International Journal of Scientific Research in Biological Sciences
Vol.9, Issue.4, pp.10-13, August (2022)
Research Paper
E-ISSN: 2347-7520

Antimicrobial Activities and Phytochemical Screening of Five Nigerian Chewing Sticks

Stephanie Ogechi Nwaiwu[1]*, Helen Adekanmbi[2]. Dolapo Igbari[3], Stephen Oluwanifise Oyejide[4], Obiyo Nwaiwu[5], Omowunmi Amao[6]

[1,2,3]Department of Botany, University of Lagos, Akoka, Lagos State, Nigeria
[5,6]Department of Pharmacology, University of Lagos, Idi-araba, Lagos State, Nigeria
[4]Department of Cell and Biology Genetics, University of Lagos, Akoka, Lagos State, Nigeria

Corresponding Author: nwaiwustephnie@gmail.com, Tel.: +2348134027468

Available online at: www.isroset.org

Received: 30/Jun/2022, Accepted: 02/Aug/2022, Online: 31/Aug/2022

Abstract—Chewing sticks are important non-timber forest products widely used for dental cleaning in Nigeria. This work was intended to study the antimicrobial activities and phytochemical screening of some indigenous Nigerian chewing sticks which will serve as scientific support for the use of chewing sticks for oral hygiene in rural areas. Ethanol and aqueous extracts of *Zanthoxylum zanthoxyloides, Massularia acuminata, Khaya ivorensis, Terminalia glaucescens* and *Azadirachta indica* were obtained through soxhlet and maceration respectively. Qualitative and quantitative testing of bioactive compounds using standard procedures revealed the presence of tannins, reducing compounds, alkaloids, phenols, flavonoids, saponins and cardiac glycosides. Antimicrobial screening of the extracts was carried out on *Staphylococcus aureus* and *Candida albicans* which were isolated from mouth swabs. The ethanolic extract of *K. ivorensis* and *A. indica* had the highest antibacterial activity against *Candida albicans* and *Staphylococcus aureus* respectively. The aqueous extract of *Massularia acuminata* exerted an antimicrobial effect only on *Candida albicans.* The variation in the antimicrobial activities of the extracts could be attributed to the concentration of the various bioactive compounds present in the chewing sticks. This study has confirmed the effectiveness of these chewing sticks and toothbrushes against certain microorganisms.

Keywords— Phytochemical screening, Chewing sticks, antimicrobial screening, Oral microbes

I. INTRODUCTION

Chewing sticks are stems or roots of certain plants that are chewed continuously for oral hygiene [1]. Plants used as chewing sticks reportedly contain bioactive compounds that benefit the health of gums and teeth and prevent the growth of oral microbes and dental plaques [2]. These plants are abundant and diverse in Nigerian rural communities and their extracts, are also used in the ethnomedical treatment of oral infections [3]. As reported by Ndukwe [4], in certain communities, there is the use of chewing sticks to counteract pathogenic microorganisms' resistance to commercially available anti-microbial drugs. Over 180 plant species that can be used as a natural toothbrush exist which differ based on appearance, scent, texture and taste [5]. Some of the most commonly used species are *Salvadora persica* (peelu), *Azadirachta indica* (neem), *Zanthoxylum zanthoxyloides*, and *Massularia accuminata*, *Terminalia glauscens* and *Khaya ivorensis* etc. To date, many in rural areas in Nigeria, make use of chewing sticks for brushing every morning. It is believed that they possess anti-microbial properties and keep the gum healthy [6].

Chewing sticks are important Non-Timber Forest Products (NTFP) widely used for dental cleaning in Tropical West Africa [7]. This research aimed at documenting the phytochemical screening and antimicrobial activities of the stems of *Zanthoxylum zanthoxyloides, Massularia acuminata, Khaya ivorensis, Terminalia glaucescens* and *Azadirachta indica* which are regularly used as chewing sticks in Lagos, Nigeria.

II. RELATED WORK

Osamudiamen [8] evaluated the crude extracts of *Fagara zanthoxyloides, Butyrospermum paradoxum, Distemonanthus benthamianus* and *Nauclea latifolia* for anti-cancer cell lines using Sulforhodamine B (SRB) assay. *F. zanthoxyloides* extracts had the highest broad-spectrum activity which posits that they could be a good source of anti-cancer drugs. Also, Yakubu [9] reported the mineral content of *Prosopis africanai, Massularia accuminata, Angoeissus leiocapan, Terminalia glaucescens* and *Zanthoxylum zanthoxyloides* commonly used as chewing sticks in the southwestern part of Nigeria to determine their suitability for human use. The analysis revealed higher percentage composition of sodium, potassium, calcium and zinc ions in *M. accuminata* coupled with a minute quantity

of lead in *T. glaucescens* which overall is beneficial to the user's health. Similarly, Orire [10] reported the use of *Jatropha curcas* in traditional medicine as a lactagogue, rubefacient, suppurative, purgative, abortifacient, hemostatic etc. Also, the treatment and prevention of fevers, convulsions, venereal diseases, constipation, skin diseases, rheumatism, malaria, diabetes, wounds, snakebites, jaundice, haemorrhoids, amenorrhoea and oligomenorrhoea have been associated with the use of *J. curcas*. *Jatropha curcas* has been used to prepare juices, pastes, decoctions or other preparations for the prevention and treatment of toothaches, mouth ulcers, cracked lips, bleeding gums etc. [11-13]. However, there is a paucity of scientific data to validate the folkloric use of these chewing sticks for oral hygiene.

III. METHODOLOGY

Collection of The Samples

Zanthoxylum zanthoxyloides, Massularia acuminata, Khaya ivorensis, Terminalia glaucescens, and *Azadirachta indica* plants were purchased at the Oyingbo market (6.4790° N, 3.3918° E), Lagos state, Nigeria. They were identified at the Herbarium unit, Department of Botany, University of Lagos, Nigeria.

Extract Preparation

Aqueous and ethanolic extraction was performed using maceration and soxhlet extraction techniques respectively [14]. Stems from each plant were air dried, at room temperature for 5 days, and ground into a coarsely powdered form using a mechanical grinder. 125g of each sample was soaked in 2.5L of distilled water in a container for 3 days with frequent agitation. After decantation and filtration, using No. 1 Whatman filter paper, the filtrates were concentrated in a rotary evaporator and stored as the aqueous extracts at 4^{o}C for further use.

For the ethanolic extraction, stems from each plant were placed in a thimble made from thick filter paper which was loaded into the main chamber of the Soxhlet extractor. The extractor was placed onto a flask containing ethanol and then equipped with a condenser. The solvent was heated to reflux, and the vapour travelled up a distillation arm and flooded the chamber housing the thimble. The chamber contains the desired compound dissolved in the warm solvent, and. is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was repeated at least two times for each plant material. The extract oven-dried at 40^{o}C for 24 h to dry and stored as the ethanol extracts at 4^{o}C for further use

Phytochemical Screening

The ethanolic extracts of the plant stems were qualitatively and quantitatively tested for the presence of saponin, tannin, phenol, flavonoid, reducing sugar, alkaloid and cardiac glycosides according to procedures by Trease and Evans [15].

Test Organisms

Pure cultures of *Staphylococcus aureus* and *Candida albicans* isolated from mouth swabs and confirmed according to modified methods of Cowan and Steel [16] were obtained from the Department of Microbiology, the University of Lagos for this study.

Antimicrobial Activity

S. aureus was cultured on Muller Hilton agar plates and *Candida albicans* on sabouraud dextrose agar (SDA) plates for the antimicrobial sensitivity tests according to Vlietinck [17]. Wells were dug into the agar using a cork borer of 6mm diameter, into which 100mg/ml of the extracts were introduced followed by incubation for 24 h at 37^{o}C. The plates were done in duplicates, and the mean and standard deviation of the zones of inhibition were recorded.

IV. RESULTS AND DISCUSSION

Phytochemical Analysis

Results from the qualitative and quantitative analysis of the ethanolic extracts of the selected plants are shown in Tables 1-2. The results show that tannins, saponins, steroids, flavonoids, cardiac glycosides and reducing sugar as bioactive compounds were found in the stems of *Zanthoxylum zanthoxyloides, Massularia acuminata, Khaya ivorensis, Terminalia glaucescens* and *Azadirachta indica*. However, the absence of alkaloids in the ethanolic extracts of *Z. zanthoxyloides* and *T. glaucescens* is consistent with the findings of Mann [18] but in contrast to the findings of Eliajh and Ola [19]. Also, in contrast to our reports, flavonoids were not detected in the ethanolic extract of *M. acuminata* in the findings of Essien [20]. A reason for the differences in the screenings could be attributed to different phytochemical screening protocols used, the strength of the organic solvents used or other environmental factors. It is also pertinent to note that chemistry laboratory techniques such as the use of high-performance liquid chromatography (HPLC) or Gas chromatography Mass Spectrometry (GC-MS) which was not employed by our study will give more definite and concise results of the presence of bioactive compounds.

Table 1: Qualitative Investigation of The Ethanolic Extracts of The Selected Plant's Stems.

Plants	Saponin	Tannin	Phenol	Flavonoid	Reducing Sugar	Alkaloid	Cardiac glycosides
Zanthoxylum zanthoxyloides	+	+	+	+	+	–	+
Massularia acuminate	+	+	+	+	+	+	+
Terminalia glaucescens	+	+	+	+	+	–	+
Khaya Ivorensis	+	+	+	+	+	+	+
Azadirachta indica	+	+	+	+	+	+	+

KEY: (+) Present, **(-)** Absent

Table 2: Quantitative Investigation of The Ethanolic Extracts of The Selected Plant's Stems (100mg/ml)

Plants	Saponin	Tannin	Phenol	Flavonoid	Reducing Sugar	Alkaloid	Cardiac glycoside
Zanthoxylum zanthoxyloides	45.60	20.76	28.53	33.06	45.63	–	31.21
Mazzularia acuminate	37;19	25.92	35.64	15.71	41.97	36.55	33.33
Terminalia glaucescens	40.21	51.79	72.19	52.13	56.69	–	35.70
Khaya ivorensis	42.18	29.90	42.10	74.61	39.84	33.10	32.19
Azadirachta indica	47.04	23.25	31.97	57.45	52.03	32.21	30.96

Antimicrobial Assay

The chewing sticks tested in this study are widely used for the maintenance of oral hygiene, and our findings agree with the study of Adesanya [21] about their antibacterial inhibitory effect. The results of our antimicrobial testing are presented in table 3. The ethanolic extracts of all the plant stem exhibited higher antimicrobial activity against the test organisms compared to the aqueous extracts which suggest that the use of ethanol increases the potency and spectrum activity of the extracts and could be considered a better solvent for extraction than water. Only the aqueous extract of *M. acuminata* exerted an antimicrobial effect and this was only on *Candida albicans* (0.16mm). This agrees with the finding of Ojo [22]. The ethanolic extracts of *Z. zanthoxyloides* had a higher antimicrobial effect on *Candida albicans* (0.20mm) compared to *Staphylococcus aureus* (0.18mm). This was also true for the ethanolic extract of *M. acuminate* (0.27mm vs 0.14mm), and *K. ivorensis* (0.37mm vs 0.28mm) which suggests that these plants contain bioactive compounds which are more effective on fungal isolates than bacterial isolates. Our study also showed that the ethanolic extract of *T. glaucescens* had the same inhibitory effect on both *S. aureus* and *C. albicans* (0.24mm), while the ethanolic extract of *A. indica* had a higher effect on *S. aureus* compared to *C. albicans* (0.32mm vs 0.19mm). Ethanolic extract of *Khaya ivorensis* showed the highest antimicrobial activity against *Candida albicans*, while ethanolic extract of *Azadirachta indica* showed the highest antimicrobial activity against *Staphylococcus aureus*. Overall, *K. ivorensis* had the highest zone of inhibition of 0.37mm while *M. acuminata* had the least zone. The variation in activity among the chewing sticks tested could be attributed to the varying concentration of the bioactive compounds they possess [23].

Table 3: Mean and Standard Deviation Susceptibility of The Plant Extracts At 100mg/ml

Organisms	*Zanthoxylum zanthoxyloides*		*Massularia acuminata*		*Terminalia glaucescens*		*Khaya ivorensis*		*Azadirachta indica*	
	EE	AE	EE	AE	EE	AE	EE	AE	EE	AE
Staphylococcus aureus	0.18±0	-	0.14±0	-	0.24±0	-	0.28±0	-	0.32±0.3	-
Candida albicans	0.20 ±0	-	0.27±0.1	0.16±0	0.24±0	-	0.37±0.2	-	0.19±0.1	-

Key: EE = Ethanol Extracts, AE = Aqueous Extracts

V. CONCLUSION AND FUTURE SCOPE

This research has shown the antimicrobial potency of five indigenous Nigerian chewing sticks in dental hygiene. The ethanolic extracts had a higher antimicrobial potency compared to the aqueous extracts, and generally had a higher effect on *C. albicans*, compared to *S. aureus*. This indicates that these locally-used chewing sticks can help in preventing pathogenic oral microbes. However, it is recommended that further analysis should be done using advanced chemistry techniques to evaluate the bio-active compounds in the plant stems. Also, the range of antimicrobial potency across several bacterial and fungal isolates should be evaluated

ACKNOWLEDGMENT

We acknowledge the effort of the Department of Microbiology for providing the microbial cultures, utensils and apparatus used in this study

REFERENCES

[1]. K. Baruah, V.K. Thumpala, P. Khetami, Q. Baruah, R. V. Tiwari, H. Dixit, "A review of toothbrushes and toothbrushing methods," *International Journal of Pharmaceutical Science Invention*, **Vol.6, Issue.5, pp.29-38, 2017.**

[2]. M. Abhary, A. Al-Hazmi, "Antibacterial activity of Miswak (Salvadora persica L.) extracts on oral hygiene," *Journal of Taibah University for Science*, **Vol.10, Issue.4, pp. 513-520, 2016.**

[3]. O. Taiwo, H.X. Xu, S.F. Lee," Antibacterial activities of extracts from Nigerian chewing sticks," *Phytother Res*, **Vol.13, Issue.8, pp. 675-9, 1999.**

[4]. K.C. Ndukwe, I.N. Okeke, A. Lamikanra, S.K. A.O. Adesina, "Antibacterial activity of aqueous extract of selected chewing sticks, "Journal *of Contemporary Dental Practice*, **Vol.6, pp.86-94, 2005.**

[5]. A. Asase, T. Kokubun, R.J. Grayer, G. Kite, M.S.J. Simmonds, A.A. Oteng-Yeboah, et al, "Chemical constituents and antimicrobial activity of medicinal plants from Ghana: Cassia sieberiana, Haematostaphis barteri, Mitragyna inermis and Pseudocedrela kotschyi," *Phytotherapy Research*, **Vol.22, pp.1013-1016, 2008**.

[6]. A. Sukkarwalla, S.M. Ali, P. Lundberg F. Tanwir, "Efficacy of miswak on oral pathogens," *Dent Res J (Isfahan)*, **Vol.10, Issue.3, pp.314-20, 2013**.

[7]. J.A. Akande, Y. Hayashi, "The potency of extract contents from selected tropical chewing sticks against Staphylococcus aureus and Staphylococcus auricularis," *World Journal of Microbiology and Biotechnology*, **Vol.14, pp.235-238, 1998**.

[8]. P.M. Osamudiamen, O.O. Aiyelaagbe, S. Vaid, A.K. Saxena, "Comparative Evaluation of the Anti-cancer Activities of the Crude Extracts of Four Nigerian Chewing Sticks," *Journal of Biologically Active Products from Nature*, **Vol.8, Issue.3, pp.201-207, 2018.**

[9]. F. B. Yakubu, J. Akoun, E. S. Anamayi, O. P. Duyilemi, "Mineral Composition Of Selected Tree Species Used For Chewing Stick In Southwestern Nigeria," *Journal of Agriculture, Forestry and the Social Sciences*, **Vol.6, Issue.1, pp.1-5, 2008.**

[10]. A.M. Orire, G. E. Ebonyi, S.Y. Daniyan, "Effects of Replacement of Vegetable oil with Detoxified Jatropha curcas oil in the Diet of Clarias gariepinus (Burchell, 1822) fingerlings," *Nigerian Journal of Fisheries and Aquaculture*, **Vol.6, Issue.1, pp.7 –14, 2018.**

[11]. O. Anyanwu, P. Eze, I. Nnaoma, K. Ngwoke, "Antimicrobial Properties of Jatropha curcas L. against Dental Pathogens," *Global Journal of Medical Research: J Dentistry & Otolaryngology*, **Vol.18, Issue.2, pp.1-7, 2018**. **DOI: 10.13140/RG.2.2.20593.74088.**

[12]. S. Verma, N.S. Chauhan, "Indigenous medicinal plants knowledge of Kunihar forest division, district Solan," *Indian Journal of Traditional Knowledge*, **Vol.6, pp.494–497, 2007.**

[13]. S.K. Jain, S. Srivastava, "Traditional use of some Indian plants among islanders of the Indian Ocean," *Indian Journal of Traditional Knowledge,* **Vol.4, pp.345–357, 2005.**

[14]. N. Raaman, "Phytochemical techniques," **New India Publishing, pp.1-320, 2006**

[15]. G. E. Trease, W. C. Evans, "A textbook of Pharmacognosy," 11th edition Bailliere Tindall **London. p. 530**, **2013.**

[16]. G. Barrow, R. Feltham, "Cowan and Steel's Manual for the Identification of Medical Bacteria," (3rd ed.). Cambridge: **Cambridge University Press**, **1993, doi:10.1017/CBO9780511527104**

[17]. A. J. Vlietinck, L. Vanhoof, J. Totte, A. Lasure, B. D. Vanden, P. C. Rwangabo, J. Mvukiyumwami, "Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties," *Journal of Ethnopharmacology*, **Vol.46, pp.31-47, 1995.**

[18]. A. Mann, J. Yisa, L. A. Fadipe, J. A. Samuel, "The phytochemical and anti-termite activity of the stem bark and leaves of Zanthoxylum zanthoxyloides," *Journal of Chemical Society of Nigeria*, Vol.**38, Issue.2, pp.114-119, 2013.**

[19]. A. Eliajh, I. Ola, "Phytochemical and antimicrobial screening of crude extracts from the root, stem bark, and leaves of Terminalia glaucescens," *African Journal of Pharmacy and Pharmacology***, Vol.3, pp.217-221, 2009.**

[20]. E. Essien, "Toxicity, hypoglycemic and antioxidant potentials of Massularia acuminata Stem," *Journal of Pharmacognosy and Phytochemistry*, **Vol.7, pp.1222-1226, 2018.**

[21]. O.O. Adesanya, A.K. Asekunowo. O. T. Asekun, "Phytoconstituents analyses and antimicrobial activity of root extracts of *Musa paradisiacal Linn.* (Musaceae)," *University of Lagos Journal of Basic Medical Sciences,* **Vol.5, Issue.9, pp.1-5,2017.**

[22]. O.O. Ojo, A.O. Ajayi. I. I. Anibijuwon, "Antibacterial properties of extracts of some chewing sticks commonly used in southwestern Nigeria," *Journal of Pure and Applied Microbiology,* **Vol.1, Issue.1, pp.33-38, 2007.**

[23]. E. S. Akpata, E. O. Akinrimisi, "Antibacterial activity of extracts from some African chewing sticks," *Journal of Oral Surgery,* **Vol.44, pp.712-722, 1987.**

