The Journal of Phytopharmacology 2013; 2(6): 1-7
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2013; 2(6): 1-7

Itemire Oghenekevwe Anne
Department of Microbiology, University of Benin, PMB 1154, Benin City, Nigeria

Ogbimi Oroboheru Andrew
Department of Microbiology, University of Benin, PMB 1154, Benin City, Nigeria

MacDonald Idu
Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin City, Nigeria

Correspondence:
MacDonald Idu
Department of Plant Biology and Biotechnology, University of Benin, PMB 1154, Benin City, Nigeria
Tel: +2348050607009
E-mail: mcdonald.idu@gmail.com

Phytochemistry and antimicrobial activity of *Zanthoxylum zanthoxyloides* root used as chewing stick in Nigeria

*Itemire Oghenekevwe Anne, Ogbimi Oroboheru Andrew, MacDonald Idu**

Abstract

This study examined the antimicrobial activity of aqueous and ethanolic extracts of *Zanthoxylum zanthoxyloides* root fractioned with chloroform against oral microbial isolates. Oral swabs were collected from 25 patients with dental problems attending the Dental clinic of UBTH Benin City. The streak plate method was used to culture on Blood, Chocolate, MacConkey and Sabouraud agar plates and were incubated at 37°C for 24 h for bacteria and room temperature for 72 h for fungi. The paper disc diffusion method was used at 100 mg/ml, 200 mg/ml, 400 mg/ml and 800 mg/ml for antimicrobial determination. The percentage occurrences of the microbial isolates were *N. catarrhalis* (48%), *S. aureus* (44%), *P. aeruginosa* (28%), *S. epidermidis* (24%), *L. acidophilus* (24%), *K. rhinoscleromatis* (20%) and *C. albicans* (12%). At 800 mg/ml the pre- fractioned and post- fractioned aqueous extracts inhibited all the microbial isolates. Ethanol-chloroform extract at 800 mg/ml inhibited all the microbial isolates except *P. aeruginosa* that had 42.9%. In all the extracts, *C. albicans* had 100% inhibition at 400 mg/ml. There was significant difference between the zones of inhibition of the difference microorganisms as $P<0.05$ and between pre- fractioned and post- fractioned aqueous extracts as $P<0.05$. The results showed that aqueous extracts had better antimicrobial activity when compared to the ethanol extracts.

Keywords: Phytochemistry, Antimicrobial, *Zanthoxylum zanthoxyloides*, Oral microbial isolates.

Introduction

Plants have very effective and important role to play in oral hygiene. A number of popular plant parts are prepared into chewing sticks, most of which have different substances in them that can keep the buccal cavity healthy. The most popular chewing sticks are those having good flavour, texture and recognized effect on the teeth and supporting tissues. Freshly cut plants are more desirable because they are more easily chewed into brushes.[1]

Some plants possess antimicrobial activity against oral microbial flora. This indicates that these chewing sticks (plant), in addition to providing mechanical stimulation to the gums also destroy microbes present in the mouth.[2, 3]

Zanthoxylum zanthoxyloides belongs to the genus Zanthoxylum, sub-family Rutoideae, family Rutaceae and tribe Xanthoxylaea. An indigenous plant used widely as chewing sticks for teeth cleansing in West Africa.[4] It is well known for its varied

uses in trado- medical practice, the root, root-bark and other parts of the plant are used in treating dental diseases, various medical problems and biopesticide for stored food protection.[5-10]

The aim of this study therefore is to evaluate the antimicrobial activity of the *Zanthoxylum zanthoxyloides* against oral aerobic microorganisms as well as compare the antimicrobial activities of the different extracts: aqueous, aqueous-chloroform, ethanol, ethanol-chloroform extract which are the common solvents used by traditional medicine practitioners

Materials and method

Plant extract: *Zanthoxylum zanthoxyloides* roots were purchased from the Yoruba and Hausa herbal plant's sellers in Lagos Street Market, Benin City, Nigeria and were identified by Prof. M. Idu of Department of Plant Biology and Biotechnology, University of Benin. Benin City, Nigeria. The roots were washed and dried. The aqueous and ethanol extracts were carried out using the modified method.[11] Ten (10) grams of the pre-fractioned aqueous extract was dissolved in 20 ml of water and poured into a separating funnel, followed by 50 ml of chloroform and shaken very well. It was allowed to stand undisturbed for proper separation of the chloroform from the mixture. The separating funnel tap was then opened to collect the chloroform fraction. Another 50 ml of chloroform was added and the process was repeated until the chloroform was clear. The chloroform fraction was left to dry at room temperature (aqueous-chloroform extract) while the water fraction was dried at 60°C (post-fractioned aqueous extract).

Ten grams of ethanol extract was dissolved in 20 ml of ethanol and poured into a separating funnel, 50 ml of chloroform was added and shaken very well. It was allowed to stand undisturbed and the separated chloroform fraction removed by opening the separating funnel tap. The process was repeated by adding another 50 ml of chloroform until chloroform fraction was clear. Ethanol fraction (post-fractioned ethanol extract) and the chloroform fraction (ethanol-chloroform extract) were left at room temperature to dry.

Preparation of discs and disc extracts concentration: Whatman No 4 filter paper was cut into disc size of 6.0 mm diameter. The discs were counted in fifties into bottles and sterilized using hot air oven at 140°C for one hour.

The following extracts disc concentrations: 100 mg/ml, 200 mg/ml, 400 mg/ml and 800 mg/ml of all extracts were prepared in sterile distilled water for aqueous extracts and 0.2% tween 80 for ethanol extracts. Discs were also impregnated with 0.2% tween 80 to serve as control.

The isolated organisms were inoculated into 10 ml nutrient broth and sabouraud broth and were incubated at 37°C and room temperature respectively for 24 h. The minimum inhibitory concentrations were determined using a method.[12]

Study population: Fifteen (15) females and ten (10) males between the ages of sixteen (16) and sixty five (65) years with dental problems attending the Dental Clinic of the University of Benin Teaching Hospital, Benin City, were recruited between February and July, 2010 and used for this study. Sterile swab sticks moistened with quarter strength Ringer's solution were used for sample collection.

Preparation of culture agar and biochemical reagents: All media and reagents used were prepared according to the manufacturer's direction and Cruickshank (1970) standard methods. The cultural and identification methods were done.[13, 14]

Results

A total of 50 microbial isolates were obtained from the 25 samples. The following results were obtained 34% were Gram positive cocci (GPC), followed by Gram negative cocci (GNC) and Gram negative bacilli (GNB) 24% each; Gram positive bacilli (GPB) 12% and Gram positive large ovoid cells 6%.

Sensitivity tests

Pre-fractioned aqueous extract: At 100 mg/ml, all micro-organisms were resistant, at 200 mg/ml, *Staphylococcus aureus* 18.2% and *Candida albicans* 66.6% were sensitive; at 400 mg/ml, *Staphylococcus aureus* 63.3%, *Staphylococcus epidermidis* 66.7%, Neisseria catarrhalis 50%, *Lactobacillus acidophilus* 16.7% and *Candida albicans* 100% were sensitive and at 800 mg/ml, all microbial isolates were sensitive. This is shown in Table 1.

Table 1: The percentage of microorganisms sensitive to the pre-fractioned aqueous extract

Organisms	Concentrations of pre-fractioned aqueous extract (mg/ml)			
	100 %	**200 %**	**400 %**	**800 %**
S. aureus	-	18.2 (2)	63.6 (7)	100.0 (11)
S. epidermidis	-	-	66.7 (4)	100.0 (6)
L. acidophilus	-	-	16.7 (1)	100.0 (6)
N. catarrhalis	-	-	50.0 (6)	100.0 (12)
P. aeruginosa	-	-	-	100.0 (7)
K. rhinoscleromatis	-	-	-	100.0 (5)
C. albicans	-	66.7 (2)	100.0 (3)	100.0 (3)

Post-fractioned aqueous extract: Table 2 shows the results of the post-fractioned aqueous extract. At 100 mg/ml, all microbial isolates were resistant; at 200 mg/ml, *S. aureus* 18.2%, *K. rhinoscleromatis* 16.7% and *C. albicans* 100% were sensitive; at 400 mg/ml, *S. aureus* 63.6%, *S. epidermidis* 66.7%, *L. acidophilus* 50%, *N. catarrhalis* 91.7%, *P. aeruginosa* 42%, *K. rhinoscleromatis* 20% and *C. albicans* 100% were sensitive and 800 mg/ml concentration had 100% sensitivity.

Table 2: The percentage of microorganisms' sensitive of the post-fractioned aqueous extract

Organisms	Concentrations of pre-fractioned aqueous extract (mg/ml)			
	100 %	**200 %**	**400 %**	**800 %**
S. aureus	-	18.2 (2)	63.6 (7)	100.0 (11)
S. epidermidis	-	-	66.7 (4)	100.0 (6)
L. acidophilus	-	-	50.0 (3)	100.0 (6)
N. catarrhalis	-	-	91.7 (11)	100.0 (12)
P. aeruginosa	-	-	42.9 (3)	100.0 (7)
K. rhinoscleromatis	-	20.0 (1)	20.0 (1)	100.0 (5)
C. albicans	-	100.0 (3)	100.0 (3)	100.0 (3)

Aqueous-chloroform extract

Aqueous chloroform extract had no antimicrobial activity as all micro-organisms grew and no zone of inhibition was recorded.

Pre-fractioned ethanol extract: The results of this extract on the microbial isolates are shown in Table 3. At 100 mg/ml, no zone of inhibition was recorded while at 200 mg/ml *S. aureus* had 27.3%, *L. acidophilus* 50 % and *C. albicans* 66.7. At 400 mg/ml, *S. aureus* 90.9%, *L. acidophilus* 100%, *N. catarrhalis* 16.7% and *C. albicans* 100% were sensitive; at 800 mg/ml, *S. aureus* 100%, *S. epidermidis* 16.7%, *L. acidophilus* 100%, *N. catarrhalis* 41.7%, *K. rhinoscleromatis* 20% and *C. albicans* 100% were sensitive.

Table 3: The percentage of sensitive microorganisms to the pre-fractioned ethanol extract

Organisms	Concentrations of pre-fractioned aqueous extract (mg/ml)			
	100 %	**200 %**	**400 %**	**800 %**
S. aureus	-	27.3 (3)	90.9 (10)	100.0 (11)
S. epidermidis	-	-	-	16.7 (1)
L. acidophilus	-	50.0 (3)	100.0 (6)	100.0 (6)
N. catarrhalis	-	-	16.7 (2)	41.7 (5)
P. aeruginosa	-	-	-	-
K. rhinoscleromatis	-	-	-	20.0 (1)
C. albicans	-	66.7 (2)	100.0 (3)	100.0 (3)

Post-fractioned ethanol extract

Table 4 show the results of the ethanol extract after chloroform fractionation. At 100 mg/ml, all microbial isolates were resistant; at 200 mg/ml, *S. aureus* 9.1% and *C. albicans* 100% were sensitive; at 400 mg/ml, *S. aureus* 27.3%, *L. acidophilus* 100% and *C. albicans* 100%; at 800 mg/ml, *S. aureus* 54.5%, *S. epidermidis* 16.7%, *L. acidophilus* 100% and *C. albicans* 100% were sensitive.

Table 4: The percentage of microorganisms sensitive to the post-fractioned ethanol extract

Organisms	**Concentrations of pre-fractioned aqueous extract (mg/ml)**			
	100 %	**200 %**	**400 %**	**800 %**
S. aureus	-	9.1 (1)	27.3 (3)	54.5 (6)
S. epidermidis	-	-	-	16.7 (1)
L. acidophilus	-	-	100.0 (6)	100.0 (6)
N. catarrhalis	-	-	-	-
P. aeruginosa	-	-	-	-
K. rhinoscleromatis	-	-	-	-
C. albicans	-	100.0 (3)	100.0 (3)	100.0 (3)

Ethanol-chloroform extract: At 100 mg/ml, no sensitivity was recorded; at 200 mg/ml, *S. aureus* 36.4%, *L. acidophilus* 16.7% and *C. albicans* 100%; at 400 mg/ml, *S. aureus* 90.9%, *S. epidermidis* 16.7%, *L. acidophilus* 50%, *N. catarrhalis* 66.7%, *K. rhinoscleromatis* 20% and *C. albicans* 100% were sensitive and at 800 mg/ml, all micro-organisms had 100% sensitivity except *P. aeruginosa* that recorded 42% sensitivity. This result is shown in table 5.

Table 5: The percentage of sensitive microorganisms to the ethanol chloroform extract

Organisms	**Concentrations of pre-fractioned aqueous extract (mg/ml)**			
	100 %	**200 %**	**400 %**	**800 %**
S. aureus	-	36.4 (4)	90.9 (10)	100.0 (11)
S. epidermidis	-	-	16.7 (1)	100.0 (6)
L. acidophilus	-	16.7 (1)	50.0 (3)	100.0 (6)
N. catarrhalis	-	-	66.7 (8)	100.0 (12)
P. aeruginosa	-	-	-	42.9 (3)
K. rhinoscleormatis	-	-	20.0 (1)	100.0 (5)
C. albicans	-	100.0 (3)	100.0 (3)	100.0 (3)

Average zones of inhibition

The results of the average zones of inhibition of the dental isolates showed that *C. albicans* had the widest zone of inhibition in both aqueous and ethanol extracts. Statistical analysis of the zones inhibition of the pre-fractioned and post-fractioned aqueous extracts using two-way anova without replication shows significant difference as $P<0.05$ but there was no significant difference between the ethanol extracts as $P>0.05$. However there was significant difference in the zones of inhibition between microorganisms $P<0.05$ in all extracts.

Phytochemical result

Table 6 shows the phytochemical substances present in aqueous and ethanol extracts, it revealed the presence of polysaccharides, alkaloids, tannins, saponins, flavonoids, phenolic compounds and phytosteroids.

Table 6: Phytochemical result of water and ethanol extracts

Phytochemical	Water extract	Ethanol extract
Polysaccharide	+	+
Reducing sugar	-	-
Alkaloids	-	+
Tannins	+	+
Saponins	+	-
Flavonoids	+	+
Phenolic compounds	+	+
Anthraquinonederivaties	-	-
Phytosterols	-	+

Key: + = Positive, - = Negative.

Discussion

The human oral cavity harbors diverse ranges of bacteria, fungi and protozoa as normal flora but may cause dental diseases in poor oral hygiene and in immune suppressed individuals.[3] These cause the disintegration of the organic substances of the tooth resulting in dental plaque, tartar, gingivitis, caries and periodontitis depending on the tissue affected.[1]

The microorganisms isolated in this study are normal oral flora: *S. aureus*, *S. epidermidis*, *L. acidophilus*, *N. catarrhalis*, *K. rhinoscleromatis* and *C. albicans* except *P. aeroginosa* which is not a known commercial of the mouth. These were also reported as causing dental diseases.[8, 15]

The use of root, stem and twig of numerous plants as chewing sticks are effective in cleaning the tooth surface, gingival massage, oral asepsis and stimulation of periodontal structures.[16] The results of this work showed that the pre-fractioned aqueous, post-fractioned aqueous, pre-fractioned ethanol, post-fractioned ethanol and ethanol-chloroform extracts of *Zanthoxylum zanthxyloides* root used as chewing stick in many African countries have antimicrobial activity against the dental isolates. At 400 mg/ml, the post-fractioned aqueous extract had better results than the pre-fractioned aqueous extract but at 800 mg/ml, both the pre-fractioned aqueous and post-fractioned aqueous extracts inhibited all the microorganisms, with the zones of inhibition wider in post-fractioned aqueous extract than pre-fractioned aqueous extract and no inhibition of the microorganisms (all the microorganisms grew) in aqueous chloroform extract. The statistical analysis of the pre-fractioned aqueous and post-fractioned aqueous extract using a two-way anova showed a significant difference between the two extracts zones of inhibition as $P < 0.05$ showing that the post-fractioned aqueous extract had a better antimicrobial activity than the initial aqueous extract. Fractionation with chloroform would have reduced the impurities in the post-fractioned crude aqueous extract making it to be more active. The post-fractioned ethanol extract did not inhibit the microorganisms as much as the pre-fractioned ethanol and ethanol-chloroform extract.

The percentage of sensitive organisms at 400 mg /ml and 800 mg/ml concentrations of pre-fractioned aqueous and post-fractioned aqueous extracts had better results when compared with that of the pre-fractioned ethanol, post-fractioned ethanol and ethanol-chloroform. This showed that the aqueous extract had more antimicrobial activity than the ethanol extract. In previous reports, aqueous soluble components of plants were found to be most effective against pathogens.[17] However; the ethanol-chloroform extract had antimicrobial activity against the microorganisms at 400 mg/ml and 800 mg/ml while the aqueous chloroform extract had no antimicrobial activity. The water solvent was able to extract water soluble antimicrobial components of the plant root which were not soluble in chloroform but ethanol was able to extract from the plant root antimicrobial components that are also soluble in chloroform. The result also showed that the percentage inhibition of the ethanol-chloroform was better than that of ethanol.

The aqueous and ethanol extracts had a high antimicrobial effect against *C. albicans* because as low as 200 mg/*ml C. albicans* was inhibited indicating that *Z. zanthoxyloides* has high activity against yeast cell. Reports of strong antimicrobial activity of alkaloids against yeast cells were documented by earlier researchers.[18]

The phytochemical analysis of the aqueous extract revealed the presence of polysaccharides, saponins, flavonoids, tannins and phenolic compounds while the ethanol extract revealed the presence of polysaccharides, alkaloids, tannins, flavonoids, phenolic compounds and phytosterol. Ethanolic extract had more phytochemical compounds than the aqueous extracts. This is in agreement with a study carried out before this study.[8] The strong antimicrobial and antibacterial activity of alkaloids reported in this study have also been reported.[19, 20] Flavonoid was isolated from the aqueous and ethanol extracts. Isolated flavonoids were synthesized by plants in response to microbial infection and were observed to be effective antimicrobial substances against a wide array of microorganisms.[21] Tannins and Phenol compounds that were isolated are reported to have antibacterial and antimicrobial activities.[22-25]

Conclusion

It is obvious from this and previous studies, the reason Africans using Z. zanthoxyloides and other plants as chewing sticks, have stronger teeth and better oral hygiene than those using toothpaste and brushes. The use of Z. zanthoxyloides root and other plant parts as chewing stick for oral hygiene should therefore be encourage in our society as large number of these plants possess in addition to their antimicrobial activity antitumor, anti-inflammatory and analgesic properties. The plant used as chewing sticks are less expensive when compared with the price of modern toothpaste and brushes. However, more work on plants used as chewing should be done with more emphasis on the mode and site of action of the antimicrobial component on the microorganisms.

Acknowledgement

The authors wish to acknowledge Miss Ovuakporie-Uvo Oghale for assisting to arrange this research article in this journal style.

References

1. Muhammad S., Lawal M.T. Oral hygiene and the use plants. Sci. Res. Essays 2010; 5:1788-95.

2. Lewis M.E. Plant and dental health. J. Prevent Dent 1990; 6: 75- 8.

3. Adenike A.O., Olubukonla G., Odumes N.D. Indigenous teeth cleansing agents. Afr. J. Exper. Microbiol 2010; 11:184-194.

4. Adebiyi A.O., Koekemoer T., Adebiye A.P., Simth N., Baxter E., Naude R.J. et al. Antimicrobial and antioxidant activities of crude extracts of two Nigerian chewing sticks. Pharm. Biol 2009; 47: 320-327.

5. Ogwal – Okeng J.W. The in-vitro study of the anthelmintic activity of crude extracts of two Uganda medicinal plants: *Cissus adenocaulis* and *Fagara zanthoxyloides*. [Master's thesis]. Makerere University M. Sc. Thesis; 1990.

6. Sofowora E.A. Medicinal plants and Traditional Medicine in Africa. Ibadan-Owerri-Kaduna-Lagos: Spectrum books ltd;1993.

7. Rotimi V.O., Laughton B.E., Bartlet J.S., Mesadomi H.A. Activities of Nigerian chewing stick extracts against *Bacteriodes gingivalis* and *Bacteriodes melaninogenicus*. Antimicrob. Agents Chemothier.1998; 32: 598- 600.

8. Taiwo O., Xu H.X., Lee S.F. Antibacterial activities of extracts from Nigerian chewing sticks. Phytother 1999; 13: 675-679.

9. Patel B., Das S., Prakash R., Yashir M. Natural bioactive compound with anticancer potential. Int. J. Adv. Pharm. Sci. 2010; 1: 32-41.

10. Udo I.O. Potentials of *Zanthoxylum zanthoxyloides* (lam) for the control of stored products insect pest. J. Stored Prod Post Harvest Res. 2011; 2: 40-44.

11. Sexena J., Methela C.S. Antifungal activity of new compound from *Nepeta leucophylla* and *Nepeta clarkei*. Appl. Environ. Microbiol.1996; 62: 702-4.

12. Irobi O.N., Daramola S.O. Antifungal activities of crude extracts of *Mitracarpus villosus* (Rubiaceae). J. Ethnopharm. 1994; 38: 604-10.

13. Cruickshank R. Medical Microbiology. 11th edition, E. and S. Livingstone London; 1970.

14. Cheesbrough M. District laboratory practice in tropical countries. Low price edition, United Kingdom; 2000.

15. Adekunle A.A., Odukoya, K.A. Antifungal activities of ethanol and aqueous crude extracts of four Nigerian chewing sticks. Ethnobot. Leaflets; 2006.

16. Djossou C.V. La brossevégétale: moyen traditional d'hygienebucco-devitaire des africains; 1985.

17. Harbone J.B. Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd Edition, Chapman and Hall. London; 1998.

18. Wanjala C.C., Juma B.F., Bojase G., Gashe B.A., Majinda R.R. Erythrinaline alkaloids and antimicrobial flavonoids from Erythrina latissima. Planta Medica.2002; 68: 640-642.

19. Faizi S., Khan R.A., Azher S., Khan S.A., Ahamd A. New antimicrobial alkaloids from root of *Polyalthia longifolia* varpendua. Planta Medica. 2003; 69: 350 -355.

20. Gonzaga W.A., Weber A.D., Giacomeli S.R., Dalcol I.I., Hoelzel S.C.S., Morel A.F. Antibacteria alkaloids from *Zanthoxylum rhoifolium*. PlantaMedica.2003; 69: 371-4.

21. Dixon R.A., Dey P.M., Lamb C.J. Phytoalexins enzymology and molecular biology. Advance Enzymol. 1983; 55: 61-9.

22. Hou A.J., Liu Y.Z., Yang H., Lin Z.W., Sun H.D. Hydrolyzable tannins and related polyphenols from *Eucalyptus globules*. J. Asian Nat Prod. Res. 2000: 205-212.

23. Yoshida T., Hatano T., Ito H. Chemistry and function of vegetable polyphenols with high molecular weight. Biofactors.2000; 13:121-125

24. Puepponen–Pimia R., Nohynek L., Meier C., Kahkoneu M., Heinonen M., Hopia A. Compounds from Berries. J. Appl Microbiol.2001; 90: 494-507.

25. Urs N.R.R., Dunleavy J.M. Enhancement of bacteria activity of a peroxidase by phenolic compounds (*Xanthomonas phaseoli* var Sojensis soyabeans). Phytopath. 1975; 65: 686-90.