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Physicochemical Evaluation and Antibacterial Activity of Massularia Acuminata Herbal Toothpaste

Olutayo Ademola Adeleye[1], Oluyemisi Bamiro[1], Mark Akpotu[2], Modupe Adebowale[3], John Daodu[4], Mariam Adeola Sodeinde[3]
[1]Department of Pharmaceutics and Pharmaceutical Technology, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria
[2]Department of Pharmaceutical Microbiology, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria
[3]Department of Pharmacognosy, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria
[4]Department of Pharmaceutical Chemistry, Olabisi Onabanjo University, Ago Iwoye, Ogun State, Nigeria

Corresponding Author Information
Ademola Adeleye
olutayoadeleye@yahoo.com
+234 8056112885
orcid.org/0000-0001-8716-4064
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ABSTRACT
INTRODUCTION: Oral hygiene being an integral part of the body's general well-being requires maintenance to prevent dental problems. This study was carried out to incorporate the ethanol extract of Massularia acuminata twig in the formulation of herbal toothpaste and to evaluate its antibacterial activity in comparison with a commercially available herbal toothpaste against two dental pathogens - Staphylococcus aureus and Streptococcus mutans.
METHODS: The dried twig of Massularia acuminata was extracted with ethanol and was used to formulate toothpaste containing 1%, 2%, 3%, 4% and 5% Massularia acuminata extract. The sensory and physicochemical properties of the toothpaste were evaluated. Agar well diffusion method was used to evaluate the antibacterial susceptibility of the toothpaste against Staphylococcus aureus and Streptococcus mutans. Data were analyzed using one-way ANOVA and t-test.
RESULTS: All the toothpastes were smooth in texture, pleasant in odour, sweet to taste. They all had good retention ability on the bristle of toothbrush with a pH range of 7.18 - 7.83. The toothpastes were active against the test organisms at all extract concentrations. The antibacterial activity of the formulated toothpastes increased significantly with increase in extract concentration. Formulation F5 containing 5% extract had the highest activity with a zone of inhibition of 19.30±0.17mm and 12.60±0.52mm on Staphylococcus aureus and Streptococcus mutans respectively even when compared to the commercially available herbal toothpaste.
DISCUSSION AND CONCLUSION: The incorporation of the extract of Massularia acuminata in the formulation of herbal toothpaste prevented the growth of Staphylococcus aureus and

Streptococcus mutans. The use of this extract in toothpaste formulation will satisfactorily maintain the oral hygiene desired to prevent dental caries and periodontal diseases.
Keywords: Toothpaste, Staphylococcus aureus, Streptococcus mutans, dental caries, antibacterial

INTRODUCTION

Oral hygiene is an integral part of the body's general well-being which begins with clean teeth. Cleaning of the teeth is a cultural habit from generation to generation which is usually done as a daily morning routine. It is regarded as an indispensable component of oral health.[1] There are various techniques by which cleaning of teeth are performed by different populations. The modern - conventional technique which involves the use of toothpaste and toothbrush which has been in use for decades,[2,3] and traditional techniques involves prominently the use of chewing sticks and local toothpaste.[4] Other methods of tradition teeth cleaning involve the use of finger to rub various substances - natural powders, bark of plants, ash, charcoal, oil and salt - onto their teeth.[5,6] (Josefine Hirschfeld, Not all cultures use toothbrushes. But how effective are alternative methods? The Conversation 7 July 2019.)

Poor oral hygiene could lead to the development of dental caries and periodontal diseases. Dental caries commonly known as tooth decay is an infectious disease caused primarily by *Streptococcus mutans*.[7,8] Periodontal disease, also known as gum disease is an inflammatory condition of the gum (known as gingivitis) or the bone and tissues of the teeth (known as periodontitis).[9] Some bacteria associated with periodontal diseases are *Aggregatibacter actinomycetemcomitans, Streptococcus mutans, Bacteroides forsythus, Staphylococcus intermedius, Lactobacillus acidophilus Porphyromonas gingivalis, Prevotella nigrescens, Treponema denticola* etc.[10,11]

Chewing sticks, a traditional method of cleaning the teeth to maintain oral hygiene has been practiced for thousands of years and is still being widely used in Africa, Asia and the Middle East.[12,13] Some studies have reported the effectiveness of chewing stick usually due to the presence of antibacterial bioactive compounds present in them in removing dental plaque thereby preventing dental caries and periodontal diseases.[14,15] Some of the chewing sticks that have been studied are, *Terminalia glaucescens, Sorindeia warneckei, Vitex doniana, Vernonia amygdalina, Fagara zanthoxyloides, Xanthoxylum zanthoxyloides, Massularia acuminata, Pseudocedrela kotschyi, Anogeissus schimperi, Anogeissus leiocarpus and Azadirachta indica*.[16-18]

Massularia acuminata (G. Don) Bullock ex Hoyle. is a shrub as shown in Figure 1 belonging to the family Rubiaceae widely distributed in West Africa. It is commonly known as chewing stick tree. The root, leaf, back and twig has several medicinal values. It is used traditionally in some West African regions for the treatment of diarrhea, dysentery, muscular pains venereal diseases and as an aphrodisiac.[19] Pharmacological studies have shown the plant to possess a strong antibacterial property against oral pathogens.[20-22]

This study was carried out to incorporate the extract of this plant in the formulation of a herbal toothpaste and to evaluate its antibacterial activity against two pathogens associated with dental caries and periodontal diseases.

MATERIALS AND METHOD

Plant Collection

The twig of *Massularia acuminata* was collected from the wild in Onigambari forest, Ibadan, Oyo State in the month of February and authenticated at Forestry Research Institute of Nigeria, Ibadan, Oyo State, Ogun State by Mr Odewo (Voucher Number: FHI. 112857).

Test organisms

Clinical isolates of *Staphylococcus aureus* and *Streptococcus mutans* obtained from Microbiology Laboratory of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State were used for the antibacterial study.

Preparation of extract

The twig of the plant was cut into pieces and air dried for 30 days. The dried twig was then ground into powder. About 150g of the powder sample was macerated in 750mL of 95% ethanol (BHD chemicals, Poole England) for 72 h at room temperature with intermittent agitation according to the procedure of Tedwins, et al. (2016).[21] Thereafter; it was filtered with Whatman's filter paper. The filtrate was concentrated to dryness using a rotary evaporator and stored in a refrigerator prior to use.

Phytochemical screening

Phytochemical screening of the extract was performed to determine the bioactive compounds present in the extract using the procedure of Trease and Evans.[23]

Antibacterial screening of plant extract

The antibacterial activity of 100mg/mL of *Massularia acuminata* extract in distilled water was determined by the agar diffusion method. A suspension of an overnight culture of *Staphylococcus aureus* and *Streptococcus mutans* in nutrient broth was standardized to 0.5 McFarland standards (10^6 cfu mL^{-1}). Nutrient agar plates were prepared in a petri dish by inoculating with 0.2mL of the standardized culture of the test organisms and were allowed to set. Wells of 6.0mm were bored in the nutrient agar and each filled with 0.5mL of the extracts, the positive control (gentamicin) and the negative control (distilled water). The plates were allowed to stand for 30 minutes for proper diffusion of the extract before incubating at 37^{o}C for 24 hours. The diameters of zones of growth inhibition were then measured in millimetre. The experimental procedure was done in triplicate.

Preparation of Massularia acuminata extract herbal toothpaste

The quantity of the ingredients required to make 100g of toothpaste is shown in Table 1. Tragacanth gum was mixed with a little quantity of distilled water in a mortar with a pestle to form a paste. Glycerin was added and triturated vigorously followed by slow addition of calcium carbonated with continuous trituration. *Massularia acuminata* extract was then added to the content in the mortar and thoroughly mixed for even distribution. Sodium lauryl sulphate (SLS) was added with slow stirring to prevent foaming. Saccharine and peppermint oil were added and then the paste was made to the required weight with addition of distilled water.

Evaluation of Massularia acuminata extract herbal toothpaste

Determination of organoleptic properties

The colour, appearance, texture, odour and taste of each formulation were determined by sensory and physical evaluation.

Determination pH

One gram of each toothpaste formulations dispersed in 10 ml of purified water (with pH 6.98) and the pH was measured in triplicate with a digital pH meter (pH600, Milwaukee).[24]

Determination of foaming ability

Five (5) gram of toothpaste formulation was dispersed in 10mL of water in a 100 mL glass beaker. The beaker was covered with a watch glass and allowed to stand for 30 minutes. The mixture was stirred with a glass rod to break up lumps and was transferred into a 250mL graduated measuring cylinder ensuring no formation of foam of more than 2mL. The beaker was rinsed with 5-6mL of water into the measuring cylinder. The cylinder was made up to 50mL with water, covered with a stopper, maintained at 30°C and shaken for few seconds. The cylinder was then allowed to stand for 5 minutes. The volume of foam with water (V_1) and water only (V) was recorded. [25]

Foaming ability was calculated as; $V_1 - V_2$

Determinations were done in triplicate

Determination of moisture

The moisture content was determined by weighing accurately 5 g (W_O) each of the formulation into an evaporating dish of 6-8 cm in diameter and 2- 4 cm depth. It was dried in an oven at 105°C ±2°C until the weight remained constant and was noted as W_1.[25]

Percentage loss by mass = $(W_O - W_1 / W_0) \times 100\%$

The mean of three determinations was calculated.

Determination of spreadability

One (1) gram of toothpaste was placed on a glass plate of 10cm x 10cm size and covered with another glass plate of same size. A weight of 1kg was placed on the top glass plate and allowed to stand for 10 minutes after which it was removed. The diameter of spread on the plate was measured and mean of three determinations was taken. [25]

Determination of viscosity

The viscosities of the formulation were measured at 20, 50 and 100 rpm at 25°C using a Brookfield viscometer (Model - DV - 11 + Pro, Brookfield Eng. Labs Inc Midddleboro, MA, USA) with spindle no. 4.

Antibacterial screening of toothpastes

The antibacterial activity of the commercially available toothpaste and different concentration of the formulated *Massularia acuminata* toothpaste was determined by the agar diffusion method. The method used for the screening of the *Massularia acuminata* extract against *Staphylococcus aureus* and *Streptococcus mutans* was adopted.

Statistical analysis

Statistical analysis was performed by subjecting data to Student's *t*-test and one-way analysis of variance (ANOVA) using GraphPad Prism version 5.01 software. P-values <0.05 were considered to be statistically significant.

RESULTS

The phytochemical constituents present in the ethanol extract of twig of *Massularia acuminata* as presented in Table 2 are anthraquinones, saponins, flavonoids, alkaloids, tannins and the flavonoids.

The antibacterial activity of the ethanol extract of twig of *Massularia acuminata* (100mg/mL) is presented in Table 3. The extract and the positive control (gentamicin) had activity on both organisms – *Staphylococcus aureus* and *Streptococcus mutans* while the negative control (distilled water) had no activity.

The results of the sensory and physical evaluation, and the pH of the formulated toothpaste without herbal extract (F0), formulated herbal toothpaste containing different concentrations of the extract of *Massularia acuminata* twig (F1 - F5) and the commercially available herbal

toothpaste (F6) are presented in Table 4. F0 had an off white colouration, F1 – F5 colours varied between light brown and brown while F6 had a green colouration. All formulations had a pleasant odour and a sweet taste; they were all smooth in texture and paste-like in appearance. The moisture content of F0 – F5 ranged from 24.22% to 28.25% while F6 was 31.36%. The spreadability and foam ability of all toothpaste was from 5.7cm to 7.2cm and 51.0cm to 63.0cm respectively. The pH of the toothpastes range was 7.18 - 7.83.

The viscosity of the toothpastes as measured by different spindle speed at a temperature of 25^0C is shown in Figure 2. The result showed a significant decrease in viscosity as the spindle speed increased.

The antibacterial activity of the toothpastes presented in Table 5 revealed that all the toothpastes had activity on both *Staphylococcus aureus* and *Streptococcus mutans* except F0 and F1 which did not show any activity on *Streptococcus mutans.*

DISCUSSION

The phytochemical constituents of plants are secondary metabolites which are the bioactive components possessing the pharmacological activity of plants. The phytochemical constituents of the ethanol extract of *Massularia acuminata* twig are anthraquinones, saponins, flavonoids, alkaloids, tannins and the flavonoids. This is similar to some studies documented in literatures.[20-22] Tannin, saponin and flavonoids are bioactive compounds which have been reported to possess antimicrobial activities in herbs.[26,27]

The test organisms employed in this study - *Staphylococcus aureus* and *Streptococcus mutans* – are among the main organisms associated with dental caries and periodontal diseases respectively.[11,28-32] The antibacterial activity of the ethanol extract indicated a strong activity against these organisms. This result corroborates the result obtained by several other researchers.[20,21,22] The ethanol extract had a significantly higher activity against *Staphylococcus aureus* than *Streptococcus mutans* but the activity of the positive control – gentamicin was significantly higher against the test organisms. The antibacterial activity of the extract could be due to the presence of bioactives such as tannin, saponin and flavonoids.

The sensory and physical evaluation of the toothpastes as shown in Table 4 indicates that they all have smooth texture, pleasant odour, sweet taste but different colours. The pleasant odour and sweet taste is as a result of the presence of flavouring agent (peppermint oil) and sweetener (saccharin) respectively in all the toothpastes including the commercially available one. Despite the high concentration of *Massularia acuminata* extract in formulations F4 and F5, the sweetener was able to mask the bitter taste of the extract. The formulated toothpaste without herbal extract (F0) was off white in colour since it contained no herbal extract. Formulated herbal toothpaste (F1 - F5) had colours varying between light brown and brown. Colour intensity deepens with the concentration of the extract. The commercially available herbal toothpaste (F6) was green. All these parameters will enhance consumer acceptability of the product. Formulation F6 had the highest moisture content (28.25%) in the following order F6 > F0 > F1 > F2 > F3 > F4 > F5. This is reflected in the quantity of water content in the formulations as shown in Table 1. This could in turn affect spreadability, foaming ability and viscosity. While the spreadabilty of the formulated toothpastes decreased with increase in concentration of extract in the following order F0 > F1 > F2 > F3 > F4 > F5, foaming ability and viscosity increased in the order F0 < F1 < F2 < F3 < F4 < F5. Viscosity is a factor which determines the spreadability,[33] thickness and ability of the toothpaste to retain its ribbon shape when extruded from the tube on the toothbrush.[33] The ribbon shape retention of toothpastes is the ability to retain its ribbon shape on the bristle of a toothbrush without collapsing.[34] All the toothpaste had good retention ability. Spreadability

measures the extent of the area the toothpastes can spread on teeth, gum, gum lines and other areas, and also the extent of penetration into infected tooth and gum. The spreadability of the commercially available toothpaste was significantly higher than the formulated toothpaste. Foaming ability is the measure of the cleansing power of toothpastes which is impacted by the presence of surfactants (SLS). Sodium lauryl sulphate produces foam which lowers surface tension of the surface film on the tooth thereby suspending and removing debris. Toothpaste with good foaming ability will provide good cleansing action of the teeth. There was a significant difference in the foaming ability of the toothpastes with F5 having the greatest cleansing action. It was observed that the presence of the extract progressively increased the foaming ability of the formulated toothpaste. This may be due to the frothing properties of saponin present in the extract.[35]

The oral microbial flora when compromised usually due to reduction in pH caused by carbohydrate metabolism by these organisms could cause dental caries and other periodontal diseases and dental plaque.[31,36,37] Maintaining the microbial flora is desirable for the well-being of individuals which could be achieved by proper oral hygiene by cleaning of the teeth. Keeping the pH of the teeth at an alkaline range may prevent the development these dental problems. The pH of the toothpaste used in this study were all in the alkaline range.

The antibacterial activity of the toothpastes was evaluated against *Staphylococcus aureus* and *Streptococcus mutans*. These bacteria are among the most implicated pathogens in dental caries and periodontal diseases. The result revealed that the toothpastes were active against the test organisms at all extract concentrations and also, antibacterial activities increased significantly with increase in extract concentration ($P < 0.05$). Formulation F5 containing 5% extract had the highest activity with a zone of inhibition of 19.30±0.17 and 12.60±0.52 on *Staphylococcus aureus* and *Streptococcus mutans* respectively even when compared to the commercially available herbal toothpaste. The increase in activity could be as a result of increase in the concentration of the bioactive phytochemical component of the extract which was similar to the trend observed with the antibacterial evaluation of the crude extract during preformulation. The antibacterial activity against *Staphylococcus aureus* was significantly higher than *Streptococcus mutans* ($P < 0.05$). Formulation F0 showed little activity because SLS and peppermint oil contained in the composition of the paste are known to possess antimicrobial activity against *Staphylococcus aureus* and *Streptococcus mutans*.[38,39,40] The antibacterial activities of the formulated toothpaste above 2% concentration of *Massularia acuminata* extract were significantly more active than the commercially available herbal toothpaste against both test organisms ($P < 0.05$). Further research is required to isolate the bioactive compound in the extract responsible for the antibacterial activity of this plant.

CONCLUSION

Poor oral hygiene is associated with the development of dental caries and periodontal diseases. However, the use of toothpastes has a role in maintaining the oral hygiene to prevent its consequences. Fortifying toothpastes with herbal antibacterial agents such as methanolic extract of *Massularia acuminata* has been demonstrated in this study to be more active against some of the pathogens implicated in the development of dental caries and periodontal diseases *in vitro*. The use of the extract of this plant as an ingredient in toothpaste formulation will enhance satisfactory maintenance of the oral hygiene that is desired to prevent dental caries and periodontal diseases.

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Table 1: Composition of toothpaste formulation (100g)

Ingredients (g)	F0	F1	F2	F3	F4	F5
M. acuminate	0.0	1.0	2.0	3.0	4.0	5.0
Calcium carbonate	20.0	20.0	20.0	20.0	20.0	20.0
Sodium lauryl sulphate	1.5	1.5	1.5	1.5	1.5	1.5
Glycerin	30.0	30.0	30.0	30.0	30.0	30.0
Tragacanth gum	1.2	1.2	1.2	1.2	1.2	1.2
Saccharine	0.5	0.5	0.5	0.5	0.5	0.5
Peppermint oil	1.0	1.0	1.0	1.0	1.0	1.0
Distilled water	45.8	44.8	43.8	42.8	41.8	40.8

Table 2: Phytochemical screening of extracts

Phytochemical	Result
Anthraquinones	+
Saponins	+
Flavonoids	+
Alkaloids	+
Tannins	+
Favanoids	+

+ present , – absent

Table 3: Antibacterial activity of extract

Test organisms	Zones of inhibition (mm)		
	Extract	Positive control	Negative control
Staphylococcus aureus	25.50±0.10	33.00±0.37	-
Streptococcus mutans	20.20±0.28	29.50±0.81	-

Mean ± SD, n=3, Extract = *Massularia acuminate* 100mg/mL, Positive control = Gentamicin 80mg/2mL and Negative control = distilled water

Table 4: Physical evaluation and pH of formulations

Parameters	F0	F1	F2	F3	F4	F5	F6
Colour	Off White	Light Brown	Light Brown	Brown	Brown	Brown	Green
Appearance	Paste-like	Paste-like	Paste-like	Paste-like	Paste-like	Paste-like	Paste-like
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Odour	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Taste	Sweet	Sweet	Sweet	Sweet	Slightly Sweet	Slightly Sweet	Sweet
Moisture	28.25±1.23	26.94±0.75	26.15±0.13	25.66±1.90	24.81±2.06	24.22±0.11	31.36±1.83

content (%)							
Spreadability (cm)	6.90±0.06	6.50±0.02	6.50±0.15	6.20±0.04	6.00±0.11	5.70±0.61	7.20±0.12
Foaming ability (cm)	51.00±0.22	55.00±0.20	56.00±0.06	60.00±0.13	62.00±0.10	63.00±0.24	58.00±0.22
pH	7.18±0.04	7.32±0.12	7.34±0.10	7.41±0.22	7.44±0.43	7.57±0.25	7.83±0.54

Mean ± SD, n=3, F0 = formulated toothpaste without herbal extract, F1 - F5 = formulated herbal toothpaste containing 1%, 2%, 3%, 4% and 5% *Massularia acuminate* extract respectively F6 = commercially available herbal toothpate

Table 5: Antibacterial activity of toothpaste formulations

Test organisms	Zones of inhibition (mm)	
	Staphylococcus aureus	*Streptococcus mutans*
F0	3.10±0.23	-
F1	7.50±0.55	-
F2	11.00±0.61	6.70±0.40
F3	13.60±0.22	9.20±0.32
F4	17.70±0.19	10.60±0.20
F5	19.30±0.17	12.60±0.52
F6	11.50±0.42	7.80±0.72

Mean ± SD, n=3, F0 = formulated toothpaste without herbal extract, F1 - F5 = formulated herbal toothpaste containing 1%, 2%, 3%, 4% and 5% *Massularia acuminate* extract respectively F6 = commercially available herbal toothpate