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COMPARATIVE ANTIBACTERIAL ACTIVITY OF CHEWINGSTICKS AND TOOTPASTE COMMONLY USED IN KANO (NIGERIA) ON CLINICAL ISOLATES OF STAPHYLOCOCCUS AND STREPTOCOCCUS SPECIES

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ABSTRACT: A number of plants and their part are used as chewing sticks in Kano, Nigeria. Different researches have been carried out on the antimicrobial effect of chewing sticks on oral micro organisms. This research work was aimed to determine the antibacterial activity of aqueous and ethanolic extract of plants and five different types of conventional toothpaste, commonly used in Kano on clinical isolate of staphylococcus and streptococcus species obtained from dental problem with a view to find the most efficacious one among them, sensitivity disc method was used to test the antibacterial activity of chewing sticks, *A. lebbeck*, *J. curcas, A. Indica, N. latifolia and V. amydalina* were the plants and Toothpaste are Dabur, Florish, Close up, Maclean and Mymy. It was found that none of the plants 'aqueous extract had activity on the two species of the bacterial isolate at various concentrations. But in ethanolic extract were active against all the test bacterial isolate obtained from dental problem with greater zone of inhibition in *N. latifolia*, followed by *A. Indica* and smaller zone of inhibition in *J. curcas*. In comparism with conventional toothpaste, chewing sticks produced similar or greater zone of inhibition than Mymy (toothpaste). Some of the secondary metabolites were all presents with high content in ethanolic extract. The extracts of these plants and toothpastes may serve as sources for chemotherapeutic agents of the management of orofacial infection.

KEYWORDS: *Staphylococcus, streptococcus,* chewing stick, toothpaste, infection.

INTRODUCTION

In Nigeria, as in other developing countries, a very significant proportion of orofacial diseases are due to microbial infections. (Adekeye and Praphu, 2002). 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 (Okeke et al, 1999) the need to conserve antibiotics in order to prevent the selection of antibiotics resistance organisms has now been recognized (levy, 1997) 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.

Good oral hygiene is necessary for healthy teeth, gums and fresh breath. A number of methods are used in oral hygiene to prevent and cure oral disease. It is of pertinent importance to look at the roles plants and toothpastes play in oral hygiene as a number of them are believed to have antimicrobial properties.

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Chewing sticks are used widely in Africa and Asia as a means of maintaining oral hygiene (Otuyemei and wu, 2001). They are made from roots or stem of plants. The prepared part is cleaned to remove dirt and cut to convenient length which varies from 15-30cm long. The user put one end directly into his mouth to scrub the surface of the teeth. A combination of vertical and horizontal stroke of the "brush" on teeth surfaces removing plaque the tongue is scrubbed as well. Cleansing movement is directed away from the gingival merging to avoid damage to the gums. Chewing sticks are used in the morning before breakfast and at night after supper for daily oral hygiene maintenance. About five minutes of completed devotion to this exercise is deemed adequate to achieve good cleansing. According to sote and Wilson, 1995. Chewing sticks obtained from a variety of selected plants are used as a traditional method of mechanical oral hygiene by up to 80-90% of Nigerians. Studies of Danielsent et al, 1989, varpalentstein helderman et al, 1992, aderinokun et al, 1999, and alzied, 2004 have demonstrated chewing sticks are at least as effective as tooth brushes in maintaining oral hygiene. Sathananthan et al, 1996 reported Africans that use chewing sticks have fewer carious lesions than those that use tooth brushes, and their use has been encouraged by the world health organization (Almas and Al-lafi, 1995). Toothpaste has a history that stretched back nearly 4000 years. Different abrasives, green lead, increase were use to clean strain from teeth until mid nineteenth century. In Middle Ages, fine sand and pumice were the primary ingredients the tooth cleaning formulas used by Arabs. In 950AD, Dr. Washington wentworh Sheffield, a dental surgeon and chemist, invented the first toothpaste (Lee et al, 2004) then, the market of the toothpaste have never been slowed down. Modern toothpaste was invented to aid in the removal of foreign particle and food substances in addition of cleaning of tooth. During 1940-60AD, fluoride was added which aided in protection from tooth decay. Many of the innovations were in toothpaste after the fluoride break through which involved the addition of ingredient with special ability to toothpaste and toothpaste packaging (Hawkins and Clarke, 2003). Dental problems are the most common health problem in the human communities (Locsche, 1996). Dental infections are mainly of three (3) types, viz:- formation of dental plaques, dental caries and periodontal diseases (Larke, 1942). Dental plaque is material adhering to teeth, which consists of bacterial cell (60-70% of the volume of plaque) salivary polymers and bacterial extracellular products. Plaque is a naturally constructed biofilms of bacteria, which may reach thickness of 300-500 cells on the teeth. The very normal flora of the oral activity, S. mutants and S. sanguis are the most dominate bacterial species in dental plaque. After initial weak attachment of streptococcal cells to salivary glycoprotein, stronger attachment takes place by polymer of glucose (glucan) synthesized by bacteria. A dental carier is the destruction of enamel, denting or cement of teeth due to bacterial activities. Caries are initiated by demineralization of the enamel of teeth due to lactic- acid bacteria, Actinomyces and various proteolytic bacteria are commonly found in human caries as secondary invaders, contribution to the progression of the lesion. Periodontal disease are bacterial infections that affect the supporting structure of the (gingival, cementum, periodontal membrane and alveolar bone) the endotoxins, hydrolytic enzymes and toxic bacterial metabolites are involved in this disease. Gingivitis, an inflammatory condition of gums, is the most common form of periodontal disease. Serious forms of periodontal disease that affect the periodontal membrane and alveolar bone may result in tooth loss. Streptococci, actinomycetes, spirochetes and bacteria's are the possible micro organisms responsible for the disease.

MATERIALS AND METHOD

The fact that, there exist high incident of tooth extractions among Kano population even though, the use of chewing sticks and tooth paste is very common among the populations. This research was aimed to determine the antibacterial activity of aqueous and ethanol extract from five (5) different type of tooth pastes commonly use in Kano on clinical isolates of staphylococcus and streptococcus specie obtained from dental problems with a view to find the most efficacious ones among them.

SOURCE OF PLANT MATERIALS

The chewing sticks selected were obtained from the branch of *Azadirechta indica* (neem tree), *Jatropha curcas* (termite plant) *Nauclear diderrichii or nauclear latifoia* (African peach) and *Vernonia amygdalina* (bitter leaf) and the root of *Albizia lebbeck* (lebbeck tree or fry wood). They were all collected from kiru local government area of Kano state, Nigeria the plants were authenticated by a botanist at the department of biological sciences, bayero university, Kano Nigeria.

SOURCE OF TOOTHPASTE

The toothpaste selected were obtained from jujin- labu stores Kano, Nigeria, these toothpastes are dabur, florish, maclean close up and my-my.

EXTRACT PREPARATION

The root and branch of the test plants where well dried at the room temperature and then grinded 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 distill 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 permit 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 fridge prior to use. The residues of both aqueous and ethanolic extract for each plant were in to (4000, 2000, 1000 and 500) ug concentrations needed for the bioassay. (mukhtar and tukur, 2001).

PREPARATION OF CONCENTRATIONS

PREPARATION OF PLANT EXTRACTION CONCENTRATION

0.5g of each of the residue were weighted using an electronic weight balance, 0.5ml of distill water and dimethyl sulphoxide (DMSO) was then added to both aqueous and ethanol extract respectively.

For 4000ug/ml, 0.2ml of stock solution was withdrawn using 1ml syringe and 0.05ml of distill water and dimethyl sulphoxide (DMSO) was added for the each extracts.

For 1000ug/ml, 0.5ml of the stock solution was withdrawn using 1ml syringe and 0.2ml of distill water and dimethyl sulphoxide (DMSO) was added for the each extracts

For 500ug/ml, 0.5ml of stock solution was taken from separate prepared 1000ug/ml and 0.5ml of fresh dimethyl sulphoxide (DMSO) was added for the each extracts.

25 discs 6mm in diameter were punched using whatman no 1 filter paper, and sterilized bottle was used by dry heat at 140^0C for 60mm. each successive sterile 25 discs were poured into each of the preferred concentrations. Discs were assumed to have absorbed appropriate potency of 4000ug/disc, 2000ug/disc, 1000ug/disc and 500ug/dics

PREPARATION OF TOOTHPASTE CONCENTRATION

1g of each toothpaste were weight using an electronic weight balnce,1ml of distil water was then added to each of the toothpastes.

For 4000ug/ml, 0.4ml of stock solution was withdrawn and 0.6ml of distill water added to each.

For 2000ug/ml, 0.2 of stock solution was withdrawn and 0.8ml of distill water was added to each

For 1000ug/ml, 0.1ml of stock solution was withdrawn and 0.9ml of distill of water was added to each.

For 500ug/ml, 0.5ml was withdrawn from preferred 1000ug/ ml and 0.5 ml of fresh dim ethyl sulphoxide (DMSO) was added to each.

100discs were punched using whatman No 1 filter paper, and sterilized in the oven at 140^0C for 60min. each prepared concentrations. disc were assumed to have absorbed appropriated potency of 4000ug/disk, 200ug/disk, 1000ug/ disc and 500ug / dick.

TEST ORGANISMS

Clinical isolate were recovered from patient with dental disease were cultured in the medical microbiology laboratory, pathology department Murtala Muhammad specialist hospital (NMSH) Kano, Nigeria and were identified and confirmed by conventional biochemical techniques as described by cheise brough, 2004. The staphylococcus was maintained on nutrient agar slant and streptococcus was maintained on blood agar slant.

ANTIMICROBIAL SUSCEPTIBILITY TESTING

DISC DIFFUSION TECHNIQUES

Nutrient agar was prepared using standard microbiological procedure and carefully poured in to sterile Petri dishes to solidify, they were then streak with clinical isolates as described by Kirby – Bauer, 1996 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 letter was set up with disc containing DMSO. The plates were incubated aerobically at 35^0C for 16-18 hours.

PHYTOCHEMICAL SCREENING

The extracts were subjected to phytochemicals tests to determine their groups of secondary metabolites present in the plant material.

TEST FOR ALKALOIDS

To 10.ml of each extract in two separate test tubes, 2 3 drops of dragendoffs and Meyers reagents were separately added. An orange red precipitate/turbidy with dragendoff's reagent or white precipitate with meyer's with reagent would indicated the presence of alkaloids (Cuilci, 1994).

TEST FOR FLAVONOIDS

To 4mg/ml each of the extracts, a piece of magnesium ribbon was added followed by cone. HCL drop wise A colour ranging from orange to red indicated flavones; red to crimson indicates flavonod while crimson to magenta indicated flavonoids (Sofowara, 1993).

TEST FOR SAPONINS

0.3ml of extracts was taken in a test tube to which 3.0ml of distilled of water was added and vigorously shaken. A present froth that lasted for at least 15minutes indicate the presence of saponins (Brain and turner, 1975).

TEST FOR REDUCING SUGAR

One ml of each extracts was taken in separate test tube, these were diluted with 2.0ml of distill water followed by additional of Fehling's solution (A+B) and mixture warmed, brick-red precipitate at the bottom of the test tubes indicated the presence of reducing sugars (Brains and turner, 1975)

TEST FOR STEROIDS

Two ml of each of the extracts was taken into separately test tube and evaporated to dryness. The residues were dissolved in acetic anhydride, and chloroform was then added. By means of a pipette concentrated sulphuric acid was added by the side of the test tube. A brown ring at the interface of the two liquids and the appearance of violet color in the supernatant layer indicated the presence of steroid (Cuilci 1994).

TEST FOR TANNINS

Two ml of each of the extracts was diluted with distilled water in separate test tubes and 2-3 drops of 5% ferric chloride ($FeCl_2$) solution was added. A green-black or blue-black colouration indicated the presence of tannins (Ciulci, 1994).

RESULTS AND DISCUSSION

Table 1. Physical characterastics of the aqueous extracts

Extract	Original weight perculated (g)	Weigth recovered (g)	Coloured	appearance	Texture
A. lebbeck	10	1.5	Brown	Solid	Soft
A. indica	10	1.10	Brown	Solid	Bristle
J. curcas	10	1.72	Milk	Creamy	soft
N. latifolia	10	0.96	Orange	Solid	Gummy
V. amygdalina	10	0.62	Brown	solid	bristle

Table 2. Physical characteristics of ethanolic extracts

Extract	Original weight perculated (g)	Weight recovered (g)	Coloured	appearance	Texture
A. lebbeck	10	1.5	Brown	Solid	Soft
A. indica	10	1.28	Brown	Solid	Bristle
J.curcas	10	0.78	Milk	Creamy	soft
N. latifolia	10	1.05	Orange	Solid	Gummy
V.amygdalina	10	0.89	Brown	solid	bristle

Table 3. Sensitivity (mm) of staphylococcal isolates of dental dieses to conventional toothpaste

	Disc potency (µg/Disk			
toothpaste	500	1000	2000	4000
Dabur	00	07	08	13
Florish	07	11	14	18
Close up	00	00	09	13
Macleans	07	10	14	18
mymy	00	00	00	09

Table 4. Sensitivity (mm) of streptococcal isolates of dental disease to conventional toothpastes

	Disc potency (µg/Disk			
toothpaste	500	1000	2000	4000
Dabur	00	07	10	12
Florish	07	12	16	20
Close up	00	07	09	12
Macleans	08	11	14	18
mymy	00	00	07	11

Table 5. Sensitivity (mm) of streptococcus isolates of dental disease to aqueous plants extracts

Extract	Disc potency (µg/disc)			
	500	1000	2000	4000
A. lebbeck	0	0	0	0
A. indica	0	0	0	0
J. curcas	0	0	0	0
N. latifolia	0	0	0	0
V.amygdalina	0	0	0	0

Table 6. Sensitivity (mm) of streptococcus isolates of dental disease to aqueous plants extracts

Extract	Disc potency (µg/disc)			
	500	1000	2000	4000
A. lebbeck	0	0	0	0
A. indica	0	0	0	0
J. curcas	0	0	0	0
N. latifolia	0	0	0	0
V. amygdalina	0	0	0	0

Table 7. Sensitivity (mm) of staphylococcal isolates of dental dieses to ethanolic plants extracts

Plant extracts	Disc potency (µg/disc)			
	500	1000	2000	4000
A. lebbeck	00	00	08	12
A. indica	00	07	09	13
J. curcas	00	00	08	10
N. latifolia	00	07	09	13
V.amygdalina	00	00	07	11

Table 8. Sensitivity (mm) of streptococcus isolates of dental dieses to ethanolic plants extracts

Plant extracts	Disc potency (µg/disc)			
	500	1000	2000	4000
A. lebbeck	00	00	08	10
A. indica	00	00	07	10
J. curcas	00	00	00	00
N. latifolia	00	00	07	12
V. amygdalina	00	00	07	10

Table 9. Phytochemical constituent of aqueous extract

sample	***Alkaloid***	***Flavanoid***	***Saponim***	***Reducing sugar***	***Tannins***
A. lebbeck	+	+	-	-	-
A. indica	-	-	-	+	+
J. curcas	+	-	-	+	-
N. latifolia	-	+	+	+	+
V. amygdalina	-	+	-	+	+

+ implies present
- implies not detected

Table 10. Phytochemical constituents of ethanol extract

sample	***Alkaloid***	***Flavanoid***	***saponim***	***Reducing sugar***	***Tannins***
A. lebbeck	+	+	-	-	-
A. indica	+	+	-	-	+
J. curcas	+	-	-	+	+
N. latifolia	+	-	+	+	+
V.amygdalina	-	+	-	+	+

+ implies present
- implies not detected

The physical characteristics of aqueous extract shown in tabled among five *J.curcas* different chewing sticks A. *indica* yield more extract than *A. leebbeck*, *N. natifolia* and *J. curcas* where as *V. amydalina* was the least. In ethanol extracts also *A. indica* showed higher yield of extract than th rest were as was the least as shown in table 2.

The antibacterial activitities of the tested toothpastes against staphylococcus and streptococcus specie shown in table 3 and 4 among the five tested toothpaste, florish showed a higher antibacterial activity and followed by Maclean, dabur, close up while my-my was the least effective. The sensitivity of staphylococcus and streptococcus specie to the aqueous extract of the tested chewing sticks had no activity as shown in table 5 and 6. Even though they were 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 very low. While these tested chewing sticks in ethanol extract revealed that the antibacterial compounds. In addition, the result reveal that the antibacterial activities of the five different tested chewing sticks vary and are target- microbe specific of the five extract, that of *N. latifolia* was the most effective against *staphylococcus* and *streptococcus* specie, followed by the *A. indica, A. lebbeck* and then *V. amygdalina* where as *J. curcas* has least activity to all test bacterial isolates bioactivities of these chewing sticks would be consequent upon the phytochemicals they contained in table 10. In comparism to conventional toothpaste, chewing sticks produced similar or greater zone on inhibition in *N. latifolia* and *A. indica* than florish (toothpaste).

CONCLUSION

Aqueous extracts of five specific type of chewing sticks used in this research showed no activity on the isolates tested but, ethanolic extracts showed activity on all test bacterial isolates of dental diseases with greater zone of inhibition in *N.latifolia.* The five different conventional thoothpaste used showed activity against all bacterial isolates of dendal diseases with greater zone of inhibition in florish. Thus gave reason why these toothpastes and extract of plants are suitable for better dental care.

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