



Comparative Analysis of Ethanolic Extract of Local Chewing Stick (*Massularia Acuminata*) and Commonly Used Close-Up on Mouth Isolate

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ABSTRACT

Good oral hygiene is necessary for healthy teeth, gums and fresh breath. In West Africa, chewing sticks and tooth paste are commonly used methods for oral hygiene. This research aimed at comparing the efficacy of local chewing stick (*Massularia acuminata*) with commonly used tooth paste (close-up) on mouth isolate from Ukwa Eburutu in Odukpani Local Government Area of Cross River State. The isolated Micro-organisms were, Staphylococcus, Streptococcus, Actinomycetes, Lactobacillus, Bacillus, Pseudomonas and Aspergillus species using modified agar well diffusion method. It was found that ethanolic extract of *M. acuminata* was active against staphylococcus with zone of inhibiting (3mm) while others were resistance to both ethanolic and distill water (negative control). However, close-up shows sensitivity of (2.7) for streptococcus, and 1.5mm, 0.9mm for Aspergillus respectively. Considering the degree of inhabitation expressed by *M. acuminata* and close-up, upward reviews and high constituent of *M. acuminata* is encouraged for continuous use for oral hygiene.

Introduction

Oral cavity consists of large number of gram positive and negative microorganisms. It act as a paradise because of its warm and moist environment, nutrients and unique anatomical feature for the growth, proliferation and multiplication of microorganisms (Roopavathi, et al, 2015). This is also where food digestion such as carbohydrate, fat and oil begins. Bacteria feed on leftover food (debris) attached to the gum in the mouth to create particles called volatile sulfur molecules, acids feed into the enamel and build up coating film on the teeth (plaque thereby encouraging the growth of harmful bacteria (Odenthopathogene) (Nwakanma et.al 2014).

According to Bankole et al (2012), dental plaque occur as a result of diverse microbial polymers growing on the teeth as a Biofilm. Microorganisms commonly found in the mouth environment include Staphylococcus, Salivarius, Neiseria Meningitides, Streptococcus species, Enterococcus fecalis, Pseumonas aeruginosa, Actinomycetes, Lactobacilluse etc. (Oyarekua, et al, 2015).

Oral hygiene is the practice of keeping the mouth and teeth clean to prevent dental problems ranging from dental plaque to periodontal disease and Halitosis, (Doweet al, 2016). In Africa, Nigeria inclusive, two major methods are usually adopted to maintain oral hygiene. These are the use of local chewing sticks and too brush with paste. Research have shown that about 80-90% of Nigeria population use chewing sticks because they are readily available, cheap and efficacious(Kareem et al, 2012).

One of the commonly used chewing sticks is *Massularia acumanita*. It is locally called Oko-edi in Efik dialect of Cross River State and Anang in Akwa Ibom State and "poko Ijebu" in Yoruba of Western Nigeria (Yakubu and Omoniwa, 2012; Ukekpe et al; 2015). According to Ukekpe et al (2015), its phytochemical screening contain Alkaloids, Tannins, Saponins, Steroids, Anthnaquinous, and cardiac glycosides. Biologically, both the aqueous and ethanolic extracts of this plant have antibacterial activity (Dowe et al; 2016).

Similarly, toothpaste is used to maintain the oral hygiene as well it aid the improvement of the aesthetic appearance and health of the earth (Nwakanma et al, 2014). It contains an active ingredient such as abrasive, fluoride, humectants, surfactants, and tartar control (Maryman, 2014; Nwakanma et al 2014).

Statement of the problems

Micro-organism associated with mouth has long ago been identified by scientists. Because of the diverse species of these organisms, researchers hold that same of which are yet to be identified. In our present day society and depending on the hard economy, communities depend on *M. acuminata* (local chewing sticks) instead of going for close-up. Evidence abound that the use of close-up is more effective in removing the organisms associated with the mouth while some studies strongly support the fact that both the local chewing stick and close-up are all functioning in the same direction. The problem of the present study is to examine therefore the efficacy of *M. acuminata* and close-up on micro-organism associated with mouth in Ukwa Ibom in Odukpani Local Government Area.

Aims and objectives

The aims and objectives of this present study is to:

- a) Identify and isolate the common pathogenic organisms (Odonthapathogenes) that can potentially cause dental carriers.
- b) Investigate and compare the efficacy of commonly used toothpaste (close-up) and local chewing stick (*M. acuminata*) on oral Rathognes literatures review

Streptococcus species: With particular references to *S. mutans* are gram positive, spherical in shape that form pairs of chain during growth. They are the most destructive bacterial strain in the oral cavity. It ferment sugar in the diet to produce lactic acid which destroys the enamel of the teeth. Jain and Sharma (2012) states that *S. Mutans* are also known to form an insoluble glucan for adhesive, aggregation that results in biofilm formation. Al.Jumaly et al (2014) said this glucan is synthesized from the glucose moiety of sucrose and plays an important role in the ability to potentiate the formation of dental carriers.

Staphylococcus aureus: Is an gram positive, non-spor forming non-motile and facultative anerobic. Poeschl et al, (2011) review that staphylococcus aureus is a putative pathogen of many oral disease, such as oral mucositis, periodontitis, endodontic infection and dental carrier. Buries et al (2005) said it is a common human pathogen that causes various skin and mucosal infection.

Aspergillus species: Aspergillus species are known to produce a broad spectrum of Mycotoxins including aflatoxins, sterigmatocystin and achratoxin. There are several Immure suppressive health issues (Gautan and Bhadauria, 2012). Aspergillus belong to a group of filamentous deutromycetes with no sexual reproductive phase (Nyongesa et al 2015).

Actinomycetes: Actinomycetes are filamentous gram positive bacteria, characterized by a complex life cycle which belong to the phylum, Actinobacteriales, which represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain bacterial (Sharma, 2014). They are non-motile, non-spor forming, non-acid fast positive pleomorphic, anaerobic-to microaerophilic filamentous bacterial rods which causes actinomycetes.

Common dental diseases

Dental plague: it is caused by a complex and dynamic processes that involves the progressive destruction of tooth enamel, dentine and cementum by bacteria (Nishimuna et al, 2012). It is commonly caused by streptococcus mutans (Jain and Sharma, 2012), a complex biofilm found on the tooth surface (Otoikhian & Okoror, 2012).

Periodontal Diseases: it is also called gum disease, the most common health problem in the human communities (Vahabi et al, 2011). It is a primary etiological factor of periodontal diseases (Ahaphanchi et al 2015).

Halitosis: It refers to us bad breath that emanates from the oral cavity either intra-oral extra-oral with different nomenclature ranging from oral malodor, factor ex-ore, bad breath and foul smell (KABIR T AL ; 2013). Zurcher et al ; (2014) describes it as offensive and unpleasant smell of the breath.

Active ingredients of tooth paste

Abrasive: it perform the primary functions removing plague and stains from teeth. Example are broking soda, sodium bicarbonate, which neutralizer acids in the saliva and provides a foaming action in the mouth (Dange et al, 2014).

Other active ingredients are fluoride which is effective in strengthening and increasing resistance of enamel solution as well as anti surfactant and tartar control additive.

General description of local chewing stick (*M. acuminata*). *Massularia acuminata* locally called 'Okok edi' in Efik dialect of Cross River State and Anang dialect of Akwa Ibom State (Ukekpe, et.al.2015). The leaves are large, practically stalks, elliptic and acuminate. The flower usually red, borne in short auxiliary cymes, appear around January. The fruits which are narrowly void, beaked and yellowish-white in colour are 5cm long (Yakubu and Omoiwa, 2012). *M. acuminata* belong to a family Rubiacene (Dowe, et al. ; 2016). According to Wang (2014), it is a tropical plant commonly found in west Africa and predominately use from oral hygiene. According to Akande and Ajao (2011), *M. acuminata* are known for their diverse use traditional medical practice. The root, bark, according to Ukekpe et al (2015) are used to treat skin infections, dental diseases and various medical problems. Yakubu and

Omoniwa (2012) said the phytochemical constituent of *M. acuminata* have been reported that its aqueous extract contain alkaloids 0.22%, saponins 1.18%, anthraquinones 0.048%, flavonoids, tannins 0.75% and phenolics 0.066%. More so, alkaloid content of the extract have anti-inflammatory activity and is effective in preventing against gingivitis and periodontitis.

Materials and methods

Collection of clinical sample

Twenty(20) people will randomly selected. Each was given a brand new toothbrush and a universal sample bottle. They will instructed to brush their mouth early in the morning with brush provided without paste, before brushing with their usual toothpaste. The mouth content will be emptied empty into the universal bottle that will be given to them. Their names were alphabetically written on the specimen bottle with ages. The collected samples will immediately be put in an ice-cool pack and transported to Microbiology Department in the University of Cross River State for analysis.

Isolation and Identification of Microorganisms Association with Mouth.

Early mouth brush samples collected were serially diluted incubated at 37⁰c for 24hrs. Colonies on the overnight culture plate were identified. Biochemical test was carried out for further identification such as catalase, oxidase, coagulate and triple sugar iron agar tests.

Collection and Identification of *Massularian Acuminata*

The fresh stem of *M. acuminata* was collected from Ukwa Ibom, in Odukpani Local Government Area, Cross River State and taken to Botanical garden of the UNICROSS for identification by Prof. Samuel E.Udoh and Mr Akiba E.E. in the department of Biological Sciences.

Preparation and extraction of local chewing stick (*Massularian acuminata*)

Sexwet extraction was done using two different solvent, ethanolic and aqueous. The fresh stem of *Massularia acuminata* will first be washed with tap water and rinse with distill water, cut into pieces and even dried at 40⁰c. After pulverization about 25g was weighted and turn into Sexwet extractor column, and 125ml 95% ethanol was measured into round bottom flask and introduced antipump to avoid cracking, thus was heated for extraction. Same will done in aqueous. The extracts was filtered using What man paper and concentration using rotary, evaporated and stored for further water to serves as inoculum.

A sterile wire loop was used to pick a little quantity of each stick culture of the isolate and emulsify in 1ml of distill water to served as innoculum.

Sensitivity Test of *M. acuminata* and Tooth paste

The modified agar-well diffusion described by Uduak and Kola (2010) was used for these tests. Well was made in diameter of 8mm using cork borer to cut uniform well on the surface of solidified agar plates. Each well was filled with 1ml of the extract and diluted toothpaste at different concentrations. The same quantity of sterile water was used as negative control. All plates was alphabetically and carefully covered and allowed for some minutes for complete diffusion of extract and diluted toothpaste in the walls. All bacterial plates was incubated at 37⁰c for 24hrs.

Preparation of tooth paste:

1g of close-up was dissolved into 1ml of dilute water. This was serially serial diluted to power minus three (10⁻³). One mole 10⁻² and 10⁻³ was introduced into each bore on the agar plate.

Result

Percentage of occurrence of isolates

Noticeable pathogenic organisms such as *Streptococcus*, *Actinomycetes*, and *Aspergillus spp* were carefully isolated in their percentages and prevalence rate of 6(30.00), 4(20.00), and 1(5.00) respectively.

Antimicrobial Sensitivity of Ethanolic Extract of *M. acuminata* and commonly used Toothpaste(close-up).

Table

	Zone of inhibition				
	Ethanolic extraction of <i>M.acuminata</i>			Close -up	
	10 ⁰	10 ⁻³	10 ⁻²	10 ⁻³	R(0)
<i>Staphylococcus spp-</i>	S(3mm)	R(0)	R(0)	R(0)	R(0)
<i>Streptococcus spp</i>	R(0)	R(0)	S(2.7mm)	R(0)	R(0)
<i>Aspergillus spp</i>	R(0)	R(0)	S(1.8mm)	S(1.8mm)	R(0)
<i>Actinomycetes spp</i>	R(0)	R(0)	R(0)	R(0)	R(0)

Discussion

Results of the study shows that microbial isolation from mouth swab samples from the study area. The Isolated Organisms were identified based on gram reactions, morphological appearance, and biochemical test such as catalase, coagulase, oxidase, triple sugar fermentation testes. Noticeable isolates were Staphylococcus, Streptococcus, Actinomycetes and Aspergillus species.

Percentage occurrence and prevalence rate of oral isolates for Staphylococcus, Streptococcus Species were far high 6(30.00) compare to the isolates for Actinomycetes and Aspergillus special with 4(20.00) and 1(5.00) respectively. High occurrence of Staphylococcus, Streptococcus species from the oral specimen of these pathogens in the mouth of the villagers indicates high risk of dental diseases.

Moreso, the antimicrobial activity of ethanolic extracts of *M. acuminata* and also that of the toothpaste shows sensitivity on Staphylococcus species having zone of inhibition 3mm at concentration of 0.01mg/ml. Others shows resistance at 0,001 and 0.001 mg/ml respectively. One-in-ten mile dilution of commonly used close-up at factor 10¹ and 10 shows sensitivity of (2.7mm, 1.5mm and 0.9mm) against Streptococcus and Aspergillus at different concentration. The trace activity of *M. acuminata* against staphylococcus species, the most threatening- pathogen that cause dental carrier proved that *M. acuminata* poses degree of Pharmacological properties due to the phytochemical constituent present

Conclusion

Considering the degree of sensitivity (3mm) expresses by *M. acuminata* to commonly used close-up (2.7mm), 1.5mm and 0.9mm), *M. acuminata* is hereby recommended for upward review of high phytochemical concentration of continuous use in the maintenance of oral hygiene.

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