



## ***In vitro* Antimicrobial Activities of Ethanol Extract of *Distemonanthus benthamianus* (Aayan) Baillon (Fabaceae) on *Streptococcus mutans***

O. O. Mebude<sup>1\*</sup>, B. A. Adeniyi<sup>1</sup> and T. O. Lawal<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Ibadan.

### **Authors' contributions**

This work was carried out in collaboration between all authors. Authors OOM and BAA designed the study. Author OOM wrote the protocol and wrote the first draft of the manuscript. Author OOM managed the literature searches and analyses of the study performed. Author BAA read and corrected the draft. Author TOL monitored the experimental process. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Objectives:** The objective of this research was to evaluate the antimicrobial potential of *Distemonanthus benthamianus* on *Streptococcus mutans*.

**Methods:** The antimicrobial activities of ethanol extract of *Distemonanthus benthamianus* was done on *Streptococcus mutans* using the agar well diffusion technique.

**Results:** The ethanol extract exhibited significant antibacterial activity against the tested bacteria with zones of inhibition between 15±0.1 mm to 20±0.2 mm at 6 mg/ml. The phytochemical analysis of the plant revealed the presence of flavonoids, phenol, steroids, and tannin. The MIC and MBC of the extract against the sensitive organisms was determined and was in the range of 15.6 µg/ml to 1000 µg/ml and 31.25 µg/ml to 1000 µg/ml. The ratio of MIC to MBC falls within 1 and 2, which is an indication of the bactericidal activity of the plant extract. The kinetic study revealed a reduction in the number of viable organisms with increase in contact time between the organisms and the extract.

**Conclusion:** This result justifies the use of *Distemonanthus benthamianus* in folkloric medicine for the treatment of dental caries diseases caused by *Streptococcus mutans* in Nigeria.

\*Corresponding author: E-mail: docky1ng@gmail.com;

**Keywords:** *Distemonanthus benthamianus*; antibacterial property; phytochemical; minimum inhibitory concentration; minimum bactericidal concentration; kinetic study.

## 1. INTRODUCTION

The mouth is colonized by 200-300 bacteria species, but only a limited number of these species participate in dental decay. Dental decay is due to the irreversible solubilization of the tooth mineral by acid produced by certain bacteria that adhere to the tooth surface in bacterial communities known as plaque. *Streptococcus mutans* is the main cause of dental decay [1]. When fermentable foods are eaten frequently, low P<sup>H</sup> in the plaque is sustained which avails aciduric organisms such as *Streptococcus mutans* [1].

*Distemonanthus benthamianus* is one of the perennial trees of the evergreen, semi-deciduous and secondary forest of West Africa tropics mainly in Cameroon, Ghana and Nigeria [2]. It belongs to the family *Leguminosae* and commonly known as Ayan in Yoruba language and rich in flavonoids compounds such as Oxyayanin A, Oxyayanin B, Ayanin and distemonanthin [3]. Recent interest in chewing stick and their extracts has focused on organisms that are involved in oral infections. Africans that use chewing stick have fewer carious lesions than those that use toothbrush and has been encouraged by World Health Organization [4]. In this study, we assess the *in-vitro* antibacterial activities of ethanol crude extract of the twig of *D. benthamianus* against *Streptococcus mutans* which is a major culprit in dental caries. In view of the importance of *D. benthamianus* in ethno botany as health remedy and its use as chewing stick locally, we investigated the antibacterial activity of the twig extract against *Streptococcus mutans*.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection

Fresh twig of *Distemonanthus benthamianus* (*Bailon*) (*Fabaceae*) were collected from Bode in Ibadan (Latitude 7° 22' N and Longitude 3° 55' E) and was authenticated at the Department of Botany in the University Of Ibadan, Nigeria and voucher specimen deposited at the Herbarium of Botany department. The twig was air dried, pulverized and stored in an air tight container.

### 2.2 Microorganism

Sterile swab was used to collect oral samples from volunteers at the 30<sup>th</sup> anniversary exhibition

of the Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria. Samples were immediately taken to the Department of Pharmaceutical Microbiology laboratory for culturing on nutrient agar, bacitracin and sucrose and sheep blood. This combination is selective for the growth of *Streptococcus mutans*. Organisms were sub-cultured to obtain pure culture, biochemically identified and stored at -4°C for further evaluation.

### 2.3 Preliminary Phytochemical Studies

Preliminary phytochemical screening tests were carried out for the presence of tannin, saponin, steroids, flavonoids and alkaloid.

### 2.4 Extract Preparation

A 1000 g of the dried pulverized twig of *D. benthamianus* was exhaustively extracted with soxhlet apparatus using ethanol. The solvent was recovered and the extract was evaporated to dryness in a water bath and the residue of the extracted plant was stored at -4°C.

### 2.5 Antibacterial Activity Assay

The antibacterial activity of the crude ethanolic extract of the twig of *D. benthamianus* was determined using the agar well diffusion technique [5]. An overnight broth culture of the test bacterial isolates in nutrient broth was standardized to 0.5 McFarland standards (10<sup>6</sup> CFU/ml). Nutrient agar plates were seeded with the bacteria. Using Pasteur pipette, 0.2 ml of the 10<sup>-2</sup> dilution of the organism in Nutrient broth was placed on the surface of each NA plate. This was uniformly spread with the aid of a sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 min. A standard cork borer of 8 mm in diameter was used to cut equidistant wells on the surface of the agar into which was added 0.2 ml solution of the extract at concentrations of 1.2, 0.8, 0.4, 0.2, and 0.1 mg/ml. The effect of the crude extract was compared with that of standard drug (Gentamycin) in separate hole. The plates were incubated at room temperature for 24 hrs after which diameters zone of inhibition were measured. All assays were carried out in triplicates.

## 2.6 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extract was determined using agar dilution method [6]. A 19 ml of a sterile molten nutrient agar maintained at 45°C was added to 1 ml volume of the dissolved extract of the following concentration 2.00 mg/ml, 1.00 mg/ml, 0.50 mg/ml, 0.25 mg/ml, 0.125 mg/ml, 0.0625 mg/ml, and 0.03125 mg/ml. They were properly mixed for even distribution of the extract within the agar medium. The mixtures were poured and allowed to set. The plates were then dried at 37°C for 30 min. A 100 µL of the cell suspensions were inoculated to each concentration of the solidified agar-extract mixtures in duplicates. The controls were prepared by inoculating plates without extracts with the cell suspensions. The plates were then examined for the presence of colonies after the incubation period of 48 hrs at 37°C. The least concentration that gave no visible colonies was taken as the MIC of the extract for the particular dilution of the organism.

## 2.7 Determination of the Minimum Bactericidal Concentration (MBC)

This was determined by a modification of [7]. To a 0.5 ml at different concentrations as used in the MIC assay that shows no visible growth on the agar plate was added 0.5 ml of test organism in tubes. These were incubated at 25°C for 24-48 hrs. Samples were streaked out from the tubes on to Nutrient agar to determine the Minimum Concentration of the extract required to kill the organism. These concentrations were indicated by failure of the organism to grow on transfer to these media plates. The lowest concentration that prevented bacterial growth after days of incubation was recorded as the minimum bactericidal concentration. The entire tests were performed in duplicates to ensure accuracy. Agar plates without extract and another without organism were also incubated to serve as positive and negative control.

## 2.8 Determination of Mechanisms of Antibiosis (Bactericidal/Bacteriostatic)

The mechanism of antibiosis of the extracts was calculated using the ratio of MFC/MIC or MIC index [8] to elucidate whether the observed antibacterial effect were bactericidal or bacteriostatic. When the ratio of MFC/MIC is  $\leq 2.0$ , the extract is considered bactericidal or

bacteriostatic. If the ratio is  $\geq 16.0$ , the extract is considered ineffective.

## 2.9 Time Kill Kinetics Assay

The 0.5 McFarland standard was used in this experiment and corresponds to bacteria density of  $1.5 \times 10^8$  cfu/ml. Assays for the rate of killing bacteria by the ethanol extract were assessed using the modified pour plate technique of [9]. Extract were incorporated into 18 ml Nutrient agar (NA) in plate. One Nutrient agar without extract inoculated with test organisms and another incorporated with the extracts at the test concentrations without test organisms, were used as positive and negative controls. The plates were inoculated at 0sec, 30 min, 60 min, 90 min, 120 min, 150 min, 180 min, 210 min, 240 min, and 24 hrs intervals and incubated for 24-36 hrs. The procedure was carried in duplicates to ensure reproducibility. Plates were incubated at 25°C for 48-72 h before counting the colonies. The positive control plates were also incubated. Colonies were counted by the plate count technique. The values obtained were then used to construct curves to demonstrate changes in colony counts with time as a function of exposure to the antimicrobials.

## 3. RESULTS

The 1000 g pulverized twig of *D. benthamianus* yielded 13.03% of extract with ethanol. Table 1 revealed the presence of saponin, tannins, steroids alkaloids and flavonoids. Twenty one (21) of Sixty (60) strains of oral swabs were biochemically characterized as *Streptococcus mutans* (Table 2). The antibiogram assay revealed varied resistant pattern to the drug of use with resistant pattern predominant in most of the tested drugs. Chloramphenicol had the highest rate of susceptibility while cloxacillin had the highest rate of resistance (Table 3). The antibacterial activity of the twig ethanol extract of *D. benthamianus* evaluated by agar diffusion assay against *Streptococcus mutans* shows that the bacteria exhibited varied susceptibility to the extract at different concentrations used. The zones of inhibition obtained ranged between  $20 \pm 0.2$  mm (Table 4). At the highest concentration of the extract (6 mg/ml), *S. mutans* (PHM50, PHM22, PHM40, and PHM06) had the widest zone of inhibition ( $20 \pm 0.2$  mm) while at the lowest concentration used (0.5 mg/ml), *S. mutans* (PHM46) had the least zone of inhibition ( $09 \pm 0.1$  mm). The bacteria were not susceptible to 10% ethanol used in the

control assay. All *S. mutans* had MICs less than or equal to 100 µg/ml, except *S. mutans* (PHM47 and PHM05) that had MICs greater than or equal to 1000 µg/ml.

**Table 1. Phytochemical screenings of ethanol extract of *Distemonanthus benthamianus***

Test	<i>Distemonanthus benthamianus</i>
Saponin	++
Tannin	++
Steroids	+
Alkaloids	++
Flavonoids	++

+: Present; ++: Very positive  
(reaction positive within sec)

The MICs of all the bacteria isolates ranged between 15.6 and 1000 µg/ml while the MBC ranged between 31.25 to 1000 µg/ml (Table 5).

In the time kill assay, the result indicated that the extract exhibited a significant bactericidal activity. The results are presented in Fig. 1.

This figure reveals that after 2hrs of incubating bacteria at 1/2× MIC, 1× MIC, 2× MIC and reduction in the viable count ranged between 40-50%. At 4hrs of incubation, the reduction in the viable count ranged between 80-90% while at 24 hrs incubation, there is a total reduction in the population of viable count of the bacterial isolates. This is graphically represented in Fig. 1.

#### 4. DISCUSSION

Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery [10]. Tannins are reported to possess broad antimicrobial properties by means of different mechanisms that include enzyme inhibition, oxidative phosphorylation reduction and iron deprivation; among others. The very positive response of tannin in the phytochemical screening of *Distemonanthus benthamianus* may be responsible for its activities on the tested organism. Phytochemical analysis also reveals the presence of Saponin, alkaloids and steroids; these are also antimicrobial attributes as described by [11].

**Table 2. Biochemical identification of *Streptococcus mutans***

Organisms	NA+Bacitracin+ Sucrose+Sheep blood	Gram staining	Catalase test	Fermentation of mannose	Suspected organism
PHM05	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM06	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM07	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM08	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM11	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM14	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM22	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM23	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM25	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM28	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM29	G	+ve	+ve	+ve	<i>S. mutans</i>
PHM40	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM44	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM45	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM46	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM47	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM50	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM52	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM53	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM56	G	+ve	-ve	+ve	<i>S. mutans</i>
PHM57	G	+ve	-ve	+ve	<i>S. mutans</i>

Key-G-Growth, +ve Positive, -ve- Negative, PHM- Pharmaceutical Microbiology

Table 3. Antibiogram of tested *Streptococcus mutans*

Antibiotics	Aug	Amx	Ery	Tet	Cxc	Gen	Cot	Chl
<b>Organism</b>								
PHM05	R	R	R	16	R	R	R	R
PHM06	R	R	R	11	R	12	R	12
PHM07	R	R	R	15	R	14	R	18
PHM08	R	R	R	R	R	R	R	20
PHM11	R	R	R	13	R	14	R	16
PHM14	R	R	15	18	R	18	10	12
PHM22	R	R	R	18	R	16	R	17
PHM23	R	R	R	R	R	15	R	15
PHM25	R	R	R	12	R	15	14	18
PHM28	R	R	R	15	12	R	R	15
PHM29	R	R	R	R	R	18	R	R
PHM40	R	R	R	R	R	R	R	18
PHM44	12	18	16	12	R	15	12	12
PHM45	R	R	R	R	R	R	R	14
PHM46	R	R	15	12	R	10	R	14
PHM47	R	R	R	R	R	R	R	23
PHM50	R	R	14	12	R	10	R	14
PHM52	R	R	18	17	R	16	R	18
PHM53	16	15	13	12	18	17	14	15
PHM56	18	17	20	19	R	15	20	21
PHM57	R	R	R	10	R	16	12	10

Aug – Augumetin (30 mg), Amx – amoxicillin (25 mg), Ery – Erythromycin (5 mg), Tet – Tetracycline (10 mg), Cxc – Cloxacillin (5 mg), Gen – Gentamycin (10 mg), Cot – Cotrimaxole (25 mg), Chl – Chloramphenicol (30 mg), PHM – Pharmaceutical Microbiology

Table 4. Effect of ethanol extract of *Distemonanthus benthamianus* on *S. mutans*

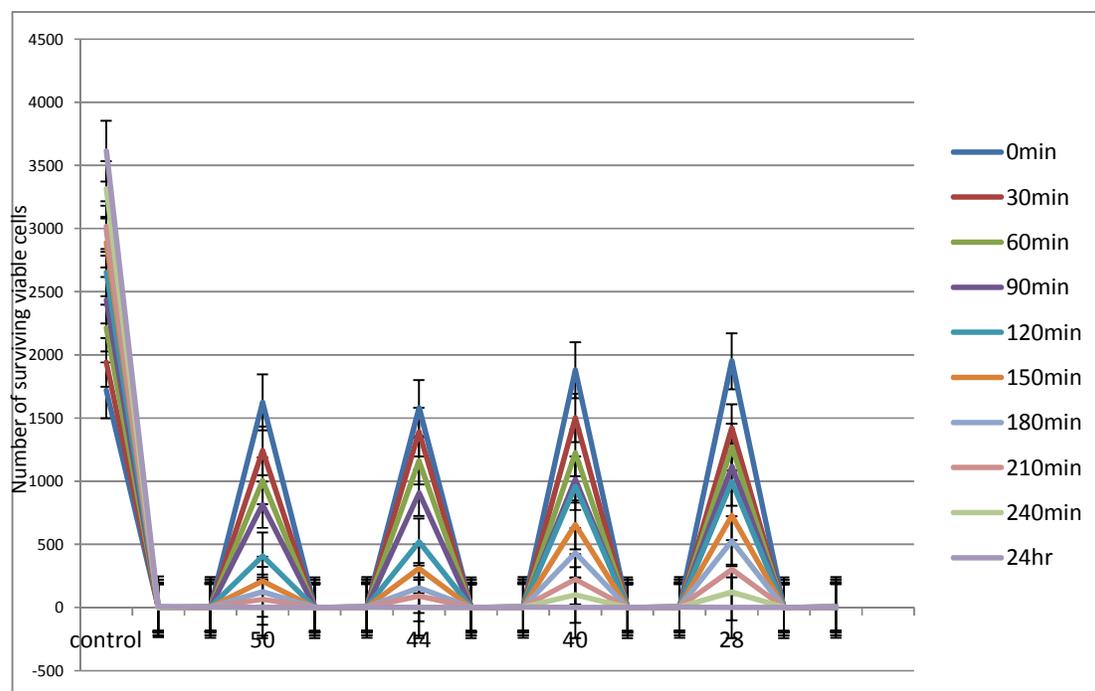
Organisms	6 mg/ml	4 mg/ml	2 mg/ml	1 mg/ml	0.5 mg/ml	Chloramphenicol (30 mg/ml)
PHM05	15±0.2	10±0.1	10±0.1	-	-	20±0.2
PHM06	20±0.2	16±0.1	14±0.1	12±0.1	10±0.2	15±0.1
PHM07	13±0.2	12±0.1	11±0.1	10±0.1	10±0.1	15±0.2
PHM08	14±0.1	12±0.1	10±0.1	10±0.1	10±0.1	18±0.2
PHM11	12±0.1	11±0.1	11±0.1	11±0.1	10±0.1	14±0.1
PHM14	10±0.1	10±0.1	-	-	-	15±0.2
PHM22	20±0.2	18±0.2	12±0.1	10±0.1	10±0.1	22±0.2
PHM23	17±0.1	13±0.1	10±0.1	10±0.1	10±0.1	22±0.2
PHM25	14±0.1	13±0.1	12±0.1	11±0.1	11±0.1	20±0.2
PHM28	15±0.1	13±0.1	11±0.1	10±0.1	10±0.1	18±0.2
PHM29	16±0.1	12±0.1	12±0.1	10±0.1	10±0.1	20±0.2
PHM40	20±0.1	12±0.1	10±0.1	10±0.1	10±0.1	16±0.2
PHM44	14±0.1	12±0.1	11±0.1	10±0.1	-	12±0.2
PHM45	15±0.2	13±0.2	10±0.2	10±0.1	10±0.1	14±0.1
PHM46	12±0.2	11±0.1	11±0.1	10±0.1	09±0.1	18±0.2
PHM47	12±0.2	10±0.1	10±0.1	-	-	25±0.2
PHM50	20±0.2	14±0.1	10±0.1	10±0.1	10±0.1	16±0.2
PHM52	18±0.2	16±0.2	12±0.1	12±0.1	10±0.1	21±0.2
PHM53	12±0.2	10±0.1	10±0.1	10±0.1	10±0.1	22±0.2
PHM56	16±0.2	14±0.1	13±0.1	12±0.1	10±0.1	18±0.2
PHM57	12±0.2	12±0.2	11±0.1	11±0.1	10±0.1	20±0.2

\*Mean ±SD, n = 3; \* 6 mg/ml, 4 mg/ml, 2 mg/ml, 1 mg/ml, 0.5 mg/ml – Various concentrations used in the experiment \* Chloramphenicol (30 mg) - Control drug

**Table 5. Minimum inhibitory concentration/minimum bactericidal concentration (MIC/MBC) of *Distemonanthus benthamianus* on *Streptococcus mutans***

Organisms	MIC (µg/ml)	MBC (µg/ml)	Antibiosis
PHM05	1000 ± 32.15	1000 ± 32.15	1
PHM06	31.25 ± 0.66	31.25 ± 0.65	1
PHM07	31.25 ± 0.65	31.25 ± 0.66	1
PHM08	31.25 ± 0.67	62.5 ± 1.32	2
PHM11	31.25 ± 0.66	31.25 ± 0.64	1
PHM14	15.6 ± 0.65	31.25 ± 0.66	2
PHM22	31.25 ± 0.66	62.5 ± 1.32	2
PHM23	31.25 ± 0.65	62.5 ± 1.32	2
PHM25	31.25 ± 0.67	62.5 ± 1.32	2
PHM28	15.6 ± 0.35	31.25 ± 0.65	2
PHM29	31.25 ± 0.66	62.5 ± 1.33	2
PHM40	31.25 ± 0.65	62.5 ± 1.33	2
PHM44	15.6 ± 0.33	31.25 ± 0.66	2
PHM45	31.25 ± 0.65	62.5 ± 1.33	2
PHM46	15.6 ± 0.34	31.25 ± 0.65	2
PHM47	1000 ± 32.15	1000 ± 32.15	1
PHM50	15.6 ± 0.33	31.25 ± 0.66	2
PHM52	31.25 ± 0.67	62.5 ± 1.34	2
PHM53	31.25 ± 0.66	31.25 ± 0.65	1
PHM56	15.6 ± 0.34	31.25 ± 0.66	2
PHM57	31.25 ± 0.65	62.5 ± 1.32	2

Mean ± SD, n = 3, PHM- Pharmaceutical microbiology, MIC- Minimum inhibitory concentration, MBC- Minimum bactericidal concentration, Antibiosis- MBC/MIC



**Fig. 1. Time kill assay of *Distemonanthus benthamianus* on *Streptococcus mutans* at 1 MIC**

n =3

Representation of tested organism (50, 44, 40, 28)

The use of ethanol as extracting solvent and its effectiveness agrees with the report of [12] which stated that the active components of plants are more soluble in organic solvent. It also corroborates the work of [13] which states that the best solvent for extracting most active component and principles of medicinal plant is ethanol. Hence, the activities of *Distemonanthus benthamianus* can be attributed to the solvent used for extracting the principles and its effectiveness against the tested bacterial isolates.

This study recorded relatively higher inhibitory activities of *Distemonanthus benthamianus* and the study of [14] supported the usage of this local chewing stick as natural means of aiding mastication, being the only oral hygiene agent that can be daily chewed over time. Since the overall aim of mouth cleansing is to achieve oral hygiene and dental health, this study further reveal the veracity of the twig of *Distemonanthus benthamianus* in achieving oral hygiene.

The study of [15] reported the antibacterial activity of the crude extract of the stem bark of *Distemonanthus benthamianus* against bacterial isolates implicated in oro-dental infections and that of [16] that reported the antifungal activity of this plant corroborates the findings of this research work and shows that the plant can be used in the treatment of the organism that is associated with dental caries caused by *Streptococcus mutans*.

## 5. CONCLUSION

This study has shown that the ethanol twig extract of *Distemonanthus benthamianus* possesses antimicrobial activities against bacterial isolates implicated in oro-dental infections. The Minimum inhibitory concentration and Minimum bactericidal concentration values of the extract against these microbes ranged from 15.6 µg/ml to 1000 µg/ml and 31.25 µg/ml to 1000 µg/ml respectively. The kinetic study also revealed a reduction in the number of viable organisms with increase in contact time between organisms and the extract. This study therefore suggests that *Distemonanthus benthamianus* may be a good source of combating dental caries caused by *Streptococcus mutans*.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Walter J. Microbiology of dental decay and periodontal diseases. Medical Microbiology. 2004;99.
2. Harborne JB. Phytochemical methods. A Guide to Modern Techniques of Plant Analysis; 1998.
3. Trease GE, Evans WC. Textbook of Pharmacognosy. 1983;21-22.
4. Ndukw C, Okeke IN, Lamikanra A, Adesina SK, Aboderin O. Antibacterial activity of aqueous extract of selected chewing sticks. Journal of Contemporary Dental Practice. 2005;86-94.
5. Adeniyi BA, Odelola HA, Oso BA. Antimicrobial potentials of *Diopyros mespiliformis*. Africa J Medi Sa. 1996;25: 179-184.
6. Lajubutu B, Pinney RJ, Robert MF, Odelola HA, Oso BA. Antimicrobial activity of diosquinone and plumbagin from *D. mespiliformis* (Hostch) (Ebenaceae). Phytother Res. 1995;9:346-350.
7. Adeniyi BA, Obasi Oleh John, Lawal Temitope O. *In vitro* antifungal activities of *Distemonanthus benthamianus* stem. International Journal of Pharmacy and Pharmaceutical Sciences. 2011;suppl 4:3.
8. Shanmughapriya SA, Manilal A, Sujith S, Selvin J, Kiran GS, Natarajaseenivasan K. Antimicrobial activity of seaweeds extracts against multi-resistant pathogens. Ann Microbiol. 2008;58:535-541.
9. Adeniyi BA, Odufowora RO. *In-Vitro* antimicrobial properties of *Aspilla africana* (Compositae). African Journal of Biomedical Research. 2000;3:167-170.
10. Dharmananda S. Gallnuts and the uses of tannins in Chinese medicine. Institute for Traditional Medicine, Portland, Oregon; 2003.
11. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999;12:564-582.
12. Kareem SO, Akpan I, Ojo OP. Antimicrobial activities of *Calotropis procera* on selected pathogenic

- microorganisms. African Journal of Biomedical Research. 2005;11:105-110.
13. Obi VI, Onuoha C. Extraction and characterization methods of plants and plants products In: Biological and Agricultural Techniques. 2005;271-286.
  14. Ogunshe AAO, Odumesi OG. Studies on the antimycotic potentials of toothpaste on human oral yeasts. African J Clin Experim Microbiol. 2010;3:182-191.
  15. Aiyegoro OA, Akinpelu DA, Afolayan AJ, Okoh AI. Antibacterial activities of crude stem bark extracts of *Distemonanthus benthamianus* Bail. Journal of Biological Sciences. 2008;2:356-361.
  16. Nguelefack Emp, Ngu Kp, Atchade A, Dimo T, Tsabang T, Mbafor Jt. Phytochemical composition and *in vitro* effects of the ethyl acetate bark extract of *Distemonanthus Benthamianus* Baillon (Caesalpiniaceae) On *Staphylococcus aureus* and *Streptococcus agalactiae*. Cameroon J Exp Biol. 2005;1:50-53.

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