Antimicrobial Activity of *Pseudocedrela kotschyi* Schweinf Harms (Meliaceae) and Longrich Branded Tooth Paste on *Streptococcus mutans* from Dental Caries

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ABSTRACT

Background: Oral health has a significant impact on overall quality of human life. The rising prevalence of dental caries, demand for effective and cost-efficient treatment products.
Objective: This study determined the antimicrobial activities of crude aqueous and ethanol extracts of *Pseudocederalla kotschyi* and Longrich branded toothpastes on isolates of *Streptococcus mutans* from dental caries.
Methods: Fifteen (15) Isolates of biochemically confirmed *Streptococcus mutans* were challenged with aqueous and methanol extract of *Pseudocedrela kotschyi* using broth dilution method to determine heir minimum inhibitory concentration and minimum bactericidal concentrations of the aqueous extract of Longrich branded toothpastes. The chewing sticks extract were also screened for secondary metabolite using standard methods.
Results: The aqueous extract of *Pseudocedrela kotschyi* and Longrich branded toothpaste elicited minimum inhibitory concentration range from 25 µg/mL – 100 µg/mL while the minimum inhibitory concentration of 3 µg/mL -100 µg/mL and 25µg/mL - 100 µg/mL were recorded for *Pseudocedrala kotschyi* and Longrich branded toothpaste respectively. Each of the sample investigated elicited antimicrobial activity but methanol extract of *Pseudocedrela kotschyi* was remarkably higher than the antimicrobial potency of the Longrich branded toothpastes.
Conclusion: Although, the aqueous extract of the Longrich branded toothpasteexhibited antimicrobial activity,the methanol extracts of *Pseudocedrela kotschyi* holds the potential for clinical application if incorporated in conventional tooth paste that are readily available in the market.

Keywords: Dental caries, Longrich branded toothpaste, *Pseudocedrela kotschyi*

INTRODUCTION

Dental caries (tooth decay) is a major oral health problem in most industrialized countries, affecting 60–90% of schoolchildren and the vast majority of adults. The early manifestation of the caries process is a small patch of demineralized (softened) enamel at the tooth surface, often hidden from sight in the fissures (grooves) of teeth or in between the teeth. The destruction spreads into the softer, sensitive part of the tooth beneath the enamel (dentine). The weakened enamel then collapses to form a cavity and the tooth is progressively destroyed[1]

Dental caries is caused by the action of acids on the enamel surface. The acid is produced when sugars (mainly sucrose) in foods or drinks react with bacteria present in the dental biofilm (plaque) on the tooth surface. The acid produced leads to a loss of calcium and phosphate from the enamel; this process is called demineralization. When demineralization occurs frequently, there is a breakdown of the enamel surface leading to a cavity[2]

Cavities, even in children who do not yet have their permanent teeth, can have serious and lasting complications such as pain, tooth abscess, tooth loss, broken teeth, chewing problems and serious infection. Caries can also attack the roots of teeth should they become exposed by gum recession. This is more common in older adults. Dental caries are preventable childhood diseases but people can be susceptible to this tooth deformity throughout their lifetime[3].

Pseudocedrela kotschyi (Schweinf.) Harms belongs to the Meliaceae family. It is widespread in savannah woodland[4]. *P. kotschyi* is a tree of up to 20 metres high with a wide crown, fissured bark and fragrant

white flowers[5]. The bark is bitter and exudes a dark-coloured gum. In West Africa, it has been established that the root of P. kotschyi is widely used as chewing sticks for dental cleaning[6] and in North Côte d'Ivoire, it is of value in the treatment of toothache and internal wound. The root of the plant, which is also used to treat intestinal helminthosis, has been found to be a potential source of antibacterial agents[7]

Pseudocedrela kotschyii known as Orin Ayan in Yoruba language are sold in the herbalist stall called 'iso elewe omo'- paediatric herbal stalls and it can be bought from street hawkers. *Pseudocedrela kotschyii* competes better than some other common African chewing sticks which includes; *Acalypha ciliate Forssk*- Arunjeran, *Sorindeia warneckei Engl.*- Afunsese, *Alcornea laxiflora*- Pepe, *Milletia thonningii* –Itoo, *Lecaniodiscus cupanioides* – Aika, *Garcinia Kola*- Orogbo, *Anogeissus leiocarpus* – Orin Odan, /Pako Dudu, *Massularia acuminate*- Pako Ijebu, *Jarthropha curcas*- Lapalapa, *Olax subscorpiodea*- Ifon, *Baphia nitida*- Iyere osun, *Terminalia glaucescens*- Orin pupa, *Nauclea latifolia*- Egbesi, *Napoleona vogelli*–Boribori, *Veronia amygdalina*,- Ewuro, *Paullinia pinnata* –Kakasenla, *Morinda lucida*- Oruwo, *Fagara zanthoxyloides* – Orin ata, *Khaya senegalensis* – Oganwo and *Jatropha gossypiifolia*- Ogegee predominantly in southwest Nigeria mainly dominated by the Yorubas[8]

Longrich toothpaste is a Chinese brand of a fluoride free toothpaste with a blend of aloe extract and strontium chloride. This combination is powerful because, while working together, both elements can strengthen the teeth at the root and also protect the gums. The active ingredients contains water, sorbitol, hydrated silica, glycerin,sodium lauryl sulphate, xylitol, PEG-32, parfum, menthone, glycerin acetal, cellulose gum, xanthan gum, sodium saccharin, calcium glycophosphate, strontium chloride hexahydrate, trisodium phosphate, menthol, maltodextrin, aloe barbadensis leaf juice, camellia sinensis leaf extract, lactic acid, sodium benzoate, potassium sorbate. The product was launched in Nigeria in 2012[9]

Streptococcus mutans is a Gram-positive facultative anaerobe commonly found in the human oral cavity and it is one of aetiologic agent of tooth decay. This cocci bacterium, along with the closely related species *Streptococcus sobrinus*, can co-habit the mouth. *Streptococcus mutans* is most prevalent on the pits and fissures, constituting 39% of the total streptococci in the oral cavity[10]

Streptococcus *mutans* plays a major role in tooth decay, metabolizing sucrose to lactic acid. The acidic environment created in the mouth by this process is what causes the highly mineralized tooth enamel to be vulnerable to decay[11]

S. mutans is one a specialized organisms equipped with receptors that improve adhesion to the surface of teeth and enzyme glucan-sucrase that convert sucrose into a sticky, extracellular, dextran-based polysaccharide that allows them to co-here, forming plaque. The combination of plaque and acid leads to dental decay[12]

Sequel to the ethno-botanical survey, the peppermint flavor associated with Pseudocedrela kotschyi that informed its choice for this study, This study therefore compared the antimicrobial activities of aqueous and methanol extracts of *Pseudocedrela kotschyi* and Longrich branded toothpaste on *Streptococcus mutans* from dental caries .

MATERIALS AND METHODS

Collection of Plant Materials

The chewing sticks *Pseudocedrela kotschyii*– Ayan were collected from the forest of Cacao Rersearch Institute of Nigeria, Onigambari Ibadan, Oyo State. The entire plant were authenticated in the herbarium unit of the Department of Pharmacognosy with the voucher number PK-OOU3 where the voucher was archived..

Collection of branded toothpaste

Longrich tooth paste was purchased from a super market at Toyota Bus stop, Ladipo market area of Lagos State, Nigeria.

Collection of Isolates

Fifteen (15) isolates of *Streptococcus mutans* from cases of dental caries were collected from selected dental clinics routine laboratory bench in Ibadan, Oyo state of Nigeria.

Biochemical Identification

The isolates were cultured and incubated anaerobically on Sheep Blood Agar base fortified with serum and were biochemically confirmed to be catalase negative, Voges proskauer positive, urease negative, .galactose positive, glucose positive, lactose positive, inulin positive , mannitol positive, starch negative and trehalose positive *Streptococcus mutans.*

Preparation of Extracts

Pseudocedrela kotschyi stem collected were washed under running tap water to remove the dirty materials, macerated into tiny pieces and sun dried for 32 days. They were milled and stored in dry aseptic containers. A quantity of 25 grams of each milled of *Pseudocedrela kotschyi* was placed in screw-capped bottle containing 250 mL of 50% methanol and sterile distilled water. It was soaked for 5 days after which it was filtered using Whatmann No.1 filter paper. It was then concentrated using a

rotary evaporator and kept in the refrigerator at 4°C for further use

Phytochemical Screening of the Plant Extracts

Alkaloids

Exactly 0.5g of the extract of *Pseudocedrela kotschyi* was diluted to 10 mL with acid alcohol, boiled and filtered. To 5mL of the filtrate was added 2mL of dilute ammonia and a few drops of Dragendorff's reagent (potassium bismuth iodide solution) were added to the filtrate. The formation of reddish brown precipitate was observed as positive for alkaloids.

Saponins

Exactly 0.5g of extract was added 5mL of distilled water in a test tube. The solution was shaken vigorously, and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Tannins

Exactly 0.5g of the extract was boiled in 10mL of water in a test tube and then filtered, A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black coloration. A blue black precipitate was observed.

Anthraquinones

A quantity of 0.5g of the extract was boiled with 10ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5mL of chloroform. The chloroform layer was pipetted into another test tube and 1mL of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Flavonoids

To a portion of the aqueous filtrate of the extract, 5mL of 10 % dilute ammonia solution was added followed by addition of 1mL of concentrated H_2SO_4. A yellow coloration observed indicates the presence of flavonoids

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration of the aqueous and methanol extract was determined for each of the test organisms at concentration of 3, 6, 9, 12, 15, 20, 25, 50 and 100µg/mL. The concentration were obtained by adding 1mL aliquot of the extract (aqueous and methanol) at double strength concentrations (6,12, 18, 24, 30, 40, 100 and 200 µg/mL) to test tubes containing 1mL of nutrient broth. The test organisms (*Streptococcus mutans*) previously diluted to 0.5 McFarland turbidity standard were introduced with the aid of sterilized inoculating loop to respective tubes. The procedure was also used for the branded toothpaste (Longrich) respectively. Tubes containing nutrient broth only were seeded with the test organisms to serve as negative controls. The preparations were incubated anaerobically at 37°C for 24 hours and were examined for turbidity, an indicator of microbial growth.

Determination of Minimum Bactericidal Concentration

A loopful of broth was collected from those tubes that showed no growth in the MIC determination and streaked on the sterile blood nutrient agar respectively. The plates were incubated anaerobically for 24 hours and after incubation the concentration with no visible growth was taken and recorded as MBC respectively.

RESULTS

Table 1 shows various metabolite present in extract of *Pseudocedrela kotschyi* tested. Alkaloids, tannins, saponins, flavonoids were present in the plant while anthraquinones was absent.

Table 1: Phytochemical Screening of *Pseudocedrela kotschyi* extract

Phyto-constituents	*Pseudocedrela kotschyi*
Alkaloids	+
Tannins	+
Saponins	+
Flavonoids	+
Anthraquinones	-

Figure 1: Chewable sizes of *Pseudocedrela kotschyi* stem

Figure 2: Industrially branded toothpaste

Table 2: Minimum Inhibitory Concentration (µg/mL) of Aqueous and Ethanol extract of *Pseudocedrela* kotschyi and Longrich Toothpaste

Microorganisms	*Pseudocedrela kotschyi* Extract		Longrich Toothpaste
	AQ EXTRACT	**MEOH EXTRACT**	**AQ EXTRACT**
S.mutans1	100	3	50
S.mutans2	50	50	-
S.mutans3	50	6	25
S.mutans4	100	6	50
S.mutans5	100	20	25
S.mutans6	100	20	100
S.mutans7	-	100	-
S.mutans8	-	-	-
S.mutans9	25	12	-
S.mutans10	-	-	-
S.mutans11	-	-	-
S.mutans12	50	6	-
S.mutans13	50	15	100
S.mutans14	50	25	50
S.mutans15	50	6	50

Table 3: Minimum Bactericidal Concentration (µg/mL) of Aqueous and Methanol extract of *Pseudocedrela kotschyi* and Longrich Toothpaste

Microorganisms	*Pseudocedrela kotschyi* Extract		Longrich Toothpaste
	AQ EXTRACT	**MEOH EXTRACT**	**AQ EXTRACT**
S.mutans1	-	20	100
S.mutans2	-	100	-
S.mutans3	100	20	50
S.mutans4	-	25	100
S.mutans5	-	50	50
S.mutans6	100	50	-
S.mutans7	-	-	-
S.mutans8	-	-	-
S.mutans9	25	25	-
S.mutans10	-	-	-
S.mutans11	-	-	-
S.mutans12	50	15	-
S.mutans13	50	25	100
S.mutans14	50	25	50
S.mutans15	50	20	100

DISCUSSION

Tooth brush is primarily designed for the removal of supragingival plaque and even though bristle may penetrate subgingivally to a depth of between 0.9 and 1.5mm. Although tooth brushing achieves considerable reduction in the number of viable bacterial cell, its effectiveness correlates with the brushing time, and this is not always optimal. It has been reported that individuals who regularly brush their teeth have 40% to 60% of their tooth surface covered with plaques/caries[13]

The presence of alkaloids, flavonoids, saponins and tannins in *Pseudocedrela* can be attributed to the antibacterial activity of the plants. Lawal et.al., [14] had shown that antibacterial effect of plant materials was due to the presence of alkaloids, tannins and anthraquinones[14]

The aqueous extract of *Pseudocedrela kotschyi* were recorded to be effective on 11 strains of *Streptococcus mutans* at the MIC range between 25-100µg/mL but it elicited no activity on isolates 7, 8, 10 and 11. The methanol extract of *Pseudocedrela kotschyi* at low MIC values were recorded for almost all the strains of *Streptococcus mutans* with exception of isolates 8, 10, 11, within the MIC range of 3 -100µg/mL as shown in Table 2. The aqueous extract of Longrich toothpaste within the MIC range of 25-100µg/mL on 8 isolates of the 15 *Streptococcus mutans* examined were relatively remarkable in comparison with the aqueous extract of *Pseudocedrela kotschyi*. Though isolates 2, 7,8, 9, 10, 11 and 12 elicited no growth on the aqueous extract of Longrich toothpaste which could be due to extermination by the chemical composition of this toothpaste while the methanol extract of *Pseudocedrela kotschyi* were significantly better than the aqueous extract of the toothpaste. 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[15].

The MBC range of 25 µg/mL and 100µg/mL were recorded for aqueous extract of *Pseudocederella kotschyi* while the MBC aqueous extract values of between 50 µg/mL and 100µg/mL were recorded from Longrich antibiotics. The MBC of methanol extract of *Pseudocedrela kotschyi* range between 15 as showed in isolates *Streptococcus mutans 12* µg/mL and 100µg/mL as elicited in *Streptococcus mutans* 2. The extracts of the chewing sticks produced a remarkable activity at a different concentration. This corroborates the work carried out by Antwi-boasiako *et al.* on *Garcinia kola* extract that had a greater antimicrobial effect than the Pepsodent toothpaste[16] The methanol extracts of the plant materials had a comparatively better antimicrobial activity than the aqueous extracts which was similar to the findings of Osho and Adelani (2012) on the

antimicrobial effect of some selected Nigerian chewing sticks on clinical isolates of Candida species[17] A wide range of compounds with antimicrobial potential have been incorporated into toothpaste in order to improve the levels of oral hygiene achievable by tooth brushing. The branded tooth paste (Longrich) investigated in this study, showed better antimicrobial activity against the tested organisms. Longrich toothpaste had competitive antimicrobial activity in comparison to plant extracts. This is not unexpected since chemical toothpastes owe their antimicrobial property to the presence of chemical compounds with antimicrobial / anticaries ingredients usually incorporated in their brands[18]

CONCLUSION

Pseudocedrela kotschyi chewing sticks examined for antimicrobial activity in this study, elicited competitive remarkable antimicrobial activity against the isolates of *Streptococcus mutans* in comparison with branded antibiotics. It is cheaply affordable than the Longrich branded toothpaste and could provide better oral care. The active compounds if isolated would be good caries-controlling components of herbal toothpastes. Further study is recommended on other chewing sticks with antimicrobial potential.

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