In vitro evaluation of antimicrobial activity of Distemonanthus benthamianus chewing stick extract mouthwash

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ABSTRACT

Background and Aims: Mouthwashes are oral home care product used to treat mouth infections and to maintain satisfactory oral hygiene. The use of effective mouthwash is a reasonable means to prevent dental caries and oro-dental infections. The aim of this study was to evaluate the antimicrobial potential of Distemonanthus benthamianus chewing stick extract formulated as a mouthwash against some microorganisms associated with oro-dental infections in comparison with two commercial brand mouthwashes.

Methods: The chewing stick was extracted with methanol and used to formulate mouthwash. Phytochemical screening was done to test for the presence of secondary metabolites. Antimicrobial activity of the crude extract and the formulated mouthwash on Staphylococcus aureus, Streptococcus mutans and Candida albicans was evaluated. The physical characteristics, pH and the stability of the mouthwashes were determined.

Results: The extract contains alkaloids, saponins flavonoids, steroids and tannins. The crude extract and the formulated mouthwashes showed significant difference in activity against the test microorganisms in the following order Candida albicans > Staphylococcus aureus > Streptococcus mutans. At high concentrations of the extract (0.3%W/V and 0.5%W/V) in formulations, antibacterial activities were significantly higher against Streptococcus mutans and Staphylococcus aureus when compared with the commercial brands. The pH of the mouthwashes ranged from 5.2 - 7.4. The stability of formulation F5 was maintained for 12 weeks.

Conclusion: The use of Distemonanthus benthamianus chewing stick extract mouthwash is safer, cheaper, and more effective in preventing dental caries and oro-dental infections compared to the commercial brand mouthwashes.

Keywords: Antimicrobial activity, chewing stick, microorganisms, mouthwash
INTRODUCTION
Mouthwashes are oral home care products. They are clear non-sterile aqueous solution termed medicated liquid intended to deodorize, clean and treat mouth infections. They are used to treat mouth infections and to ensure or maintain satisfactory oral hygiene to enhance oral health (Khirtika, Ramesh, & Muralidharan, 2017). Thus, the use of effective mouthwash is a reasonable means to prevent dental caries and oro-dental infections. This is achieved by the presence of chemical antimicrobial agents such as cetlypyridinium chloride, triclosan or chlorhexidine and many oils in the commercially available mouthwash brands (Masadeh, Gharaibeh, Alzoubi, Al-Azzam, & Obeidat, 2013; Dabholkar, Shah, Kathariya, Bajaj, & Doshi, 2016; Kunte, Kadam, Patel, Shah, Lodaya, & Lakde, 2018).

The use of chewing sticks have been encouraged by the WHO since 1987 due to their enormous oral health benefits such as the elimination of bad odour, good cleansing properties and wide spectrum antimicrobial activities which they possess in the general maintenance of oral hygiene (Malik, Shaukat, Qureshi, & Abdur, 2014). They are less toxic because of their natural source. They are abundant in nature which makes them readily available and cheap. For people of low economic stature, they are a cheaper and safer alternative of mouth cleansing to commercial mouthwashes with side effects such as tooth staining and vomiting (Yadav & Yadav, 2013; Parkar, Thakkar, & Shah, 2013).

Distemonanthus benthamianus Baill. is a semi deciduous tree which belongs to the family Leguminosae. It is commonly known as African satiwood in English and as movigui in French. It is widespread in Ghana and Nigeria, and known as “bonsamdua” and “ayan”, respectively (Adeniyi, Obasi, & Lawal, 2011; Asamoah, Friimpong-Mensah, & Antwi-Boasiako, 2014). It is used traditionally in the treatment of different skin conditions, rheumatism, pains, fever, uro-genital and gastrointestinal infections (Saha et al., 2014; Shittu, Aiyuu, David, Njiga, & Ishaq, 2019). The root is used in Western Nigeria as a chewing stick for oro-dental hygiene. There are experimental evidences which show that D. benthamianus possesses teeth cleansing properties and antimicrobial activity against a wide range of microorganisms associated with dental caries and oro-dental infections such as Streptococcus mutans, Staphylococcus aureus, Candida albicans etc. (Adeniyi & Odumosu, 2012; Aworinde, Eriyoso, & IkubuOnoluwa, 2016; Mebude, Adeniyi, & Lawal, 2017; Shittu et al., 2019). In view of these assertions, this study was carried out to investigate the antimicrobial potentials of the methanol extract of D. benthamianus chewing sticks against microorganisms associated with oro-dental infections and also to formulate and evaluate the antimicrobial activity of mouthwash containing different concentrations of the extract in comparison with two commercial brand mouthwashes.

MATERIALS AND METHODS

Plant collection
D. benthamianus chewing sticks (freshly uprooted root of the plant) were bought same day from Oke-Aje, Ijebu Ode, Ogun state, Nigeria in August 2019 and identified at the Forest Research Institute of Nigeria (FRIN) Ibadan, Oyo State by Mr. Samuel Odewo. The plant was air dried, cut into pieces and ground into powder using an electric blender.

Test microorganisms
The test microorganisms used in this study were clinical isolates of S. aureus, S. mutans and C. albicans collected from the Microbiology Department of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State.

Preparation of plant extract
Five hundred grams of the powdered chewing sticks were extracted by the method of Adeleye, Babalola, Femii-Oyewo, & Balogun, (2019) with 75% methanol for 72 hours at room temperature. It was filtered; the filtrate was concentrated with a rotary evaporator at 40°C and stored in a refrigerator at 4°C.

Phytochemical analysis of extract
The extract was screened for the presence of phytochemicals according to the methods of Trease & Evans, (1989).

Antimicrobial sensitivity screening
The antimicrobial sensitivity of the test microorganism to different concentrations of D. benthamianus extract prepared with distilled water was evaluated by the agar well diffusion method (Hood, Wilkinson, & Cavanagh, 2003). The bacterial test isolates were inoculated into Nutrient Broth and incubated for 24 hours at 37°C while the fungal test isolate was inoculated into Sabouraud Dextrose Broth and incubated for 48 hours at 37°C. The inoculated broths were standardized to 0.5 McFarland standards (Hood et al., 2003). A 6 mm cork borer was used to bore wells in an agar plate inoculated with microorganisms (Nutrient Agar for bacterial and Sabouraud Dextrose Agar for fungi) and filled with 0.5 mL of different concentrations of the extracts, gentamicin or ketoconazole (positive control) and distilled water (negative control). The plates were incubated at 37±2°C for 24 hours and 48 hours for Nutrient Agar Sabouraud Dextrose Agar respectively. Zones of inhibition were measured in millimeters (mm).

Preparation of Distemonanthus benthamianus mouthwash
Each formulation (F1 - F5) was prepared according to Table 3. Sodium bicarbonate was dissolved in water and added to the mixture of chloroform water, peppermint oil and propylene glycol in a calibrated bottle. The extract D. benthamianus was dissolved in water and strained through a whatman paper to remove particles before being added to the mixture in the bottle.

Evaluation of mouthwash
Evaluation of physical characteristics of the mouthwashes
Physical characteristics (appearance, colour, taste, odour and flavour) of the mouthwashes were determined by visual inspection and sensory evaluation.

Evaluation of pH of the mouthwashes
The pH of the mouthwashes was determined in triplicate using a pH meter.

Antimicrobial assay of the mouthwashes
The antimicrobial assay of the mouthwashes was performed by the agar diffusion method similar to the previous method used for antimicrobial sensitivity screening.
Panelist perception
The physical characteristics of the mouthwashes were characterized by subjective physical and sensory assessment by 70 panelists, 10 for each formulation. The panelists were instructed to rate the appearance, taste, flavour, colour, smoothness and acceptability of the mouthwash as they perceived them on two scales of either good or bad and accepted or rejected.

Stability studies of the mouthwash
The stability of formulation F5 was evaluated by taking samples at specific intervals from the product stored at 4±1°C, 25±1°C and 50±1°C for 12 weeks (Almekhlafi, Thabit, Alwossabi, Awadhth, Thabe, & Algaadari, 2014; Adeleye et al., 2019). The physical characteristics (appearance, colour, taste, odour and flavour) and antimicrobial properties of the samples were analyzed.

Statistical analysis
Significant difference in data obtained was analyzed with GraphPad Prism 5.01 using t-test and one-way ANOVA. P-values less than 0.05 were considered statistically significant.

RESULTS
The phytochemical constituents of the extract are shown in Table 1. The extract contains low concentrations of alkaloids and saponins with moderate concentrations of flavonoids, steroids and tannin.

The antimicrobial assay of different concentrations of the crude extract is presented in Table 2. The crude extract showed a significant difference in activity against the test microorganism with the highest activity at 250 mg/mL concentration on C. albicans (27.1±0.20mm) followed by S. aureus (22.5±0.10mm) and S. mutans (16.3±0.22mm). The positive control 1 (gentamicin) had activities on S. aureus (28.5±0.10mm) and S. mutans (22.8±0.06mm), and the positive control 2 (ketoconazole) had activity on C. albicans (22.8±0.06mm) while the negative control (distilled water) showed no activity against the test microorganisms.

The composition of the formulated mouthwashes is shown in Table 3, and the physical characteristics and the pH of the mouthwashes are presented in Table 4. All the mouthwashes are clear solutions in appearance with a minty flavour and with colours ranging from light red to light blue. The formulated mouthwashes are odourless and neutral in taste while the commercial brand mouthwash has a pleasant odour and a sweet taste. The pH of the mouthwashes ranges from 5.2 to 7.4.

The antimicrobial assay of the mouthwashes is presented in Table 5. All the test microorganisms are susceptible to the mouthwashes but at significantly different degrees except F1 which showed zero activity. Formulation F5 had the highest activity against all the test microorganisms with C. albicans showing the highest susceptibility (24.6±0.08mm), followed by S. aureus (17.8±0.20mm) then S. mutans (10.5±0.12mm).

The physical characterization and sensory assessment of the mouthwashes by panelists indicates that commercial brands generally had better physical and sensory characteristics, and were better accepted than the formulated mouthwashes (Table 6).

### Table 1. Phytochemicals of *Distemonanthus benthamianus* extract.

| Test     | Result |
|----------|--------|
| Alkaloids| +      |
| Flavonoids| ++  |
| Saponins | +      |
| Tannins  | ++     |
| Steroids | ++     |

* + = Low concentration; ++ = Moderate concentration

### Table 2. Antimicrobial assay of *Distemonanthus benthamianus* extract.

| Extract (mg/ml) | *Streptococcus mutans* | *Staphylococcus aureus* | *Candida albicans* |
|----------------|------------------------|------------------------|-------------------|
| 250            | 16.3±0.22              | 22.5±0.10              | 27.1±0.20         |
| 125            | 14.9±0.14              | 20.8±0.20              | 24.7±0.07         |
| 62.5           | 12.7±0.06              | 18.1±0.04              | 22.3±0.16         |
| 31.25          | 10.1±0.21              | 16.0±0.21              | 17.8±0.10         |
| 15.625         | 8.4±0.10               | 12.8±0.14              | 12.0±0.42         |
| Positive control 1 | 25.2±0.02              | 28.5±0.10              | -                 |
| Positive control 2 | -                     | -                      | 22.8±0.06         |
| Negative control | -                     | -                      | -                 |

Positive control 1 = Gentamicin, Positive control 2 = Ketoconazole, Negative control = Distilled water

### Table 3. Composition of *Distemonanthus benthamianus* extracts mouthwash.

| Ingredients                  | F1   | F2   | F3   | F4   | F5   |
|------------------------------|------|------|------|------|------|
| *D. benthamianus* extract    | -    | 0.1  | 0.2  | 0.3  | 0.5  |
| Sodium bicarbonate (g)       | 1.0  | 1.0  | 1.0  | 1.0  | 1.0  |
| Propylene glycol (mL)        | 1.0  | 1.0  | 1.0  | 1.0  | 1.0  |
| Peppermint oil (mL)          | 1.5  | 1.5  | 1.5  | 1.5  | 1.5  |
| Chloroform water (mL)        | 5.0  | 5.0  | 5.0  | 5.0  | 5.0  |
| Amaranth solution (mL)       | 5.0  | 5.0  | 5.0  | 5.0  | 5.0  |
| Water to (mL)                | 100.0| 100.0| 100.0| 100.0| 100.0|
Figures 1, 2 and 3 show the antimicrobial stability of formulation F5 for 12 weeks. The stability was maintained at all experimental temperatures for 12 weeks.
A phytochemical test was performed to find the phytochemical constituents (bioactive secondary metabolites) present in *D. benthamianus* extract. Table 1 shows that the extract contains alkaloids, steroids, flavonoids, saponins and tannins as highlighted by other researchers (Aiyegoro, Akinpelu, Afolayan, & Okoh, 2008; Shittu et al., 2019; Obiang, Misso, Atome, Ondo, Engonga, & Emvo, 2019). The presence of some of these compounds has been linked to the antimicrobial activity of the extract. Adeniyi, Adeonipekun, & Omotayo, (2014); Aworinde et al., (2016); Mebude et al., (2017), reported that the presence of tannins, alkaloids, flavonoids and steroids in plants are responsible for their antimicrobial activities.

The antimicrobial assay of the crude extract revealed activity against the three test microorganisms and the susceptibility of the microorganisms to the extract was in the following order: *C. albicans* > *S. aureus* > *S. mutans*. The antimicrobial activity increased significantly (P<0.05) as the concentration of the extract increased. This indicates that the activity of the extract is concentration-dependent. As discussed above, the presence of tannins, alkaloids, flavonoids and steroids in the extract could be the reason for the antimicrobial activity of the extract. Physical characteristics (appearance, colour, odour, taste and flavor) of the formulated mouthwashes (F1–F5) and the commercial brand mouthwashes (F6–F7) showed that the formulations are clear solutions without particulate matters. The light red colouration and the minty flavour of the formulated mouthwashes were due to the inclusion of amaranth solution and peppermint oil respectively in the composition of the mouthwash. F1–F5 are odourless and neutral in taste but the commercial brands are pleasant in odour with a sweet taste.

The ideal pH range of saliva is 6.2–7.6 with an average pH of 6.7 (Baliga, Muglikar, & Kale, 2013), which helps maintain the pH of the mouth, including keeping the pH of the gum and teeth within a range of 6.7–7.3 so as to prevent proliferation of cariogenic bacteria, tooth demineralization, tooth decay and gum diseases (Zhao, Tsoi, Wong, Chu, & Matinlinna, 2017). When the mouth pH falls below the critical pH value of 5.5, various oral health complications could arise. As a result of low oral pH, in the composition of the mouthwash, *C. albicans* had significant activity on *S. mutans* and *S. aureus* (P < 0.0001) when compared with the commercial brands, except F6, which had a significantly higher activity against *S. aureus* than formulation F4 (0.3%/v/v). However, at low concentrations of the extract in formulations F2 (0.1%/v/v) and F3 (0.2%/v/v), the antibacterial activity of these formulations were significantly lower against *S. mutans* and *S. aureus* (P<0.0001) when compared with the commercial brands.

The rating of the physical characteristics of the mouthwashes from the assessment of panelists indicated generally that more than 50% of the panelists perceived all the mouthwashes as being good in appearance, colour, odour and smoothness, without a gritty taste. Generally, more than 50% of the panelists perceived the formulated mouthwashes to have a poor flavour and taste, unlike the commercial brands, which over 70% of the panelist perceived as having a good flavour and taste. More than 70% of the panelist accepted the commercial brands while less than 50% of panelists generally rejected the formulated mouthwashes. The rejection of the formulated mouthwashes was based on its palatability in which the flavour and sweetener in the formulation was a determining factor. Sweetener was not included in the formulated mouthwash, hence its high rejection rate. However, the commercial brand mouthwashes gained a high rate of acceptance due to the presence of sweeteners: sodium saccharin and sorbitol. The acceptability of the formulated mouthwashes can be improved upon by enhancing its palatability with the inclusion of a suitable sweetener.

The physical characteristics (appearance, colour, taste, odour and flavour) of the mouthwash were stable at the selected temperature for 12 weeks. This finding is an indication that the antimicrobial stability of the mouthwash was maintained at the selected temperature for 12 weeks. This finding is an indication that the mouthwash can be stored on shelves at room temperatures without becoming compromised.

Further research is necessary to isolate and evaluate the compound in the plant responsible for the antimicrobial activity in vivo. Also, toxicological evaluation of the formulated mouthwash is necessary to ascertain its safety.
CONCLUSION
The test microorganisms were sensitive to the mouthwashes but at various degrees depending on the concentration of the extract. The formulated mouthwashes showed more remarkable activity than the commercial brands. The formulated mouthwashes were stable in physical attributes and activity but were poor in acceptability and need to be improved to be considered for commercial production. This study concludes that the use of *Distemonanthus benthamianus* chewing stick extract mouthwash would be an effective, cheap and safe means to prevent dental caries and oro-dental infections compared to the commercial brand mouthwashes.

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