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: Vücutun 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 etme ve iki dental patojen olan Staphylococcus aureus ve Streptococcus mutans’a karşı antibakteriyel aktivitesini ticari olarak var olan bir diş macunuyla karşılaştırarak değerlendirerek yapılmıştır.

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

Bulgular: Tüm diş macunları pürüzsüz ve tatlıdı ve hoş kokuyordu. Hepsinin diş fırçası kili 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. Formül eblimiş 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.
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. 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, whereas traditional techniques primarily involve the use of chewing sticks and local toothpaste. 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 (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*. 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). Bacteria that cause periodontal diseases include *Aggregatibacter actinomycetemcomitans*, *S. mutans*, *Bacteroides forsythus*, *Staphylococcus intermedius*, *Lactobacillus acidophilus*, *Porphyromonas gingivalis*, *Prevotella nigrescens*, and *Treponema denticola*.

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. 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. Some of the chewing sticks that have been investigated include *Terminalia glaucescens*, *Sorindeia warneckei*, *Vitex doniana*, *Vernonia amygdalina*, *Fagara zanthoxyloides*, *Massularia acuminata*, *Pseudocedrela kotschyi*, *Anogeissus schimperi*, *Anogeissus leiocarpus*, and *Azadirachta indica*.

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, 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, by Mr. Odewo (Voucher no. FHI.112857).

**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. 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 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\(_2\)) 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°C±2°C until the weight remained constant and was noted as W\(_1\).\(^{25}\)

Percentage loss by mass: (W\(_0\) - W\(_1\)/ W\(_0\) ) × 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, saponnins, flavonoids, alkaloids, tannins, and flavonoids.

### Table 1. Composition of toothpaste formulation (100 g)

| Ingredients (g)       | F0 | F1 | F2 | F3 | F4 | F5 |
|-----------------------|----|----|----|----|----|----|
| Massularia acuminata  | 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|
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 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. Tannins, saponins, and flavonoids in herbs are bioactive compounds that have been reported to possess antibacterial activities.

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

| 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 |
| *Staphylococcus aureus* | 25.50±0.10 | 33.00±0.37 | - |
| *Streptococcus mutans*  | 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
with dental caries and periodontal diseases, respectively. The antibacterial activity of the ethanol extract demonstrated a strong activity against these organisms. This result corroborates with those of several other studies. 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. Ribbon shape retention is defined as the ability of the toothpaste to retain its ribbon shape on the bristles of a toothbrush without collapsing. 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.

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. 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*. 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) |
|----------------|-------------------------|
|                | *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 ± standard deviation, n=3; F0: Formulated toothpaste without herbal extract, F1-F5: Formulated herbal toothpaste containing 1%, 2%, 3%, 4%, and 5% *Mussularia 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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**REFERENCES**

1. Ersoy M, Tanalp J, Ozel E, Cengizlier R, Soyman M. The allergy of toothpaste: a case report. Allergol Immunopathol (Madr). 2008;36:368-370.
2. Horseman RE. The her-story of toothpaste. J Calif Dent Assoc. 2006;34:769-770.
3. Jardim JJ, Alves LS, Maltz M. The history and global market of oral home-care products. Braz Oral Res. 2009;(Suppl 1):17-22.
4. Oke GA, Bankole OO, Denloye OO, Danfillo IS, Enwonwu CO. Traditional and emerging oral health practices in parts of Nigeria. Odontostomatol Trop. 2011;34:35-46.
5. Shah N, Mathur VP, Jain V, Logani A. Association between traditional oral hygiene methods with tooth wear, gingival bleeding, and recession: A descriptive cross-sectional study. Indian J Dent Res. 2018;29:150-154.
6. Gupta P, Shetty H. Use of natural products for oral hygiene maintenance: revisiting traditional medicine. J Complement Integr Med. 2018 Mar 27;15:[j:jcim.2018.15.issue-3/jcim-2015-0103/jcim-2015-0103.xml.
7. Bowen WH, Koo H. Biology of *Streptococcus mutans*-derived glucosyltransferases: role in extracellular matrix formation of cariogenic biofilms. Caries Res. 2011;45:69-86.
8. Fejerskov O, Nyvad B, Kidd E. Dental caries, what is it? In: Fejerskov O, Nyvad B, Kidd E, eds. Dental caries: The disease and its clinical management. Oxford, UK: Wiley Blackwell;2015:7-10.
9. Chapple IL, Van der Weijden F, Doerfer C, Herrera D, Sharipa L, Polak D, Madianos P, Louroupoulo A, Machtei E, Donos N, Greenwell H, Van Winkelhoff AJ, Eren Kuru B, Arweiler N, Teughels W, Aimetti M, Molina A, Montero E, Graziani F. Primary prevention of periodontitis: managing gingivitis. J Clin Periodontol. 2015;(Suppl 16):S71-S76.
10. Popova C, Dosseva-Panova V, Panov V. Microbiology of periodontal diseases. A review. Biotechnol Biotechnol Equip. 2013;27:3754-3759.
11. Silva N, Abusleme L, Bravo D, Dutzan N, Garcia-Sesnieh J, Vernal R, Hernández M, Gamanol J. Host response mechanisms in periodontal diseases. J Appl Oral Sci. 2015;23:329-355.
12. Darout IA, Christy AA, Skaug N, Egeberg PK. Identification and quantification of some potentially antibacterial anionic components in miswak extract. Indian J Pharmacol. 2000;32:11-14.
13. al-Otaibi M. The miswak (chewing stick) and oral health. Studies on oral hygiene practices of urban Saudi Arabians. Swed Dent J Suppl. 2004;2-75.
14. Almas K, Al-Zeid Z. The immediate antimicrobial effect of a toothbrush and miswak on cariogenic bacteria: a clinical study. J Contemp Dent Pract. 2004;5:105-114.
15. Saha S, Mohammad S, Saha S, Samadi F. Efficiency of traditional chewing stick (miswak) as an oral hygiene aid among Muslim school children in Lucknow: A cross-sectional study. J Oral Biol Craniofac Res. 2012;2:176-180.
16. Taiwo O, Xu HX, Lee SF. Antibacterial activities of extracts from Nigerian chewing sticks. Phytother Res. 1999;13:675-679.
17. Ogundiya MO, Okunade MB, Kolapo AL. Antibacterial activities of some Nigerian chewing sticks. Ethnobot Leaflets. 2006;10:265-271.
18. Oloke J, Odelade K, Oladeji O. Characterization and antibacterial analysis of flavonoids in Vernonia amygdalina: a common chewing stick in southwestern Nigeria. Bull Pharm Res. 2017;7:149-158.
19. Iwu MM. Pharmacognostical profile of selected medicinal plants: Handbook of African medicinal plants CRC Press, 2014.
20. Bankole PO, Adekunle AA, Oyedele RT, Paparusi F, Adewole A. Antibacterial activities and phytochemical screening of two tropical Nigerian chewing sticks. Int J Applied Sci Tech. 2012;2:131-138.
21. Tedwins EJO, Benjamin OJU, Ayobola ED, Goodies ME, Oghemesuvue EE. A comparative study on the effect of Massularia acuminata and mouthwash against isolates from the oral cavity. J Res Dent. 2016;4:64-68.
22. Odeleye OF, Okunye OL, Kesi C, Abatan TO. A Study of the antacaries activity of three common chewing sticks and two brands of toothpaste in south west Nigeria. Bri J Pharm Res. 2016;11:1-7.
23. Trease A, Evans WC. Pharmacognosy (13th ed). London; Balliende Tindiall; 1989.
24. Ali HS, Abdul-Rasool BK. Propolis buccal paste in treatment of aphthous ulceration: formulation and clinical evaluation. Asian J Pharm Clin Res. 2011;4:29-33.
25. Mangial T, Ravikumar M. Preparation and evaluation of herbal toothpaste and compared with commercial herbal toothpastes: An *in vitro* study. Int J Ayu Her Med. 2016;2:2266-2251.
26. Muinat AA, Mbang FON, Lateef BG, Esther TO, Olyumisi BA. Antimicrobial studies of the leaf extract of Argemone mexicana and its ointment formulation. West Afr J Pharm. 2015;26:33-40.
27. Adeleye OA, Babalola CO, Femi-Oyewo MN, Balogun GY. Antimicrobial activity and stability of Andrographis paniculata cream containing shea butter. Nig J Pharm Res. 2019;15:9-18.
28. Antimicrobial activities and phytochemical properties of crude extracts of Garcina kola heckle stems used for oral health. Res J Pharmacol. 2011;5:68-76.
29. Antiw-Boasiako C, Abubakari A. Antimicrobial and emerging oral health practices in parts of Nigeria. Odontostomatol Pract. 2004;5:105-114.
30. Maripandi A, Arun KT, Al Salamah AA. Prevalence of dental caries bacterial pathogens and evaluation of inhibitory concentration effect on different tooth pastes against *Streptococcus* spp. Afr J Microbio Res. 2011;5:1778-1783.
31. Danyian S, Abalaka M. Prevalence and sceptibility pattern of bacterial isolates of dental caries in a secondary health care institution, Nigeria. Shiraz E-Med J. 2012;12:135-139.
31. Dige I, Baelum V, Nyvad B, Schlafer S. Monitoring of extracellular pH in young dental biofilms grown in vivo in the presence and absence of sucrose. J Oral Microbiol. 2016;8:30390.

32. Adeoti OM, Adedotu SA, Adedokun EO, Olaye OJ, Abiola AO, Okesipe FO. Efficacy of chewing sticks extract on the agent of dental carries isolates. Arch Clin Microbiol. 2020;11:101-104.

33. Oladimeji F, Akinkunmi EO, Raheem A, Abiodun G, Bankole V. Evaluation of topical antimicrobial ointment formulations of essential oil of lippia multiflora moldenke. Afr J Tradit Complement Altern Med. 2015;12:135-144.

34. Ahuja A, Potanin A. Rheological and sensory properties of toothpastes. Rheol Acta. 2018;57:459-471.

35. Moghimipour E, Handali S. Saponin: properties, methods of evaluation and applications. Annu Res Rev Bio. 2014;5:207-220.

36. Welin-Neilands J, Svensater G. Acid tolerance of biofilm cells of Streptococcus mutans. Appl Environ Microbiol. 2007;73:5633-5638.

37. Sanz M, Bäumer A, Buduneli N, Domnisch H, Farina R, Kononen E, Linden G, Meyle J, Preshaw PM, Quirynen M, Roldan S, Sanchez N, Sculean A, Slot DE, Trombetti L, West N, Winkel E. Effect of professional mechanical plaque removal on secondary prevention of periodontitis and the complications of gingival and periodontal preventive measures: consensus report of group 4 of the 11th European Workshop on Periodontology on effective prevention of periodontal and peri-implant diseases. J Clin Periodontol. 2015;(Suppl 16):S214-S220.

38. Chaudhari LK, Jawale BA, Sharma S, Sharma H, Kumar CD, Kulkarni PA. Antimicrobial activity of commercially available essential oils against Streptococcus mutans. J Contemp Dent Pract. 2012;13:71-74.

39. Liang R, Xu S, Shoemaker CF, Li Y, Zhong F, Huang Q. Physical and antimicrobial properties of peppermint oil nanoemulsions. J Agric Food Chem. 2012;60:7548-7555.

40. Shen Y, Li P, Chen X, Zou Y, Li H, Yuan G, Hu H. Activity of Sodium Lauryl Sulfate, Rhamnolipids, and N-Acetylcysteine Against Biofilms of Five Common Pathogens. Microb Drug Resist. 2020;26:290-299.