British Journal of Pharmaceutical Research
11(5): 1-7, 2016, Article no.BJPR.26004
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

A Study of the Anticaries Activity of Three Common Chewing Sticks and Two Brands of Toothpaste in South West Nigeria

Odeleye Olubola Florence[1*], Okunye Olufemi Lionel[1], Kesi Christopher[1] and Abatan Temitope Olubunmi[1]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author OOF designed the study, supervised the experimental process and wrote the first manuscript. Author OOL carried out the literature search and verified the authenticity of the bacteria isolates. Author ATO sourced the chewing sticks and tooth pastes. Authors KC and ATO managed the experimental process. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/26004
Editor(s):
(1) R. Deveswaran, M.S. Ramaiah College of Pharmacy, Bangalore, India.
Reviewers:
(1) Armando Cuéllar Cuéllar, Havana University, Cuba.
(2) Miguel A. Sogorb, Miguel Hernández University, Spain.
(3) Triveni Krishnan, National Institute of Cholera and Enteric Diseases, Kolkata, India.
Complete Peer review History: http://sciencedomain.org/review-history/14473

Original Research Article

Received 29th March 2016
Accepted 21st April 2016
Published 5th May 2016

ABSTRACT

Aim: To evaluate three common chewing sticks and two brands of toothpaste in south west Nigeria for the ability to control caries-causing bacteria.
With an increase in tooth decay and gum diseases all over the world, there is need to produce oral cleaning agents that will better control caries-causing bacteria.
Study Design: Three common chewing sticks in South-West Nigeria- *Fagara zanthoxyloides* (FZ), *Vernonia amygdalina (*VA*)* and *Massularia accuminata (*MA*)*- and two brands of toothpaste- Close Up and Macleans- were evaluated for their anti-caries activities.
Fifty isolates of *Staphylococcus aureus*, one of the bacteria often implicated in dental caries, isolated from patients presenting with various dental problems at the University College Hospital (UCH), Ibadan, Nigeria were obtained from the Medical Microbiology Department of the Hospital.

**Corresponding author: E-mail: ayaode@yahoo.com;*

Methodology: The isolates were challenged with the toothpastes, undiluted, as well as ethanol and aqueous extracts of the chewing sticks using the agar cup diffusion method. The chewing sticks were also screened for secondary metabolites using standard procedures.
Results: The ethanol extracts of *Fagara zanthoxyloides* (FZ) showed the highest anti-caries activity followed by *Vernonia amygdalina* (VA) and then *Massularia acuminata* (MA). 43 isolates (86%) were sensitive to the ethanol extract of *Fagara zanthoxyloides* while 36 (72%) and 25 (50%) were sensitive to *Vernonia amygdalina* and *Massularia acuminata* respectively.
Both brands of toothpaste were inferior to the ethanol extracts of all the chewing sticks in anti-caries activity. Only 15 (30%) and 20 (40%) of the isolates were sensitive to Close up and Macleans respectively.
16 (32%), 14 (28%) and 10 (20%) of the isolates were sensitive to the aqueous extracts of FZ, MA and VA respectively.
Conclusion: The active constituents in the ethanol extracts of the chewing sticks will be useful as anti caries components of herbal toothpastes which are becoming common in the market.

Keywords: Chewing sticks; Fagara zanthoxyloides; Vernonia amygdalina; Massularia accuminata; Staphylococcus aureus; dental caries.

1. INTRODUCTION

Although there is more attention on oral and dental hygiene all over the world, tooth decay and other oral diseases are on the increase daily. This has been attributed to a change in eating habits and increased sugar consumption among people of different age groups [1].

The use of chewing sticks has been documented since ancient times [2]. They were widely used by the Israelites, Ethiopians, Egyptians and Nigerians.

In many African homes, teeth are cleaned in the morning by chewing the root or stem of certain plants, until they acquire brush like ends [3]. The fibrous end is then cleaned and it brushes the teeth thoroughly. In certain parts of West Africa, e.g. Ghana, Nigeria and Senegal, chewing sticks are used frequently during the day. These chewing sticks impact varying taste sensations which could be a tingling, peppery taste, a bitter taste or numbness [4].

Some of these chewing sticks have been shown to possess varying degrees of antimicrobial activity against oral microbial flora which indicates therefore, that the chewing sticks, in addition to providing mechanical stimulation of the gums, also destroy microbes, a feature which is absent in the common toothpaste and brush method. This advantage of the chewing sticks over the conventional toothpaste and brush could explain why many Africans have strong teeth [5]. The extracts of some chewing sticks have been demonstrated to have anti-plaque and antimicrobial activities against certain oral bacteria like *Streptococcus mutans, Bacteroides gingivitis* and oral anaerobes commonly implicated in dental caries and orodental infections [6].

Chewing sticks therefore can safeguard against dental problems, which is probably the reason why dental caries (decay) is not rampant in certain parts of Nigeria where the use of chewing sticks is frequent. Thus, the use of chewing sticks has been encouraged by the World Health Organization [7]. In Nigeria, about 80-90% of the population in rural areas use chewing sticks, mainly because they are readily available, cheap and efficacious [8]. The cleansing efficacy of chewing sticks is attributed to the mechanical effects of its fibers, the release of beneficial chemicals or a combination of both [9]. Some African chewing sticks have also been reported to contain fluoride ions, silicon, tannic acid, sodium bicarbonate and other natural plaque inhibiting substances that can reduce bacterial colonization and plaque formation [10].

The common chewing sticks in Southwest Nigeria include *Zanthoxylum zanthoxyloides (sym. Fagara zanthoxyloides), Vernonia amygdalina and Massularia acuminata*.

Zanthoxylum zanthoxyloides (*Fagara zanthoxyloides*) is a glabrous shrub or tree with an English or common name of candlewood or Senegal prickly ash. It belongs to the family Rutaceae [11]. Its chewing sticks are obtained either from the stems or the roots and they give a warm pungent and numbing effect on the palate. These plants have also been reported to possess antisickling, antiparasitic and antiseptic activities and have been known to be used to treat other

ailments including toothache, sexual impotence, gonorrhoea, malaria, dysmenorrhoea, urinary and venereal diseases and abdominal pain [12].

Vernonia amygdalina commonly called bitter leaf, is a perennial shrub belonging to the family "Asteraceae". It is mostly found in Southwestern Nigeria where it is called 'Ewuro'. The plant is used as an anti-helminth, anti-malarial, laxative, digestive tonic, appetizer and febrifuge and for the topical treatment of wounds. This plant also has a measure of anti caries activity. The roots and stems of this plant are used as chewing sticks and have been known to possess a measure of anti caries activity.

Massularia acuminata of the family Rubiaceae is known as "Pako Ijebu" especially among the rural communities in Southwestern Nigeria. It grows as a shrub or small tree. The inhibitory properties of the plant are attributed to its phytochemicals which include saponins, flavonoids, glycosides, tanins and anthraquinones [13].

This study therefore evaluated the antimicrobial activities of ethanolic and aqueous extracts of *Zanthoxylum zanthoxyloides, Vernonia amygdalina and Massularia acuminata* and two common brands of toothpaste for the prevention of tooth decay.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials and Preparation of Extracts

The chewing sticks, *(Zanthoxylum zanthozyloides, Vernonia amygdalina*, and *Massularia acuminata*) were purchased from Lafenwa market in Abeokuta, Ogun state of Nigeria. They were authenticated at the Forestry Research Institute of Nigeria (FRIN).

The chewing sticks were washed under running tap water, chopped into small pieces and sun-dried for 3 days. They were milled properly and stored in dry containers. 25 grams of each chewing stick was placed in screw-capped bottle containing 250 mls of 50% ethanol and sterile distilled water. It was soaked for 7 days after which it was filtered using Whatmann No.1 filter paper. It was then concentrated using a rotary evaporator and put in the fridge for further use.

2.1.1 Sourcing of toothpastes

Two brands of tooth paste, Macleans and Close up, were purchased from Sabo market in Sagamu, Ogun state, Nigeria.

2.2 Collection of Isolates

Stapylococcus aureus isolates from dental plaque were collected from the Microbiology laboratory of the University College Hospital (UCH), Ibadan, Oyo state of Nigeria. The study population was drawn from patients attending different clinics of the hospital, presenting with various symptoms of dental disease, such as tooth ache, hole in the teeth, and varying degrees of tooth dentures. Those patients that were already on antibiotics therapy were excluded from the study.

Gram staining and standard biochemical tests including coagulase and catalase, were used to confirm the isolates as *Staphylococcus aureus*.

2.3 Phytochemical Screening of the Plant Extracts

2.3.1 Alkaloids

Alkaloid levels were analyzed using Dragendorffs test as described by Evans [10]. Two milliliters (2 mls) of the plant extract was warmed with 2% H_2SO_4 for two minutes. It was filtered and a few drops of Dragendorff reagent (potassium bismuth iodide solution) were added to the filtrate. An orange or red precipitate indicated the presence of alkaloids.

2.3.2 Saponins

The method described by Harbone [14] was used. One milliliter (1 ml) of each plant extract was measured into test tubes and 5mls of distilled water was added and boiled. The presence of froths after standing for few minutes indicated the presence of saponins.

2.3.3 Tanins

Two milliliters (2 mls) of each plant extract was stirred into 10 mls of distilled water and filtered. A few drops of ferric chloride were then added to the filtrate. Presence of tannin was indicated by a blue-black, green or blue-green precipitate [10].

2.3.4 Anthraquinones

Two milliliters (2 mls) of each plant extract was added to 10 mls of benzene and shaken. It was filtered and 5 mls of 10% ammonium solution was added to the filtrate and shaken properly. Presence of anthraquinone was indicated by pink, red or violet colour in the lower phase [10].

2.4 Screening of Chewing Sticks for Antibacterial Activity

2.4.1 Antibacterial sensitivity bioassay

The sensitivity test was carried out using Kirby-Bauer agar well diffusion method. Wells were made in previously seeded Mueller Hinton agar plates using a sterile cork borer (6 mm in diameter). The wells were filled with different concentrations (25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml) of the plant extract. The plates were allowed to stand for about 1 hour to allow for diffusion of the extracts before incubation. The plates were incubated at 37℃ for 48 hours. Zones of inhibition were measured in millimeters (mm) after incubation.

The antimicrobial susceptibility bioassay for the toothpaste was carried out by agar well diffusion method also. Each well in previously seeded Mueller Hinton agar plate was filled with undiluted toothpaste. The plates were allowed to stand for about 1 hour for diffusion before being incubated at 37℃ for 48 hours. Zones of inhibition were measured in mm after incubation.

3. RESULTS

3.1 Phytochemical Screening of Plant Extracts

Saponins and tannins were common to the three chewing sticks while they varied in other components. Table 1 shows the various secondary metabolites in the plants.

Table 1. Phytochemical screening of plant extracts

S/N	Tests	FZ	VA	MA
1	Alkaloids	+	+	-
2	Saponins	+	+	+
3	Tannins	+	+	+
4	Anthraquinones	-	-	+

Legend: FZ: Fagara zanthoxyloides, VA: Vernonia amygdalina, MA: Massularia acuminate, (-) negative i.e. absent, (+) positive i.e. present

3.2 Confirmation of the Identity of Bacterial Isolates

All the isolates were confirmed to be Gram positive cocci arranged in clusters and were coagulase and catalase positive. The result of the microscopic and biochemical tests carried out for the identification of the bacterial isolates is shown in Table 2 below.

Table 2. Microscopic and biochemical tests results of isolates

Tests	Result
Gram staining/ shape	+/ spherical shaped
Mannitol salt	+
Catalase	+
Coagulase	+
Oxidase	-

Legend, (+) positive, (-) negative

3.3 Antibacterial Activity of Ethanol and Aqueous Extracts of Plant Materials and Toothpastes

Appreciable antibacterial activity of the extracts was obtained at 100 mg/ml.

The highest zone of inhibition was recorded for the ethanol extract of *Fagara zanthoxyloides* with 30 mm in diameter and the lowest (9 mm) was recorded for the aqueous extract of *Fagara zanthoxyloides* and macleans toothpaste. Table 3 shows the zones of inhibition in millimeters (mm) of ethanol and aqueous extracts of plant materials, the toothpastes and ethanol (control).

The sensitivity of the test isolates to extracts of the chewing sticks and toothpastes, expressed as percentage are presented in Tables 4, 5 and 6.

4. DISCUSSION

The presence of saponins and tannins in all the three plants, of anthraquinone in *Massularia acuminata* and of alkaloids in *Fagara zanthoxyloides* and *Vernonia amygdalina* can be linked to the antibacterial activity of the plants. Cowan [15] had shown that antibacterial effect of plant materials was due to the presence of alkaloids, tannins and anthraquinones.

It had also been reported by Hagerman et al. [16] that tannins form irreversible complexes with proline- rich proteins which could lead to inhibition of cell wall protein synthesis, a property that may explain the mode of action of the chewing stick extracts used in this work. In addition to its antibacterial effect, saponins also have antifungal properties.

Table 3. Zones of inhibition of ethanol extracts, aqueous extracts of plant materials and toothpastes (mm)

S/N	Zones of inhibition (mm)								
	FZ		VA		MA				
	E.E	A.E	E. E	A.E	E.E	A.E	CL	MAC	ETH
1	15	0	14	0	14	15	0	0	0
2	18	0	16	0	16	0	0	0	0
3	18	12	16	0	16	14	0	0	0
4	0	0	0	0	0	0	0	0	0
5	16	12	14	11	14	0	0	0	0
6	0	0	0	0	0	0	0	0	0
7	18	0	0	0	0	0	0	0	0
8	16	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0
11	18	0	0	0	0	0	0	14	0
12	20	0	0	0	0	0	0	0	0
13	15	0	11	0	11	0	0	0	0
14	18	0	20	0	0	0	20	0	0
15	18	0	15	0	14	0	0	15	0
16	20	0	12	0	16	0	0	0	0
17	12	0	13	0	12	15	0	0	0
18	10	0	13	0	10	0	0	0	0
19	18	0	12	0	15	0	0	20	0
20	30	0	12	0	0	0	0	0	0
21	13	0	17	0	0	0	0	0	0
22	30	10	17	0	0	0	0	0	0
23	30	9	17	0	0	0	0	0	0
24	0	12	0	10	0	10	0	0	0
25	30	0	12	0	0	0	0	0	0
26	11	0	0	0	0	0	0	0	0
27	20	0	18	0	14	0	19	9	0
28	16	0	16	0	0	0	10	10	0
29	15	11	20	0	16	0	0	17	0
30	0	9	0	0	11	0	12	10	0
31	15	10	13	0	0	0	10	13	0
32	0	0	20	0	0	0	16	9	0
33	16	10	20	11	17	11	17	20	0
34	16	0	0	0	0	0	0	0	0
35	18	0	12	0	15	0	10	10	0
36	16	10	0	0	0	0	0	17	0
37	19	10	12	0	16	0	0	16	0
38	20	0	20	0	18	0	20	17	0
39	25	10	16	0	20	0	20	15	0
40	20	0	20	10	15	10	20	16	0
41	20	0	26	0	17	0	0	17	0
42	22	10	21	0	20	0	0	20	0
43	17	0	17	0	12	0	0	0	0
44	16	0	12	0	12	0	0	0	0
45	20	0	23	0	15	0	0	0	0
46	16	10	0	9	0	0	12	14	0
47	15	0	9	0	0	0	20	0	0
48	20	10	29	0	17	10	17	0	0
49	18	10	19	0	0	0	0	23	0
50	11	0	10	0	0	0	17	20	0

Legend, FZ- Fagara zanthoxyloides, VA- Vernonia amygdalina, MA- Massularia acuminate, CL- Close up, MAC- Macleans, E.E- Ethanolic Extract

Table 4. Percentage sensitivity of isolates to ethanol extracts of plant materials

Ethanol extracts of plant material	Percentage (%)/No sensitivity
FZ	86 (43)
VA	72 (36)
MA	50 (25)

Table 5. Percentage sensitivity of isolates to aqueous extracts of plant materials

Aqueous extracts of plant material	Number / percentage sensitivity
FZ	32 (16)
VA	10 (5)
MA	14 (7)

Legend, FZ- Fagara zanthoxyloides
VA- Vernonia amygdalina
MA- Massularia acuminata

Table 6. Percentage sensitivity of isolates to toothpastes

Toothpaste	Percentage sensitivity
Close up	30 (15)
Macleans	40(20)

The test organism used for this study, *Staphylococccus aureus* is one of the organisms often implicated in dental caries. It accounts for over 70% of dental caries cases from the research works carried out by Daniyan and Abalaka [17] and had highest proportion in the work of Oluremi et al. [18]. It is a pathogenic organism that causes dental infections and are now widely isolated from the oral cavity.

The extracts of the three chewing sticks produced a greater activity at a concentration of 100mg/ml than the two brands of toothpaste. This is similar to the work carried out by Antwi-boasiako et al. [19] where the plant extracts of *Garcinia kola* had a greater antimicrobial effect than the pepsodent toothpaste used.

The ethanol extract of *Fagara zanthoxyloides* gave a better antimicrobial activity against all the fifty isolates than the ethanol extracts of *Vernonia amygdalina* and *Massularia acuminata.* 43 (86%) of the isolates were sensitive to the ethanol extracts of *Fagara zanthoxyloides* while 36(72%) and 25(50%) were sensitive to *Vernonia amygdalina* and *Massularia acuminata* respectively.

The ethanol extracts of the plant materials had a better antimicrobial action than the aqueous extracts which is in accordance with a research work by Isawumi [11] and Rotimi et al. [20].

The aqueous extracts of the plant materials showed a poor inhibitory action against the test organism. This may be due to poor solubility nature of the active principles of the plants in water.

The two brands of toothpaste had weaker antimicrobial activity than the plant extracts. This is not unexpected since chemical toothpastes owe their antimicrobial property to the presence of fluorides as part of their ingredients. Macleans however had a better inhibitory action than close up.

5. CONCLUSION

The chewing sticks used in this study showed good antimicrobial activity against the isolates and could provide better care than fluoride toothpastes. The active compounds if isolated would be good caries-controlling components of herbal toothpastes.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Farogh G, Monika R, Mohini SR, Manisha S, Neha S, Anushree V, Abhimanyu KJ. *In vitro* study to investigate the antimicrobial efficacy of different toothpastes and mouth rinses. Research J of Pharmaceutical, Biological and Chemical Sciences. 2014;5(2):245-257.
2. Osho A, Adelani OA. The antimicrobial effect of some selected Nigerian chewing sticks on clinical isolates of *Candida* species. Journal of Microbiology Research. 2012;2(1):1-5. p-ISSN 2166-5885.
3. El-Said F, Fadulu SO, Kuye JO, Sofowora A. Native Cures in Nigeria, Part II: The antimicrobial properties of the buffered

exracts of chewing sticks. Lloydia. 1971;34:172-175
4. Buada CV, Boakye-Yiadom K. The antibacterial activity of some Ghanian chewing sticks. Ghana Pharmaceutical Journal. 1973;1:150-151.
5. Ugoji E, Egwari LO, Obisesan B. Antibacterial activities of aqueous extracts of ten African chewing sticks on oral pathogens. Nig. Journal of Internal Medicine. 2000;3(1):7-11.
6. Ndukwe KC, Okeke IN, Lamikanra A, Adesina SK, Aboderin O. Antibacterial activity of aqueous extracts of selected chewing sticks. J. Contemp. Dent. Pract. 2005;3(6):086-094.
7. Almas K, Al-Lafi TR. The natural toothbrush. World Health Forum. 1995;16: 206-210.
8. Kareem TK, Alli SO, Atayese AO, Abimbola RE. Antibacterial effect of *Distemonanthus benthamianus* extract against some oral pathogen. Internationaal Journal of Applied Science and Technology. 2012;2(2):114-118.
9. Hardie J, Ahmad K. The miswak as an aid in oral hygiene. J. Phillip Dent Assoc. 1995;4:33-38.
10. Evans WC. Trease and Evans Pharmacognosy. 15th ed. W. B. Saunders, London; 2002.
11. Isawumi MA. Nigerian chewing sticks. Nigerian Field. 1978;43(2):50-58.
12. Lara O Orafidiya, Ezekiel O Akinkunmi, Fadekemi O Oginni, Ajibola Oluwamakin. Effectiveness of extracts of the root of *Zanthoxylum zanthoxyloides* (Linn) Waterman (Rutaceae) formulated as toothpastes; 2010. Available:https://www.researcgate.net/publication/233894316
13. Burkill HM. Useful plants of tropical west Africa Vol. 4 Royal Botanic Gardens, Kew. 1997;996.
14. Harbone JB. Phytochemical methods, a guide to modern techniques of plant analysis. 3rd edition, Chapmann and Hall, London, UK. 1998;278.
15. Cowan MM. Plants products as antimicrobial agents. Clinical Microbiological Review. 1991;12:564-582.
16. Hargerman AE, Butler IG. Specificity of proanthocyanidin-protein interactions. Journal of Biological Chemistry. 1981; 256:4494-4497.
17. Daniyan SY, Abalaka ME. Caries in a Secondary Health Care Institution, Nigeria. Shiraz E-Medical Journal. 2012;12(3).
18. Oluremi BB, Osungunna MO, Idowu OA, Adebolu OO. Evaluation of the anticaries activity of selected mouthwashes marketed in Nigeria. Tropical Journal of Pharmaceutical Research. 2010;19(6):581-586.
19. Antwi-boasiako C, Abubakari A. Antimicrobial and phytochemical properties of crude extracts of *Garcina kola* heckle stems used for oral health. Research Journal of Pharmacology. 2011;5(5):68-76. ISSN 1815-9362
20. Rotimi VO, Mosadomi HA. The effects of crude extracts of nine African chewing sticks on oral anaerobes. J Med Microbiol. 1987;23(1):55-60.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14473