



Physicochemical Evaluation and Antibacterial Activity of *Massularia acuminata* Herbal Toothpaste

Massularia acuminata Herbal Diş Macununun Antibakteriyel Aktivitesi ve Fizikokimyasal Değerlendirmesi

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ABSTRACT

Objectives: Oral hygiene, an integral part of the body's general well-being, should be maintained to prevent dental problems. This study was conducted to incorporate the ethanol extract of *Massularia acuminata* twigs in a formulation of herbal toothpaste and evaluate its antibacterial activity compared with a commercially available herbal toothpaste against two dental pathogens, namely, *Staphylococcus aureus* and *Streptococcus mutans*.

Materials and Methods: The content of dried *M. acuminata* twig was extracted using ethanol and used in the formulation of toothpaste containing 1%, 2%, 3%, 4%, and 5% *M. acuminata* extract. The sensory and physicochemical properties of the toothpaste were evaluated. The agar well diffusion method was used to evaluate the antibacterial susceptibility of the toothpaste against *S. aureus* and *S. mutans*. Data were analyzed using One-Way analysis of variance and Student's t-test.

Results: All toothpastes were smooth and sweet and smelled pleasant. They all had good retention ability on the bristles of toothbrush and had a pH range of 7.18-7.83. The toothpastes of the extracts of different concentration demonstrated antibacterial activities against the test organisms. The antibacterial activity of the formulated toothpastes increased significantly with an increase in the extract concentration. F5 that contained 5% extract showed the highest activity, with an inhibition zone of 19.30±0.17 mm and 12.60±0.52 mm against *S. aureus* and *S. mutans*, respectively, even when compared with the commercially available herbal toothpaste.

Conclusion: The incorporation of the *M. acuminata* extract in the formulation of herbal toothpaste prevented the growth of *S. aureus* and *S. mutans*. Incorporating this extract in toothpaste formulation will satisfactorily maintain oral hygiene, which is desirable to prevent dental caries and periodontal diseases.

Key words: Toothpaste, *Staphylococcus aureus*, *Streptococcus mutans*, dental caries, antibacterial

ÖZ

Amaç: Vücudun genel iyilik halinin bir bileşeni olan oral hijyenin dental sorunları önlemek için idamesi sağlanmalıdır. Bu çalışma *Massularia acuminata* dallarının etanol ekstresinin bir bitkisel diş macunu formülasyonuna dahil etmek ve iki dental patojen olan *Staphylococcus aureus* and *Streptococcus mutans*'a karşı antibakteriyel aktivitesini ticari olarak var olan bir diş macunuyla karşılaştırarak değerlendirmek için yapılmıştır.

Gereç ve Yöntemler: Kurutulmuş *M. acuminata* dalının içeriği etanol kullanılarak ekstre edilmiştir ve %1, %2, %3, %4 ve %5 *M. acuminata* ekstresi içeren diş macunu formülasyonlarında kullanılmıştır. Diş macununun duyuşsal ve fizikokimyasal özellikleri değerlendirilmiştir. Diş macununun *S. aureus* ve *S. mutans*'a karşı antibakteriyel duyarlılığını değerlendirmek için agar kuyusu difüzyon yöntemi kullanılmıştır. Veriler One-Way analizi ve Student t-testi kullanılarak analiz edilmiştir.

Bulgular: Tüm diş macunları pürüzsüz ve tatlıydı ve hoş kokuyordu. Hepsinin diş fırçası kılları üzerinde iyi bir tutma kabiliyeti vardı ve pH aralığı 7,18-7,83 idi. Farklı konsantrasyondaki ekstraktların diş macunları, test organizmalarına karşı antibakteriyel aktivite göstermiştir. Formüle edilmiş diş macunlarının antibakteriyel aktivitesi, ekstrakt konsantrasyonundaki artışla önemli ölçüde artmıştır. %5 ekstrakt içeren F5, ticari olarak satılan bitkisel diş macunu ile karşılaştırıldığında bile, sırasıyla *S. aureus* ve *S. mutans*'a karşı 19,30±0,17 mm ve 12,60±0,52 mm'lik bir inhibisyon bölgesi ile en yüksek aktiviteyi göstermiştir.

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Sonuç: *M. acuminata* özütünün bitkisel diş macunu formülasyonuna katılması *S. aureus* ve *S. mutans*'in büyümesini engellemiştir. Bu ekstreyi diş macunu formülasyonuna dahil etmek, diş çürüklerini ve periodontal hastalıkları önlemek için arzu edilen ağız hijyenini tatmin edici bir şekilde koruyacaktır.

Anahtar kelimeler: Diş macunu, *Staphylococcus aureus*, *Streptococcus mutans*, diş çürüğü, antibakteriyel

INTRODUCTION

Oral hygiene is an integral part of the body's general well-being, which begins with clean teeth. The cleaning of one's teeth is a cultural habit that is followed from generation to generation and is usually performed as a daily morning routine. It is regarded as an indispensable component of oral health.¹ Different populations employ various techniques when cleaning the teeth. Modern conventional techniques involve the use of toothpaste and toothbrush, which have been in use for decades,^{2,3} whereas traditional techniques primarily involve the use of chewing sticks and local toothpaste.⁴ Other traditional methods of teeth cleaning involve the use of one's finger to rub various substances, including natural powders, bark of plants, ash, charcoal, oil, and salt, onto the 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 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 Bacteria that cause periodontal diseases include *Aggregatibacter actinomycetemcomitans*, *S. mutans*, *Bacteroides forsythus*, *Staphylococcus intermedius*, *Lactobacillus acidophilus*, *Porphyromonas gingivalis*, *Prevotella nigrescens*, and *Treponema denticola*.^{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 that the effectiveness of chewing stick lies in the presence of antibacterial bioactive compounds in these sticks that help remove dental plaque, thereby preventing dental caries and periodontal diseases.^{14,15} Some of the chewing sticks that have been investigated include *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

M. acuminata (G. Don) Bullock ex Hoyle is a shrub (Figure 1) that belongs to the family Rubiaceae and is widely distributed in West Africa. It is commonly known as the chewing stick tree. Its root, leaf, bark, and twig have several medicinal values, and it is used traditionally in some West African regions for treating diarrhea, dysentery, muscular pains, and venereal diseases and as an aphrodisiac.¹⁹ Pharmacological studies have shown the plant to possess a strong antibacterial property against oral pathogens.²⁰⁻²²



Figure 1. *Massularia acuminata* plant

The present study was conducted to incorporate the extract of *M. acuminata* in a formulation of herbal toothpaste and evaluate its antibacterial activity against two pathogens associated with dental caries and periodontal diseases.

MATERIALS AND METHODS

Plant collection

M. acuminata twigs were collected from Onigambari Forest, Ibadan, Oyo State, in the month of February 2019 and authenticated at the Forestry Research Institute of Nigeria, Ibadan, Oyo State, Ogun State, by Mr. Odewo (Voucher no. FHI. 112857).

Test organisms

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

Extract preparation

Twigs were cut into pieces and air dried for 30 days. The dried twig pieces were then ground into powder. Approximately 150 g of the powdered sample was macerated in 750 mL 95% ethanol (BHD Chemicals, Poole, England) for 72 h at room temperature with intermittent agitation, as per the protocol described by Tedwins et al.²¹ Thereafter, the sample was filtered through Whatman's filter paper. The filtrate was concentrated to a dry powder 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, as per the procedure described by Trease and Evans.²³

Antibacterial screening

The antibacterial activity of 100 mg/mL *M. acuminata* extract in distilled water was determined by the agar diffusion method. A suspension of an overnight culture of *S. aureus* and *S. mutans* in nutrient broth was standardized to 0.5 McFarland standards (10^6 colony forming units mL⁻¹). Nutrient agar plates were prepared in a Petri dish by inoculating with 0.2 mL of the standardized culture of the test organisms and allowed to settle. Wells of 6.0 mm were bored in nutrient agar, and each well was filled with 0.5 mL of the extracts; the positive control was gentamicin and the negative control was distilled water. The plates were allowed to stand for 30 min for the proper diffusion of the extract before incubating at 37°C for 24 h. The diameters of the zones of growth inhibition were then measured in millimeters. The experiments were performed in triplicate.

Preparation of *M. acuminata* extract herbal toothpaste

The quantity of the ingredients required to prepare 100 g toothpaste is shown in Table 1. To prepare a paste, tragacanth gum was mixed with about 10 mL of distilled water in a mortar and pestle. Glycerin was added and triturated vigorously, followed by the slow addition of calcium carbonate with continuous trituration. *M. acuminata* extract was then added to the content in the mortar and thoroughly mixed for even distribution. Sodium lauryl sulfate (SLS) was added with slow stirring to prevent foaming. Saccharine and peppermint oil were added, and the paste was then adjusted to the required weight by adding distilled water.

Evaluation of *M. acuminata* extract herbal toothpaste

Determination of organoleptic properties

The color, appearance, texture, odor, and taste of each formulation were determined by sensory and physical evaluations.

Determination of pH

In this step, 1 g of each toothpaste formulation was dispersed in 10 mL of purified water (pH 6.98), and the pH

Table 1. Composition of toothpaste formulation (100 g)

Ingredients (g)	F0	F1	F2	F3	F4	F5
<i>Massularia acuminata</i>	0.0	1.0	2.0	3.0	4.0	5.0
Calcium carbonate	20.0	0.0	20.0	20.0	20.0	20.0
Sodium lauryl sulfate	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

was measured in triplicate with a digital pH meter (pH 600, Milwaukee).²⁴

Determination of foaming ability

Next, 5 g of toothpaste formulation was dispersed in 10 mL of water in a 100 mL glass beaker. The beaker was covered with a watch glass and allowed to stand for 30 min. The mixture was stirred with a glass rod to break up lumps and transferred into a 250 mL graduated measuring cylinder while ensuring that no foams >2 mL were formed. The beaker was rinsed with 5-6 mL of water into the measuring cylinder. The cylinder was filled with up to 50 mL of water, covered with a stopper, maintained at 30°C, and shaken for about 20 seconds. The cylinder was then allowed to stand for 5 min. The volume of foam with water (V_1) and water only (V) was recorded.²⁵

Foaming ability was calculated as $V_1 - V_2$. The experiments were performed in triplicate.

Determination of moisture

The moisture content was determined by accurately weighing 5 g (W_0) each of the formulation into an evaporating dish of 6-8 cm in diameter and 2-4 cm in depth. The formulation was then dried in an oven at $105^\circ\text{C} \pm 2^\circ\text{C}$ until the weight remained constant and was noted as W_1 .²⁵

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

The mean of three values obtained was calculated.

Determination of spreadability

In this process, 1 g of toothpaste was placed on a glass plate of 10x10 cm size and covered with another glass plate of the same size. A weight of 1 kg was placed on the top glass plate and allowed to stand for 10 min, after which it was removed. The diameter of the spread on the plate was measured, and the mean of three values was taken.²⁵

Determination of viscosity

The viscosities of the formulations were measured at 20, 50, and 100 rpm at 25°C using a Brookfield viscometer (Model DV-II+Pro, Brookfield Eng. Labs Inc., Middleboro, MA, USA) with spindle number 4.

Antibacterial screening of toothpastes

The antibacterial activities of a commercially available toothpaste and the formulated *M. acuminata* toothpaste in different concentrations were determined by the agar diffusion method. The method used for the screening of the *M. acuminata* extract against *S. aureus* and *S. mutans* was adopted.

Statistical analyses

Statistical analyses of the data were performed by Student's t-test and One-Way ANOVA using GraphPad Prism (version 5.01) software. P values <0.05 were considered significant.

RESULTS

The phytochemical constituents present in the ethanol extract of the *M. acuminata* twig (Table 2) included anthraquinones, saponins, flavonoids, alkaloids, tannins, and flavonoids.

The antibacterial activity of the ethanol extract of the *M. acuminata* twig (100 mg/mL) is presented in Table 3. The extract and positive control (gentamicin) demonstrated high antibacterial activities against *S. aureus* and *S. mutans*, whereas the negative control (distilled water) did not show any antibacterial activity.

The results of the sensory and physical evaluation as well as pH measurement of the formulated toothpaste without herbal extract (F0), formulated herbal toothpaste containing different concentrations of the *M. acuminata* twig extract (F1-F5), and the commercially available herbal toothpaste (F6) are presented in Table 4. F0 was off white in color, F1-F5 varied between light brown and brown, and F6 appeared green. All formulations had a pleasant odor and a sweet taste. They were all smooth in texture and paste-like in appearance. The moisture content of

Table 2. Phytochemical screening of extracts

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

+: Present

Table 3. Antibacterial activity of extract

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

Mean ± standard deviation, n=3, extract, *Massularia acuminata* 100 mg/mL, positive control, gentamicin 80 mg/2 mL, negative control, distilled water. -: Absent

Table 4. Physical evaluation and pH of formulations

Parameters	F0	F1	F2	F3	F4	F5	F6
Color	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
Odor	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant	Pleasant
Taste	Sweet	Sweet	Sweet	Sweet	Slightly sweet	Slightly sweet	Sweet
Moisture content (%)	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
Spreadability (cm)	6.9±0.06	6.5±0.02	6.5±0.15	6.2±0.04	6.0±0.11	5.7±0.61	7.2±0.12
Foaming ability (cm)	51±0.22	55±0.20	56±0.06	60±0.13	62±0.10	63±0.24	58±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 ± standard deviation, n=3, F0: Formulated toothpaste without herbal extract, F1-F5: Formulated herbal toothpaste containing 1%, 2%, 3%, 4%, and 5% *Massularia acuminata* extract, respectively, F6: Commercially available herbal toothpaste

F0-F5 ranged from 24.22% to 28.25%, whereas that of F6 was 31.36%. The spreadability and foaming ability of all toothpastes ranged from 5.7 cm to 7.2 cm and from 51.0 cm to 63.0 cm, respectively. The pH of the toothpastes ranged from 7.18 to 7.83.

The viscosity of the toothpastes as measured by different spindle speeds at 25°C is shown in Figure 2. The results showed that viscosity significantly decreased as the spindle speed increased.

All toothpastes demonstrated high antibacterial activities against both *S. aureus* and *S. mutans*, except F0 and F1 (Table 5). F0 and F1 did not show any antibacterial activity against *S. mutans*.

DISCUSSION

The phytochemical constituents of plants are secondary metabolites; these metabolites are bioactive components that possess pharmacological activity in plants. The phytochemical constituents of the ethanol extract of *M. acuminata* twig are anthraquinones, saponins, flavonoids, alkaloids, tannins, and flavonoids. This finding is similar to those of some previous studies.²⁰⁻²² Tannins, saponins, and flavonoids in herbs are bioactive compounds that have been reported to possess antibacterial activities.^{26,27}

In this study, the test organisms employed were *S. aureus* and *S. mutans*, which are among the main organisms associated

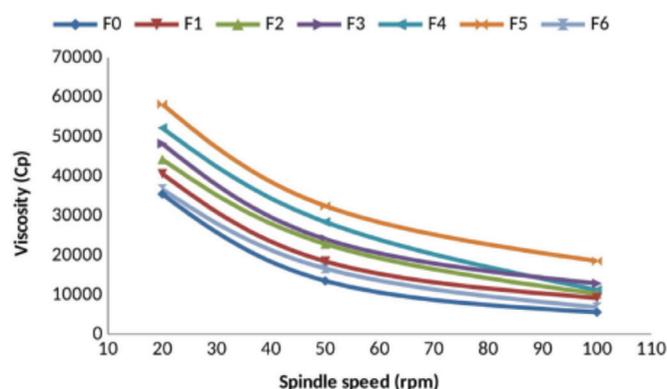


Figure 2. Viscosity of toothpaste formulations at 25°C

with dental caries and periodontal diseases, respectively.^{11,28-32} The antibacterial activity of the ethanol extract demonstrated a strong activity against these organisms. This result corroborates with those of several other studies.²⁰⁻²² The ethanol extract demonstrated a significantly higher antibacterial activity against *S. aureus* than against *S. mutans*; however, the activity of the positive control (gentamicin) was significantly higher against the test organisms. The antibacterial activity of the extract could be attributed to the presence of bioactive components, including tannins, saponins, and flavonoids.

With respect to the sensory and physical evaluation of the toothpastes (Table 4), all were smooth, smelled pleasant, and tasted sweet but were of different colors. The pleasant odor and sweet taste can be attributed to the presence of flavoring agent (peppermint oil) and sweetener (saccharin), respectively, in all toothpastes, including the commercially available one. Despite the high concentration of *M. acuminata* extract in 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 color because it did not contain the herbal extract. Formulated herbal toothpaste (F1-F5) had colors varying from light brown to brown; the color intensity deepened with the increasing concentration of the extract. The commercially available herbal toothpaste (F6) was green. All these parameters may enhance the consumer acceptability of the product. F6 had the highest moisture content (28.25%); the moisture content was ranked in the following order: F6 > F0 > F1 > F2 > F3 > F4 > F5. This is reflected in the level of water content in the formulations, as shown in Table 1. This property could, in turn, affect the spreadability, foaming ability, and viscosity of the toothpaste. While the spreadability of the formulated toothpastes decreased with increased concentration of the extract (F0 > F1 > F2 > F3 > F4 > F5), foaming ability and viscosity increased (F0 < F1 < F2 < F3 < F4 < F5).

Viscosity is a factor that determines the spreadability, thickness, and ability of the toothpaste to retain its ribbon

shape when extruded from the tube on the toothbrush.³³ Ribbon shape retention is defined as the ability of the toothpaste to retain its ribbon shape on the bristles of a toothbrush without collapsing.³⁴ All toothpastes had good retention ability. Spreadability measures the extent of the area that the toothpaste can spread, such as on the teeth, gum, gum lines, and other areas, and the extent of penetration into the infected tooth and gum. The spreadability of commercially available toothpaste was significantly higher than that of the formulated toothpastes. Foaming ability is the measure of the cleansing power of toothpastes, which is affected by the presence of surfactants (i.e., SLS). SLS produces foam that lowers the surface tension of the surface film on the tooth, thereby suspending and removing debris. Toothpaste with good foaming ability will provide a good cleansing action of the teeth. A significant difference was found in the foaming ability of the toothpastes, with F5 having the greatest cleansing action. The presence of the extract progressively increased the foaming ability of the formulated toothpastes. This may be because of the frothing properties of saponin present in the extract.³⁵

The oral microbial flora when compromised, usually by reduction in pH due to the carbohydrate metabolism of the organisms, could cause dental caries, other periodontal diseases, and dental plaque.^{31,36,37} Maintaining the microbial flora is desirable for the well-being of individuals; this can be achieved by proper oral hygiene, such as by cleaning of one's teeth. Keeping the pH of the teeth at an alkaline range may prevent the development of these dental problems. In this study, the pH of all toothpastes was in the alkaline range.

This study evaluated the antibacterial activity of the toothpastes against *S. aureus* and *S. mutans*. These bacteria are among the most implicated pathogens in dental caries and periodontal diseases, respectively. The results revealed that the toothpastes demonstrated antibacterial activities against the test organisms at all extract concentrations and antibacterial activities increased significantly with an increase in extract concentration ($p < 0.05$). F5 containing 5% extract exhibited the highest antibacterial activity, with an inhibition zone of 19.30 ± 0.17 and 12.60 ± 0.52 against *S. aureus* and *S. mutans*, respectively, even when compared with the commercially available herbal toothpaste. The increase in antibacterial activity could be attributed to the 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 *S. aureus* was significantly higher than that against *S. mutans* ($p < 0.05$). F0 showed little antibacterial activity because SLS and peppermint oil contained in the composition of the paste are known to possess antibacterial activity against *S. aureus* and *S. mutans*.³⁸⁻⁴⁰ The antibacterial activities of the formulated toothpastes with >2% concentration of *M. acuminata* extract were significantly higher than those of the commercially available herbal toothpaste against both test organisms ($p < 0.05$). However, further investigation is required to isolate

Table 5. Antibacterial activity of toothpaste formulations

Test organisms	Zones of inhibition (mm)	
	<i>Staphylococcus aureus</i>	<i>Streptococcus mutans</i>
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 ± standard deviation, n=3, F0: Formulated toothpaste without herbal extract, F1-F5: Formulated herbal toothpaste containing 1%, 2%, 3%, 4%, and 5% *Massularia acuminata* extract, respectively, F6: Commercially available herbal toothpaste, -: Absent

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 plays a role in maintaining oral hygiene and otherwise prevents the consequences of poor oral hygiene. This study demonstrated that fortifying toothpastes with herbal antibacterial agents, such as the ethanolic extract of *M. acuminata*, provides higher antibacterial activities against some of the pathogens implicated in the development of dental caries and periodontal diseases *in vitro*. The use of the *M. acuminata* extract as an ingredient in toothpaste formulation will improve the maintenance of oral hygiene to prevent dental caries and periodontal diseases.

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