Antimicrobial activity of *Pseudocedrela kotschyi* (Meliaceae) stem bark in traditional dental care

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ABSTRACT

Background: *Pseudocedrela kotschyi* (Meliaceae) stem bark is widely accepted as chewing stick in Nigeria, used as such and in management of mild mouth cavity infections.

Objective: This study was carried out to evaluate and justify the traditional use of *Pseudocedrela kotschyi* (Meliaceae) stem bark in dental care.

Methods: The methanolic stem bark extract of *P. kotschyi* and its fractions were evaluated for possible anti-microbial activities against micro-organisms isolated from the teeth cavity (*Staphlococcus aureus, Streptococcus, Lactobacillus, Actinomyces, Candida albicans and Aspergillus).* Agar diffusion bio assay method was employed for the study. Chlorhexidine gluconate and sterile water were used as the positive and negative control respectively.

Results: Data obtained shows a dose dependent zone of inhibition of test bacteria while insignificant or no inhibitory activity was observed for test fungi by both the extract and the fractions. Higher growth inhibitory effect were seen with the polar fractions (butanol and aqueous remainder) compared to the extract at concentration of 100 mg mL^{-1}. Chlorhexidine gluconate exhibited sensitivity for all tested microorganisms.

Conclusion: The study justifies the traditional use of chewing stick, *P. kotschyi* as an alternative in management of dental infections caused by susceptible organisms. Further study could be done to isolate and characterize the active phytochemicals present in the plant bark.

Key words: *Pseudocedrela kotschyi*, antimicrobial activities, dental disinfection, chewing stick

Activité antimicrobienne de l'écorce de tige de *Pseudocedrela kotschyi* (Meliaceae) dans les soins dentaires traditionnels

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RESUME

Contexte: L'écorce de tige de Pseudocedrela kotschyi (Meliaceae) est largement acceptée comme cure-dents au Nigéria, utilisée comme telle et dans le traitement des infections légères de la cavité buccale.

Objectif: Cette étude a été réalisée pour évaluer et justifier l'utilisation traditionnelle de l'écorce de tige de Pseudocedrela kotschyi (Meliaceae) dans les soins dentaires.

Méthodes: L'extrait de méthanol de l'écorce de tige de P. kotschyi et ses fractions ont été évalués pour les activités antimicrobiennes possibles contre les micro-organismes isolés de la cavité dentaire (Staphlococcus aureus, Streptococcus, Lactobacillus, Actinomyces, Candida albicans et Aspergillus). La méthode d'analyse biologique par diffusion d'agar a été utilisée pour l'étude. Le gluconate de Chlorhexidine et l'eau stérile ont été utilisés respectivement comme témoin positif et négatif.

Résultats: Les données obtenues montrent une zone dépendante de dose d'inhibition des bactéries d'essai tandis qu'une activité inhibitrice non-significative ou nulle n'a été observée pour les champignons d'essai à la fois par l'extrait et les fractions. Un effet inhibiteur de la croissance plus élevé a été observé avec les fractions polaires (butanol et résidu aqueux) par rapport à l'extrait à une concentration de 100 mg mL-1. Le gluconate de Chlorhexidine présentait une sensibilité pour tous les microorganismes testés.

Conclusion: L'étude justifie l'utilisation traditionnelle du cure-dents, P. kotschyi comme une alternative dans le traitement des infections dentaires causées par des organismes sensibles. Des études plus poussées pourraient être faites pour isoler et caractériser les phytochimiques actifs présents dans l'écorce de la plante.
Mots-clés: Pseudocedrela kotschyi, activités antimicrobiennes, désinfection dentaire, cure-dents

INTRODUCTION

Medicinal plants are unevenly distributed world-wide. They have served as sources of bioactive agents both in form of extracts and chemical entities for pharmaceutical products. One aspect of medicine where medicinal plants have played a vital role is in the development of new antimicrobial drugs. Constant inflow of new antimicrobial agents is necessitated by the rate of emergence of resistant or multi-resistant strains of microorganisms imposing the need for a permanent search. Traditional medicine, which is part of the way of life or culture of a set of people living within the same location to keep healthy is an easy place to start the search for new drugs. This involves systematic evaluation of medicinal plants used in the traditional system for physiological activities and for their bioactive phytoconstituents.[1]

Oral pathogenic microorganisms cause dental plaque, a major causative factor of gingivitis and periodontal diseases. Preventive dental practices such as effective removal of dental plaque through the use of tooth brush and paste, or by use of parts of various plants can reduce the incidence of oral infections.[2] Chewing stick, one of the several methods to reduce the amount of dental plaque has been reported as being capable of reducing pathogenic microorganisms.[3] Many primary screenings have demonstrated other properties of chewing sticks which include, cleaning, gum healing, bleeding, anti-inflammatory, analgesia effects.[3-5]

In Nigeria before the use of synthetic tooth brush and paste, the people depended on chewing sticks for their oral hygiene and herbal preparations for oral health care.[4,6,7] These chewing sticks are abundant in the environment and at little or no cost.[2,8] Elderly people are observed to rub *Nicotiana tabacum* (Linne) leaves on their gums for few minutes before washing off as prophylaxis and disease management of dental challenges.[9] The sticks, which could be the stem or the root are chewed or crushed between the teeth on one side till a rough brush like structure is obtained for cleaning the mouth. The spicy or astringent liquid extracted from the sticks while being chewed serves as mouth wash, contains potent agents for prevention or management of dental caries and any other periodontal disease.[10,11] The claim that chewing sticks strengthen the gum, kill off the worm that eats the teeth and nullifies pain has been justified by several studies demonstrating the antiplaque and antibacterial activities of extracts of Nigerian chewing sticks.[8,9,12]

In a clinical trial among adolescents in Nigeria, the results showed that the *Massularia acuminata* chewing stick was as effective in controlling and removing dental plaque as the toothbrush and paste.[12] Data obtained from various antimicrobial studies of extracts of wooden chewing sticks widely used in Nigeria against different micro-organisms have proven that the chewing sticks contain different types of principles responsible for antimicrobial activities.[13,14]

Pseudocedrela kotschyi (Schweinf.) Harms (family Meliaceae), is a common tree found in the savannah region of West Africa.[15-16] The roots, leaves and stem bark of *P. kotschyi* are used for various medicinal purposes in Nigeria. The leaves are used for the treatment of rheumatism and dysentery.[15] The root and stem bark are used for the treatment of malaria, dysentery, diarrhoea, worm and oral infection.[17,18,19] *P. kotschyi* wood is also used as a chewing stick for dental cleaning in several West Africa countries.[20-23]

The aim of this study is to evaluate the antimicrobial potential of the stem bark of *Pseudocedrela kotschyi* (Schweinf.) Harms on some oral pathogens compared with a standard mouth wash agent, chlorhexidine gluconate.

MATERIALS AND METHODS

Plant collection and identification

Stem bark of *P. kotschyi* was in the wild, at Ogun state, Western Nigeria by a Lagos state based herbal collector in May, 2015. The plant stem bark was identified and authenticated by Mr O. O. Oyebanji of the herbarium, Department of Botany, University of Lagos. The herbarium specimen was prepared and deposited with voucher number LUH 6540.

Extract preparation

The chewing stick cut stem barks were washed under running tap water to remove dirts. The cut stem barks were sun dried for five (5) days and then oven dried for 24 hours at 40°C to facilitate grinding. The dried stem barks were pulverized using a mechanical mill and weighed. The powdered stem bark (900 g) of *P. kotschyi* was macerated in absolute methanol in a big amber bottle for six (6) days. The solvent was filtered using Whatman No1 filter paper every 48 hours with concurrent replacement of methanol/solvent. This gave a total of three batches of methanol. The methanolic extract was air dried at room temperature until a fully dried solid (80.10 g) was obtained. This was stored in the refrigerator to prevent degradation and/or contamination. *P. kotschyi* methanolic extract (40 g) pre-dissolved in water (300 ml) was partitioned

by the modified Kupchan partition protocol and the resultant fractions were evaporated with Buchi rotatory evaporator to yield chloroform (1.95 g), ethyl acetate (11.46 g), n-Butanol fractions (14.85 g) and aqueous remainder (10.68 g).

A serial dilution of the standard, chlorhexidine gluconate was prepared and the following working concentrations were used: (5, 2.5, 1.25 and 0.625 % v/v). Dilute solutions of the plant methanolic extracts were prepared in the following concentrations: (400, 200, 100, 50 mg ml^{-1}).

Phytochemical analysis

Phytochemical analysis of the crude extract was carried out using the methods described by Sofowora 1993 and Abraham *et al.,* 2014.[24-25]

Collection, isolation and identification of clinical isolates

Sterile swab sticks were used to obtain teeth swabs from 15 patients who previously had or currently presenting dental caries. Two sterile swab sticks were used per patient. One set of the obtained samples were labelled and enriched in Brain Heart Infusion (BHI) Oxoid, while the other set of samples were enriched in Fluid Thioglycolate medium broth (Oxoid). These were then incubated for 72 hours. The turbid enriched media were then inoculated on agar plates. In order to obtain aerobic bacteria of the oral microflora, inoculation was done from BHI medium to Muller Hinton agar plate (MHA) Oxoid, Blood agar plate (BA) Oxoid and incubated aerobically at 37°C. In order to obtain anaerobic bacteria of the oral microflora, inoculation was done from the Thioglycolate medium to MHA plate immediately to maintain anaerobiosis and incubated anaerobically inside anaerobic jar with gas pack system (Oxoid) at 37°C. The mixed cultures were then sub-cultured onto different diagnostic media and streaked in a pattern that will yield the growth of the organisms in a distinct colony. The organisms were further purified by picking each observable single colony, and sub-culturing them again, on respective diagnostic medium. The organisms were Gram stained and observed under the microscope for their Gram reactions and characterization.

Inoculation was done from BHI to Sabouraud Dextrose Agar (SDA) Oxoid, to obtain fungal species. Growth observed after 48 hours were mixed cultures of different bacteria all Gram positive large cocci with positive Germ-tube test were presumptly confirmed as being *Candida* species.

Microbial sensitivity assay

Organisms were calibrated in McCartney bottles using the 0.5 McFarland turbidity standard for comparison. One (1) ml of the calibrated organisms was poured aseptically into petri dish culture plate and 25 ml (twenty five) of warm and molten agar was poured into the petri dish containing the organisms. The preparation was mixed by gentle agitation and left to solidify. Four wells labelled according to the concentration of extract to be used were bored into the solidified agar. Cork borer (10 mm) was used to make well on the plates. Each of these wells was filled with the appropriate concentration. Anaerobic organisms were incubated anaerobically in the anaerobic jar (Oxoid) at 37°C while those aerobic bacteria were incubated aerobically in the regulated incubator at 37°C for 48 h. The fungi containing organisms were incubated at 25°C in incubator and observed daily until growth occurs.

The same procedure was performed using sterile distilled water and Chlorohexidine gluconate, both without plant extract served as negative and positive control respectively. Inhibitory zones surrounding the agar wells were measured with ruler and diameter recorded in mm. Duplicate plates were prepared for each extract and controls.

The observed organisms that were susceptible to any of the extracts were then further tested by subjection to the fractions of the extract using the same procedure as above. The average of those zones were recorded in millimeters.

Minimum inhibitory concentration (MIC)

Varying concentrations of the methanolic extracts of *P. kotschyi* bark were prepared using a geometrical pattern: (0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.6 mg ml^{-1}).

A quantity 0.01 ml (10 µl) of the suspension of the test bacterium (standardised inoculum) was inoculated onto fresh nutrient agar plates at different extract concentrations. The plates were incubated at 37°C for 72 h and 25°C for one week for bacteria and fungi respectively after which the plates were observed for growth/turbidity. Controls were prepared by inoculating tubes without the extracts but with bacteria cell suspensions. The lowest concentration of the extract with no observable bacterial growth when compared with the control was considered as the minimum inhibitory concentration (MIC).

Statistical analysis

Two way ANOVA test was used to determine the level of

significance of the test organisms at 5% level of significance.

RESULTS

Phytochemical analysis

Result of preliminary phytochemical screening of the

Table 1: Phytochemical screening result of *P. kotschyi* stem bark

Phytochemicals	
Saponins	+
Tannin	+
Cardiac glycoside	+
Flavonoids	+
Phenols	+
Alkaloids	+
Steroids	+

+ Presence of phytochemical

Antimicrobial assay

The sensitivity (mean zones of inhibition) of *P. kotschyi* against the pathogens is shown in table 2. The result revealed that *S. aureus*, *Streptococcus sp, Lactobacillus sp. and Actinomyces sp* are susceptible to methanolic extract of *P. kotschyi*. Extract showed no zone of inhibition against the growth of *C. albican* and *Aspergillus sp* (Table 2), hence no activity. The result shows a dose dependent antimicrobial activity of *P. kotschyi* stem bark extract on the test organisms that were susceptible. The order of susceptibility (starting with widest zones of inhibition) of the organisms at 400 mg mL^{-1} is; *Actinomyces sp* > *Lactobacillus sp* > *S. aureus* > *Streptococcus sp*. Inhibitory activities of chlorhexidine gluconate (the standard drug used for the study) against all the pathogens is shown in table 3. Chlorhexidine gluconate exhibited a better activity against the bacteria than the test extract. It produced the highest significant zone of inhibition in *Actinomyces sp.* (50.00 ± 0.01 mm at 5% concentration) while its least zone of inhibition was observed for *Aspergillus sp,* 12.00 ± 0.01 mm at concentration of 0.625%. The negative control, sterile water did not inhibit growth in any of the tested microorganisms.

Zones of inhibition result for semi purified fractions of *P. kotschyi* extract shows the antibacterial potentials of the polar fractions, n-butane > aqueous remainder fractions (Table 4). At 100 mg mL^{-1}, the maximum growth inhibition for *S. aureus* was 56.00 and 46.50 mm for n-butanol and aq. reminder fractions respectively.

Table 2: Zones of inhibition at different concentration (mg/ml) of methanolic extract of *P. kotschyi* stem bark

Organisms	Zone of inhibition (mm)				
	Concentration (mg/ml)				Sterile water
	50	100	200	400	
Bacteria					
Staphylococcus aureus	... ...	20.00 ± 0.1	22.00 ± 0.2	24.00 ± 0.2	0.00
Streptococcus sp.	16.00 ± 0.1	18.00 ± 0.1	22.00 ± 0.1	24.00 ± 0.1	0.00
Lactobacillus sp.	16.00 ± 0.1	20.00 ± 0.16	24.00 ± 0.2	26.00 ± 0.3	0.00
Actinomyces sp.	18.00 ± 0.21	20.00 ± 0.45	26.00 ± 0.2	30.00 ± 0.25	0.00
Fungi					
C. albicans	0.00	0.00	0.00	0.00	0.00
Aspergillus sp.	0.00	0.00	0.00	0.00	0.00

Means zones of Inhibition ± Standard Error of Mean (SEM)

Table 3: Zones of inhibition at different concentrations of Chlorhexidine gluconate

Organisms	Zone of inhibition (mm)				
	5%	2.5%	1.25%	0.625%	Sterile water
Bacteria					
S. aureus	36.00 ± 0.00	34.00 ± 0.00	32.00 ± 0.00	32.00 ± 0.00	0.00
Streptococcus sp.	30.00 ± 0.05	30.00 ± 0.01	28.00 ± 0.02	24.00 ± 0.01	0.00
Lactobacillus sp.	26.00 ± 0.02	24.00 ± 0.01	22.00 ± 0.01	22.00 ± 0.01	0.00
Actinomyces sp.	50.00 ± 0.01	44.00 ± 0.05	40.00 ± 0.00	38.00 ± 0.02	0.00
Fungi					
C. albicans	36.00 ± 0.1	30.00 ± 0.05	26.00 ± 0.1	20.00 ± 0.1	0.00
Aspergillus sp.	22.00 ± 0.25	18.00 ± 0.00	14.00 ± 0.15	12.00 ± 0.1	0.00

Means zones of Inhibition ± Standard Error of Mean (SEM)

Table 4: Sensitivity of the pathogens to *P. kotschyi* fractions

	Zone of inhibition (mm)			
Organisms	**n-Butanol**	**Aq. Remainder**	**Ethyl acetate**	**Chloroform**
S. aureus	56.00 ± 0.1	46.50 ± 0.65	Nil	Nil
Streptococcus sp.	40.00 ± 0.16	34.00 ± 0.11	Nil	Nil
Lactobacillus sp.	50.00 ± 0.1	39.50 ± 0.05	Nil	Nil
Actinomyces sp.	52.00 ± 0.1	37.00 ± 1.25	Nil	Nil

Means zones of Inhibition ± Standard Error of Mean (SEM)

Table 5: Minimum inhibitory concentration of *P. kotschyi* methanolic bark extract.

Organism	Minimum Inhibitory Concentration (mg ml^{-1})
S. aureus	3.2
Lactobacillus sp.	6.4
Streptococcus sp.	6.2
Actinomyces sp.	3.2

Means zones of Inhibition ± Standard Error of Mean (SEM)

Minimum inhibitory concentration (MIC) of *P. kotschyi* methanolic crude extract

Minimum inhibitory concentration is the minimum concentration at which the antimicrobial agent will completely prevent the growth of susceptible microorganisms. The MIC observed (Table 5) for *S. aureus* and *Actinomyces sp.* was 3.2 mg ml^{-1} while *Streptococcus sp.* and *Lactobacillus sp.* exhibited much higher MIC value of 6.2 and 6.4 mg ml^{-1} respectively.

DISCUSSION

The phytochemical screening of methanolic crude extract showed the presence of many secondary metabolites such as saponins, alkaloids and tannins. Previous studies have reported similar phytochemical constituents being present in the stem, root and the leaves of various solvent crude extracts of *P. kotschyi*.[23,26-27] These phytoconstituents are known to be the bioactive agents of plants and may be responsible for the inhibitory effects of test extract, *P. kotschyi* on investigated oral pathogens. Different and varied microorganisms isolated from the teeth and gums

confirm the observation that the oral cavity contains varied flora that could be harmful to the body system. [28] Micro-organisms isolated include; *S. aureus, C. albicans, Actinomyces sp.*, *Streptococcus sp., Aspergillus sp.* and *Lactobacillus sp.* Isolated organisms have been documented to cause mouth infections such a pulpitis, periodontitis and bacteria inflammation of the supporting structures of the teeth.[28, 29-30] This study has shown that both the crude methanolic extract and the semi-purified polar fractions of the stem bark of *P. kotschyi* exhibit antibacterial activities against tested bacteria and not against fungi as reported by Alhassan *et al.,* 2014, who recorded both antibacterial and antifungal activities.[31] The contradiction in the results to the earlier reported research by Alhassan *et al.,* 2014 could be attributed to the microbial load used for the assay, sub-therapeutic use of the extract or the method of extraction which did not yield compound(s) with pharmaceutical effect against all the isolate.[14] It could also be attributed to multi-drug resistant and reduced susceptibility to antibiotics microbial strains commonly presented by clinical microorganisms. Indiscriminate use of broad-spectrum antibiotics by humans could easily lead to development of resistance to available antibiotics by the microorganisms. However, it was observed by Valenciennes *et al.,* (2001), that antimicrobial agent might not necessarily be effective as both antibacterial and antifungal.[32]

When compared to the reference drug, chlorohexidine gluconate, the antibacterial activity of the extract is relatively low, however the semi purification process ameliorated the activity profile (Table 4). The n-butanol and aqueous remainder fractions of *P. kotschyi* methanolic crude extract in this study showed improved activity against the isolates *S. aureus*, *Streptococcus sp.*, *Lactobacillus sp.* and *Actinomyces sp.* (Table 4) as against the effect observed at the same concentration of 100 mg mL^{-1} for the crude extract (Table 2). The ethyl acetate and chloroform fractions were completely inactive against all the microbes tested (Table 4) which may signify that the organisms were resistant to the phytoconstituents of the fractions. This observation suggests that the bioactive chemical constituents which are responsible for the observed antibacterial activities of the crude extract may reside in the polar fractions particularly in the n-butanol fraction. The n-butanol fraction gave zones of inhibition ranging between 40 and 56 mm against all the bacteria while the aqueous remainder fraction presented 34 to 46.5 mm zone of inhibition range for the same bacteria. Saponins, glycosides and flavonoids identified by the phytochemical are constituents normally present in polar fractions (aqueous and n-butanol fractions) and may be suggested as the bio-active constituents responsible for the antimicrobial activity of *P. kotschyi* stem bark. Efficacy of polar fraction of *P. kotschyi* stem bark extract on some selected pathogens was reported by Alhassan *et al.,* 2014. Microorganisms they worked on were susceptible to ethyl acetate fraction.[31]

The clinical isolates, both bacteria and the fungi were very sensitive to the reference drug chlorhexidine gluconate at the tested concentrations when compared with the methanolic crude extract. At the concentration of 100 mg mL^{-1}, the semi-purified polar fractions exhibited significantly (P > 0.05) wide zones of inhibition for the bacteria while showing no antifungal effect (resistance) (Tables 2 – 4). Antibacterial effect of the polar fractions were comparable to the effects of chlorhexidine gluconate. Most mouthwashes contain chlorhexidine gluconate because of its broad spectrum antimicrobial activity but has been shown to irritate the mucosal membrane, stain the teeth and easily inactivated by food and saliva. An alternative with less adverse effect will be highly welcomed.

Activity displayed by the methanolic crude extract against *Actinomyces sp.* > *S. aureus* > *Streptococcus sp.* > *Lactobacillus sp.* in accordance to the MIC rating 3.2, 3.2, 6.2 and 6.4 respectively justify the ethnomedicinal use of the stem bark of *P. kotschyi* in the treatment of oral infections (Table 5) and as a daily oral hygiene product. The lower the MIC, the more effective the antimicrobial agent is and a much lower concentration of the agent needed to achieve desired antimicrobial effects (Table 2). The minimum inhibitory concentration (MIC) of the methanolic crude extract ranged from 3.2 mg ml^{-1} to 6.4 mg ml^{-1}, an indicative of P. kotschyi being a potential potent antibacterial agent.

Although this research was carefully prepared, we are still aware of its limitations and shortcomings, particularly in the size of population from whom oral cavity swabs were collected. Further research is recommended to isolate, purify and characterize possible active chemical constituents with a view to supplementing conventional mouth wash.

CONCLUSION

The results presented in this study showed that the stem bark of the tested chewing stick (*P. kotschyi*) contain antibacterial phyto-compounds against the tested clinical isolated microorganisms which were all sensitive to the reference drug, chlorhexidine gluconate. In addition, the results revealed increase in antimicrobial activities of the polar fractions of

methanolic extract of *P. kotschyi* stem bark. The regular use of *P. kotschyi* as chewing sticks and patiently holding the fluid in the mouth for extraction of active compounds into the saliva (polar solvent) may decrease the incidence of dental disease cause by oral microbe.

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