	Alkaloid	Flavonoid	Soponim	Reducing sugar	Tannins
Salvadora persica	+	-	-	-	+
Azadirachta indica	+	-	+	+	+
Jatropha curcas	+	-	-	+	-
Gledistsia triacanthos	-	+	-	+	+
Eucalyptus globulus	-	+	+	+	+

(+) implies present (-) implies not detected

Table 8: Phytochemical constituents of ethanol extract

Sample	Alkaloid	Flavonoid	Soponim	Reducing sugar	Tannins
Salvadora persica	+	+	+	+	+
Azadirachta indica	+	+	+	+	+
Jatropha curcas	+	-	-	+	+
Gledistsia triacanthos	+	+	+	+	+
Eucalyptus globulus	+	+	+	+	+

(+) implies present (-) implies not detected

CONCLUSION

The present study has shown thatAqueous extracts of five specific type of chewing sticks used in this research show no activity on the isolates tested but, ethanolic extracts show activity on all test bacterial isolates of dental diseases with greater zone of inhibition in *Azadirachta indica*. Stems and bark extracts possess a broad spectrum activity against a panel of bacteria responsible for most dental diseases. This study can boast a new possibility for finding novel clinically effective antimicrobial compounds.

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