Original Research Article

Antimicrobial Potential of Ethanol Extract and Fractions of *Caesalpinia benthamianaMezoneuron benthamianum* (Caesalpinaceae) Root on Some Organisms Implicated in Oral Infections.

ABSTRACT

This study investigated the activities of ethanol root extract of *Mezoneuron benthamianum* Baill (Caesalpiniaceae) against some microbial isolates implicated in oral infections and determined the killing rate of the most active fraction. It also investigated the phytochemical properties of the root extract. This was with a view to providing scientific basis for the use of the root in the treatment of oral infections.

The plant root was collected from the wild, washed, air-dried, ground to powder and macerated using ethanol and water at ratio 7:3 (v/v) with constant shaking for 72 hours in a mechanical shaker. The filtrate was concentrated *in-vacuo* at 50 ^{o}C using a rotary evaporator and freeze dried. The crude extract was screened for phytochemical and antimicrobial properties. The extract was further partitioned into fractions using different organic solvents in order of their polarity. The antimicrobial potential of the different fractions was determined using agar-well diffusion and agar dilution method respectively. The most active fraction was used to carry out the time-kill-assay on each of the organisms namely, *Staphylococcus aureus* (NCIB 8588) clinical isolates of *Streptococcus mutans*, ***Streptococcus*** *S. pyogenes*, ***Streptococcus*** *S. salivarius*, *Staphylococcus aureus* and *Candida albicans*. The values obtained were subjected to inferential statistical analysis.

Phytochemical screening revealed the presence of flavonoids, tannins, terpenes, saponins, phenolics and phenolic glycosides and anthraquinones. The root extract showed appreciable activity against all the test organisms, with the ethyl acetate fraction demonstrating highest activity and lowest MIC (0.16 mg/**ml**mL) compared with the crude extract and the other fractions. The activity was also time and concentration dependent. At triple the MIC all cells of respective organisms were killed at 5 minutes as was the case with all the standard antibiotics and anti-fungi used as positive control.

It was concluded that *C. benthamiana****M. benthamianum*** ethanol root extract was highly active against oral isolates with its ethyl acetate fraction being the most effective.

*Keywords: Caesalpinia benthamiana****Mezoneuron benthamianum****; Antimicrobial; Anticandidal; Phytochemical; Rate of kill.*

1. INTRODUCTION

The oral cavity harbours a microbial community of very diverse microflora which inhabits various surfaces of the mouth. The organisms exist in a complex matrix of biofilm which may vary depending on the dietary constituents, illness and oral hygiene and have been implicated in oral infectious diseases (1). The **Gram positive**Gram-positive organisms happen to be the

Comment [m1]: It is very well-marked that this study is acceptable with minor revision and useful for publish in this journal.

Note: Discussion section should be develop.
NOTE:The name of the plant species is not current http://www.theplantlist.org/tpl1.1/record/ild-33983

NOTE: I'd like to see the article again after the corrections.

In addition to, please add some pape and you can use them in manuscript.

23. Sevindik M. Investigation of Antioxidant/Oxidant Status and Antimicrobial Activities of Lentinus tigrinus. Adv Pharmacol Sci. 2018; https://doi.org/10.1155/2018/1718025
24. Sevindik M. The novel biological tests on various extracts of Cerioporus varius. Fresen Environ Bull. 2019;28(5):3713-3717.
26. Sevindik M. Investigation of oxidant and antioxidant status of edible mushroom Clavariadelphus truncatus. The Journal of Fungus. 2018; 9(2):165-168.
27. Mohammed FS, Akgul H, Sevindik M, Khaled BMT. Phenolic content and biological activities of Rhus coriaria var. zebaria. Fresen Environ Bull. 2018;27(8): 5694-5702.

Comment [m2]: This name is a synonym of *Caesalpinia benthamiana* (Baill.) Herend. & Zarucchi.

early colonisers of the oral cavity. These organisms, essentially the ***Streptococcus*** *S. mutans* more efficiently metabolize sugars, carbohydrates, oral food residues and produce organic acids which result to demineralization of the enamel, thus resulting to dental caries [2, 3]. The Streptococci and other related **Gram positive**Gram-positive organisms serve as mutual precursors of root canal infections, odontogenic diseases, endocarditis and abscesses [4]. The acid produced by mutans streptococci cause decalcification of the teeth enamel thus resulting to caries. Prolonged accumulation of caries causes inflammation of the gingiva which manifest as gingivitis or periodontitis, in which case the inflammatory response result in loss of collagen attachment of the tooth to the bone and in loss of bone [1]. The acidic environment created, also promote the colonization and virulence of ***Candida*** *C. albicans,* in the oral cavity especially in persons with immune impairment, resulting from organ transplant, HIV, cancer or chemotherapy [4]. ***Candida*** *C. albicans* is the most common species of yeast isolated from patients with oral candidiasis [5]. The global need for alternative prevention and treatment option and product for oral diseases that are safe, effective and economical comes from the rise in disease incidence, especially in developing countries, increased resistance, opportunistic infections in immunocompromised individuals, and financial considerations [6]. In addition, the reported toxicity and teeth staining of other agents used in the treatment of oral diseases, such as chlorhexidine, amine fluorides or products containing such agents continue to add impetus to the search for alternative products and natural phytochemicals isolated from plants used in traditional medicine [7].

*C. benthamiana****Mezoneuron benthamianum*** is a shrub or woody climber to 8 meter high and grows in dry deciduous secondary jungle and savannah forest of West Africa, from Senegal to Nigeria[8, 9]. It is reportedly used across the West Africa sub region for the treatment of various infections of the skin, wounds and other ailments [10, 11]. Phytochemical analysis of the leaf extract revealed the presence of flavonoids tannins cardiac glycosides, anthraquinones and saponins [11]. Previous studies also showed that the leaf of the plant has antibacterial [12] antifungal [13], analgesic and antipyretic activities [11]. Various gallic acid derivatives and monoterpines, sesquiterpenes, sesquiterpinoids have been isolated from the leaf extract and oil respectively [14]. However, there is a dearth of information on the phytomedical status of the root alone. Ethno medicinal information about the use of the root as chewing stick for the treatment of tooth pain resulting from oral infections necessitated this study.

2. MATERIAL AND METHODS

2.1 Organisms used for the experiment

The standard strain used was ***Staphylococcus*** *S. aureus* (NCIB 8588) maintained in the Microbiology Laboratory of the Drug Research and Production Unit, Faculty of Pharmacy, while the clinical isolates of oral bacteria and ***Candida*** *C. albicans* were collected from the stock culture maintained in the Laboratory of the Department of Microbiology and Parasitology, College of Health Sciences, Obafemi Awolowo University, Ile-Ife. The bacteria were first sub cultured in a nutrient broth (Fluka) and incubated at 37 ^{o}C for 18 h while the *Candida albicans* was sub cultured in a sabauraud dextrose agar (SDA) (Oxoid) and incubated at 25 ^{o}C for 72 h. before use.

2.2 Collection of plant root

The root of *C. benthamiana****Mezoneuron benthamianum*** was collected in the forest along the agricultural farm road within the Obafemi Awolowo University campus in the month of March 2012. The plant was jointly identified and authenticated by Mr. Oladele of the Herbarium Section, Faculty of Pharmacy, O. A. U., Ile-Ife, (now in the Department of Forestry and Wild Life Management, Niger Delta University, Nigeria) and Prof. H. C. Illoh of the Department of

Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. Voucher specimen of the plant was deposited in the Herbarium with voucher number IFE - 11047.

2.3 **Preparation and extraction of bioactive component of the root Sample**

The root of the plant was washed clean, air dried at room temperature and subsequently activated in the oven, regulated at 45 ^{o}C and was ground into fine powder. Exactly 954 g of powdered sample was then soaked in ethanol and sterile distilled water in ratio 7:3 (v/v) for extraction. The mixture was put in the mechanical shaker and agitated intermittently for 72 hours. The extract was then filtered through Whatman No. 1 filter paper. The filtrate collected was concentrated *in vacuo* using rotary evaporator (Buchi) at 50 ^{o}C to completely drive out the ethanol solvent. The remaining aqueous portion was finally lyophilized to obtain the extract. The weight of the dried crude extract was noted.

2.4 Qualitative phytochemical screening of the root extract

The phytochemical compounds in the root extract were qualitatively analyzed using the method of Trease and Evans [15] and Harborne [16]. The test included determination of the presence of saponin, terpene, alkaloid, flavonoid, phenol and phenolic glycosides and free anthraquinones in the root extract.

2.5 Preparation of partitioned fractions of the crude extract.

Ten gram (10 g) of the dried ethanol root extract was dissolved in 120 **ml** mL of distilled water and partitioned between chloroform and water in a separating funnel. The aqueous layer was further partitioned with petroleum spirit, and later with ethyl acetate. The four fractions obtained (i.e. chloroform, petroleum spirit, ethyl acetate and aqueous fractions) were then concentrated, freeze-dried and weighed respectively. The dried fractions were stored in the refrigerator until required.

2.6 Antimicrobial sensitivity assay

The solutions of the crude extract and its different fractions at concentrations of 25 and 10 mg/**ml** mL respectively were tested against panel of organisms using agar-well diffusion method [17,18]. Tetracycline, ampicillin and amphotericin B were also tested as standard antimicrobial drugs. The bacterial strains were first grown in nutrient broth for 18 h at 37 ^{o}C, while the ***Candida*** *C. albicans* was grown in Sabouraud dextrose broth (SDB) (Oxoid). The cell populations were standardized to 0.5 McFarland concentration, approximating 1x10^{6} cfu/ml for bacteria and 1x10^{5} cfu/**ml** mL for *C. albicans* respectively. The cell suspensions (200 **µl**µL) were seeded into previously sterilized molten (45 ^{o}C) nutrient agar (Fluka Biochemical, England), gently mixed and poured into a sterile Petri dish and left to solidify. The *Candida albicans* was seeded on Sabouraud dextrose agar (Oxoid Ltd.). Wells (9 mm diameter) were made equidistant to each other with a sterile cork borer. The wells were then filled with 25 mg/**ml** mL concentration of the extract and 1mg/**ml** mL of the standard antibiotics respectively and allowed to diffuse for 45 minutes at room temperature. The plates were then incubated at 37 ^{o}C for 24 hours after which the diameter of inhibition zones formed around the wells were measured in millimeter and recorded. The procedure was repeated for each of the fractions of the root extract at a concentration of 10 mg/**ml** mL and zones of clearance recorded for each experimental set up. The readings were carried out in triplicates.

2.7 Minimum inhibitory concentrations (MIC)

The MIC test was carried out for the extract and each of the fractions respectively, using the two-fold Agar dilution method of Russell and Furr, [17]; Irobi, *et al*, [18] to give a concentration range of 0.098 to 12.5 mg/**ml** mL for the extract and 0.04 to 5 mg/ml for the fractions. Two milliliters (2 ml) of individual concentration of the extract and the different fractions was introduced into 18 ml of sterile molten agar at 45 ^{o}C, mixed gently and poured into a sterile Petri dish and allowed to solidify. Approximately 1 x 10^{6} cfu/**ml** mL of each organism was then streaked on the pre-dried surface of the nutrient agar and later incubated at 37 ^{o}C for 24 h. The *C. albicans* was streaked on the pre-dried surface of SDA and incubated at 25 ^{o}C. The least concentration inhibiting growth of the organisms was taken as the MIC.

2.8 Time-kill assay for the test organisms

The rate of kill experiment was carried out on the most active fraction (ethyl acetate) as described by Balows *et al.*, [19] with modifications. A 5 ml overnight broth culture of the test organism was centrifuged at 2000 rpm for 10 minutes. The broth supernatant was carefully decanted out and the organism washed twice with 5 ml normal saline for 10 minutes, at 2000 rpm respectively. The washed cells of each bacterial strain and ***Candida*** *C. albicans* were first standardized to approximately 1x10^{6} cfu/**ml** mL and 1x10^{5} cfu/**ml** mL respectively. A 0.5 **ml** mL aliquot of standardized cells suspension was introduced into 4.5 ml of the ethyl acetate fraction solution at the test concentrations of 0.16 mg/ml, 0.32 mg/ml and 0.48 mg/ml respectively. Exactly 0.5 **ml** mL aliquot was introduced first into a recovery broth medium containing 3 % “Tween 80” in order to wash off the residual effect of the agent on the cells. A 0.5 **ml** mL volume was serially diluted and plated out at intervals of 5, 10, 15, 20, 30, 40, 50 and 60 minutes and incubated for 24 hours at 37 ^{o}C. Controls of untreated cells were also set up alongside the experimental. Colony count was done after the incubation period to determine the viable count at the different time intervals and compared with the control. Decrease in population of growth with time indicated killing by the fraction.

2.9 Statistical analysis

All experiments were carried out in triplicates and the mean of the values was compared using the Student t-test at significant ($p < 0.05$) level. Data was analysed graphically using GraphPad PRISM.

Formatted: Font: 10 pt

3. RESULTS

The yield obtained from the powdered sample of the plant was 16.84 g (1.75 %). The extract was dark in colour. Partitioning of 10 g of the ethanol crude extract yielded 0.953 g aqueous, 1.11 g ethyl acetate, 0.94 g petroleum spirit and 0.31 g chloroform fractions. The result presented in Table 1 shows the activities of the ethanolic root extract at 25 mg/**ml** mL concentrations. The zones of inhibition exhibited by the crude extract against the test bacterial strains ranged between 16.3 mm and 20.4 mm, while for the different fractions, the activities ranged between 20.6 – 23.7mm, 14.7 – 18.7 mm, 13.7 – 18.3 and 11.3 – 15.7 for ethyl acetate, petroleum spirit, aqueous and chloroform fractions respectively at a concentration of 10 mg/**ml**mL. Tetracycline and ampicillin gave zones of inhibition range of 21.3+0.33 – 24.0+0.58 mm, 22.3+0.89 – 24.0+0.33 mm respectively, for all the bacteria while Amphotericin B exhibited a zone of inhibition of 21.3+0.33 mm against the ***Candida*** *C. albicans*.

The results of the minimum inhibitory concentration showed that the ethyl acetate fraction had an MIC of 0.16 mg/**ml** mL for all the organisms while the petroleum spirit fraction had an MIC range of 2.50 mg/ml - 5 mg/ml (Table 2). The aqueous fraction had an MIC range of 1.25 mg/**ml** mL - 5 mg/**ml** mL while chloroform fraction had a range of 0.31 mg/**ml** mL – 2.50

mg/**ml**mL. Thus, ethyl acetate fraction being the most potent, was used for further test to determine its killing rate on all the organisms.

Table 1: Antimicrobial activities of the partitioned fractions of the ethanolic root extract of *C. benthamiana*Mezoneuron benthamianum

Test organisms	Mean zone of inhibition (mm)*							
	EtOH	PSF	CLF	EAF	AQF	TET	AMP	APB
	25 mg/ml	10 mg/ml	10 mg/ml	10 mg/ml	10 mg/ml	1 mg/ml	1 mg/ml	1 mg/ml
S. aureus (NCIB 8588)	20.4±0.33	15.7±0.33	18.7±0.33	23.7±0.33	18.3±0.33	23.3±0.67	24.3±0.89	-
S. mutans (CI)	19.7±0.33	13.3±0.33	16.3±0.33	21.0±0.58	15.3±0.67	24.0±0.58	23.0±0.67	-
S. pyogenes (CI)	18.3±0.33	13.7±0.33	15.7±0.33	21.0±0.58	15.3±0.33	23.7±0.67	24.0±0.33	-
S. salivarius (CI)	20.3±0.33	11.3±0.33	14.7±0.33	20.6±0.33	13.7±0.33	21.3±0.33	22.3±0.89	-
S. aureus (CI)	18.7±0.67	13.7±0.33	16.7±0.33	20.7±0.33	16.3±0.33	22.7±0.33	23.7±0.33	-
C. albicans (CI)	16.3±0.33	14.3±0.33	17.3±0.67	21.7±0.33	16.3±0.33	-	-	21.3±0.33

*: Values are mean of three readings; EtOH: Ethanolic extract; PSF: Petroleum spirit Fraction; CLF: Chloroform Frac tion; EAF: Ethyl Acetate Fraction; AQF: Aqueous Fraction; TET: Tetracycline; AMP: Ampicillin; APB: Amphotericin B

Table 2: Minimum inhibitory concentration (MIC) of ethanolic extract and partitioned fractions of *C. benthamiana*Mezoneuron benthamianum

Test Organisms	Concentration (mg/ml)							
	ETOH	PSF	CLF	EAF	AQF	TET	AMP	APB
S. aureus (NCIB 8588)	12.5	ND	0.63	0.16	1.25	0.039	0.039	-
S. mutans (CI)	12.5	2.5	2.5	0.16	5	0.039	0.039	-
S. pyogenes (CI)	12.5	5	0.63	0.16	1.25	0.039	0.039	-
S. salivarius (CI)	6.25	ND	2.5	0.16	2.5	0.039	0.039	-
S. aureus (CI)	ND	ND	0.63	0.16	1.25	0.039	0.039	-
C. albicans (CI)	3.13	ND	0.31	0.16	2.5	-		0.078

EtOH: Ethanolic extract; PSF: Petroleum spirit Fraction; CLF: Chloroform Frac tion; EAF: Ethyl Acetate Fraction; AQF: Aqueous Fraction; TET: Tetracycline; AMP: Ampicillin; APB: Amphotericin B

The reduction in population of the test organisms by the ethyl acetate fraction with time is as revealed in the graph of the log of viable count of the organisms against time at different test concentrations (Figures 1-6). The log of viable count of *S. aureus* (NCIB 8588) against time (Fig. 1) showed that at 1 x MIC (0.16 mg/**ml**mL), 13.55 % of the organisms were killed at 5 minutes. At 10 minutes, the percentage of cells killed slightly increased to 19.41 % while no viable count was observed at the end of 15 minutes of exposure time to the ethyl acetate fraction. At 2 x MIC (0.32 mg/**ml**mL), 16.36 % of the organism were killed within 5 minutes, while no viable count was observed as time increased to 10 minutes. At concentration of 3 x MIC (0.48 mg/**ml**mL) all the organisms were killed within a short period of 5 minutes.
Figure 2 shows the graph of the log of viable count of *S. mutans* at different test concentrations and time intervals. At 1 x MIC (0.16 mg/ml) 12.81 % of the organism was killed

at the end of 5 minutes. This rose slightly to 15.02% at 20 minutes period, while no viable count was observed at the end of 30 minutes. At 2 x MIC (0.32 mg/**ml**mL) 13.79 % of the organism have been eradicated in 10 minutes, while a total kill was observed at the end of 15 minutes. When the organism was introduced to 3 x MIC (0.48 mg/**ml**mL) of the fraction no viable count was observed at the end of 5 minutes contact time.

The result also revealed that at 1 x MIC value 11.27 % of *S. pyogenes* was killed within 5 minutes of exposure to the ethyl acetate fraction (Figure 3). Not much increase in killing rate was noticed from this time up to the end of 30 minutes when 16.36 % killing was achieved, while at the end of 40 minutes no viable count was observed. When the organism was exposed to 2 x MIC of the fraction, 12.97 % was killed in five minutes, while at 10 minutes 18.79 % of the cells were killed. The rate of killing increased to 21.45 % at the end of 15 minutes, while 100 % killing was achieved at the end of 20 minutes of contact with the organism. When the concentration was increased to 3 x MIC, total elimination of the organism was achieved at the end of 5 minutes of contact. Figure 4 showed that at 1 x MIC concentration (0.16 mg/**ml**mL) 12.12 % and 15.00 % of *S. salivarus* was killed at the end of 5 and 10 minutes respectively. At the end of 15 minutes of exposure to the ethyl acetate fraction, the organism was totally eliminated. When the concentration was doubled (2 x MIC = 0.32 mg/ml) the time of total death reduced to 10 minutes. At 3 x MIC total elimination of the organism was achieved at 5 minutes of exposure.

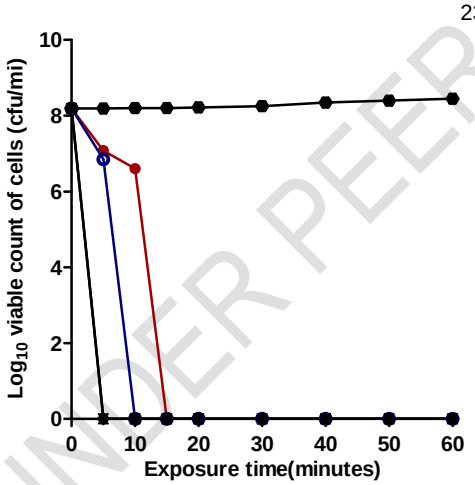

Fig. 1; The rate and extent of kill of *S. aureus* (NCIB 8588) by ethyl acetate fraction at 1 x MIC (●), 2 x MIC (○), 3 x MIC (▼), Tetracycline (▲), Ampicillin (◆) and Control (⬣) Each point represent the mean log10 survival of bacterial cells at a particular time interval in the presence of the fraction.

Formatted: Font: Italic

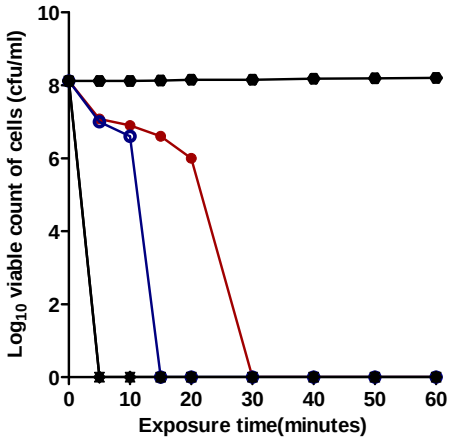

Fig. 2: The rate and extent of kill of *S. mutans* (CI) by ethyl acetate fraction at 1 x MIC (), 2 x MIC () 3 x MIC (), Tetracycline (), Ampicillin () and Control (). Each point of the represent the mean log10 survival of bacterial cells at a particular time interval in the presence fraction.

Formatted: Font: Italic

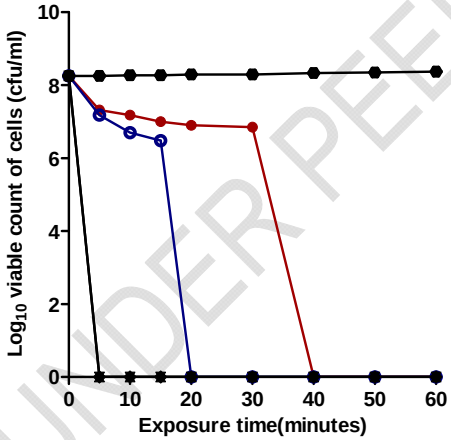

Fig. 3; The rate and extent of kill of *S. pyogenes* (CI) by ethyl acetate fraction at 1 x MIC (), 2 x MIC (), 3 x MIC (), Tetracycline (), Ampicillin () and Control (). Each point represent the mean log10 survival of bacterial cells at a particular time interval in the presence of the fraction.

Formatted: Font: Italic

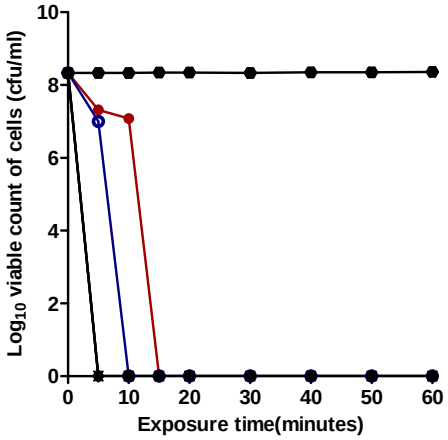

Fig. 4: The rate and extent of kill of *S. salivarus* (CI) by ethyl acetate fraction at 1 x MIC (●), 2 x MIC (●), 3 x MIC (▲), Tetracycline (▼), Ampicillin (◆) and Control (■). Each point represent the mean log_{10} survival of bacterial cells at a particular time interval in the presence of the fraction.

Formatted: Font: Italic

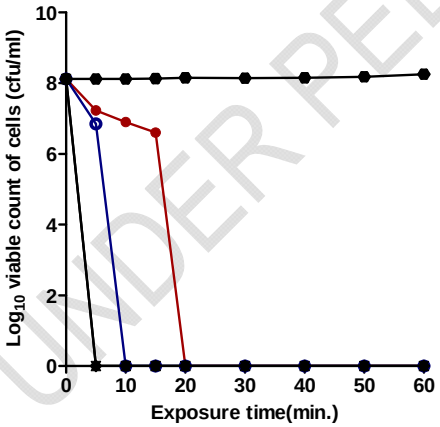

Fig. 5: The rate and extent of kill of *S. aureus* (CI) by ethyl acetate fraction at 1 x MIC (●), 2 x MIC (●), 3 x MIC (▲), Tetracycline (▼), Ampicillin (◆) and Control (■). Each point represent the mean log10 survival of bacterial cells at a particular time interval in the presence of the fraction.

Formatted: Font: Italic

The log of viable count of *S. aureus* (CI) against time (Figure 5) at 1 x MIC (0.16 mg/**ml**mL) of the fraction revealed that 10.96 % killing was achieved at 5 minutes, 15.02 % at 10 minutes and 18.72 % at 15 minutes while total elimination was achieved at 20 minutes of exposure, At 2 x MIC (0.32 mg/**ml**mL) total elimination time dropped to 10 minutes. At 3x MIC (0.48 mg/**ml**mL), all the organisms were completely eliminated at the end of 5 minutes. The log of viable count of *C. albicans* against time at different test concentrations (Figure 6) showed that no viable count was observed after exposure to the MIC of the fraction at 15 minutes, while at MIC x 2 concentration (0.32 mg/ml.), total elimination time was reduced to 10 minutes. However at a concentration of MIC x 3 (0.48 mg/**ml**mL) all the cells were killed at 5 minutes after the exposure of the organism to the fraction. For the standard anti-Candidal agent (Amphotericin B), all the organisms were killed at 5 minutes after exposure to its minimum inhibitory concentration.

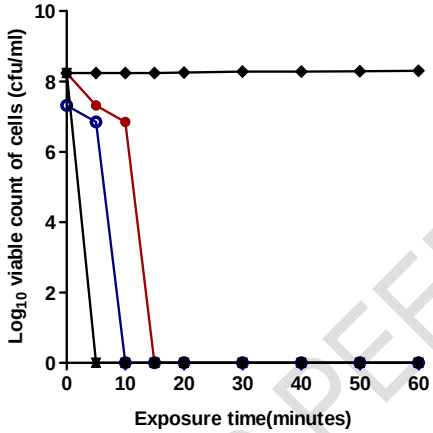

Fig. 6: The rate and extent of kill of *C. albicans* (CI) by ethyl acetate fraction at 1 x MIC ( ) 2 x MIC ( ), 3 x MIC (, Amphotericin B ( ) and Control ( ). Each point represent the mean $\log_{10}$ survival of bacterial cells at a particular time interval in the presence of the fraction.

4. DISCUSSION

The antimicrobial potential of *C. benthamiana**M. benthamianum*** was investigated against some Gram-positive bacteria and *Candida albicans* commonly implicate in human oral infections. The phytochemical property of the plant was also investigated. The root extract of *C. benthamiana* ***M. benthamianum*** and its chloroform, petroleum spirit, ethyl acetate and aqueous fractions exhibited a high level of activity against the test organisms which include *S. aureus* (NCIB) 8588 and clinical isolates of *S. mutans*, *S. pyogenes*, *S. salivarius, S. aureus and C. albicans* (Table 1) from the human oral cavity. ***Streptococcus*** *S. mutans*, *S. pyogenes*, *S. salivarius* are found in plaque while *C. albicans* is the causative agent of oral candidiasis. *S. salivarius* is also the major causative organism of periodontal disease in children as it is the organism that first colonizes the oral cavity [20]. ***Sreptococcus*** *S. mutans* has also been implicated in gingivitis and dental caries. The action of the partitioned fractions showed a trend of increasing activities from petroleum spirit <aqueous <chloroform <ethyl

acetate against all the test organisms at the test concentration of 10 mg/**ml**mL. With the exception of the ethyl acetate fraction, all the other fractions demonstrated much lesser activities against the organisms at this concentration than the ethanol crude extract. This was in agreement with the results of an earlier work by Fayemi and Osho [13] which showed that the petroleum spirit and chloroform fractions of *C. benthamiana**M. benthamianum* whole plant demonstrated weaker activities than the ethanol crude extract. A finding of this study is that the ethyl acetate fraction showed the highest activity against the test organisms at the test concentration of 10 mg/**ml** mL compared with the crude extract and all the other partitioned fractions, thus suggesting that fractionation with ethyl acetate improves the antimicrobial activity of the plant. It should be noted also that the activity of the ethyl acetate fraction was comparable with that of the standard antibiotics used.

The result of the minimum inhibitory concentration (MIC) (Table 2) showed that each of the four fractions had different MICs for the organisms. It is however evident from this study that the potential effect of the different fractions against all the organisms followed the same trend, as there was a correlation between the MICs and the sensitivities of these fractions. This also agreed with the findings of previous authors [12] who carried out sensitivity tests on the whole plant extracts, using different solvents for extraction. Furthermore, the highest activity and lowest MICs of the ethyl acetate fraction, suggested that it is the most active of all the fractions. In addition, the highest yield of fraction produced by the ethyl acetate solvent is a pointer to the fact that the putative compound(s) of the plant is (are) best extracted by this polar solvent.

The bactericidal efficacy of the ethyl acetate fraction as revealed in Figures 1 to 6 was high, and rapidly eliminated the cells in less than 60 minutes period. However, S*. aureus*, *S. mutans* and *S. pyogenes* took a longer time to be eradicated completely than the rest organisms. The reason behind this may due to the fact that being clinical isolates, they might have developed some level of resistance than their counterparts (*S. salivarus* and *C. albicans*) due to previous over exposure to antibiotics and hence do not respond quickly to the activity of the extract within the shortest time interval. It was observed however, that the absolute value of the rate of death for each of the organisms was altered by increase in the concentration of the ethyl acetate fraction, as the time of death and viable count for each organism reduced. At triple the minimum inhibitory concentration of the fraction (i.e. 0.48 mg/**ml**mL), all the organisms were equally eliminated within the same period of time as was the case with the standard antibiotics used in this study i.e. ampicillin and tetracycline for the bacteria and amphotericin B for the fungus. This shows that the activities of the plant root are both concentration and exposure time dependent, and supports claims by traditional medical practitioners that it is fast acting. Hence its use as an analgesic as earlier reported by Mbagwu *et al*., [7]. The generally accepted definition of bactericidal activity in antibiotics is a reduction in the microbial population to 99 % of the initial population of the organisms within the shortest period of time [21]. Thus the bactericidal activity of ethyl acetate fraction obtained from the ethanol root extract of *C. benthamiana**M. benthamianum* in this study showed significant therapeutic potential and hence supports its use in folkloric remedies. The high degree of antimicrobial activity obtained from the result of this study is an indication that *C. benthamiana* ***M. benthamianum*** is a good source of potent antimicrobial agent for the treatment and prevention of oral infections caused by these organisms and can also help in the reduction of dental caries. In addition, it serves as a support for the ethno medical claim of the use of the root as chewing stick for the treatment of tooth pain resulting from oral infections.

This study revealed the presence of flavonoids, tannins, terpenes, saponins, phenolics and phenolic glycosides and anthraquinones in the root extract of the plant (Table 3). These phytochemicals are known to have biological activities and hence, might have contributed to

the observed activities noted in this study. Flavonoids are known to exhibit a wide range of biological activities including antimicrobial, anti-inflammatory, analgesic, cytostatic and

Table 3: Phytochemical Screening of the root extract of *C. benthamiana*Mezoneuron *benthamianum*

Secondary Metabolite	Proportion
Saponin	++
Terpene	++
Alkaloid	-/+
Flavonoid	+++
Phenolics and phenolic glycoside	+++
Anthraquinone	+

Key: +++ = Highly present; ++ = Present; + = fairly present; +/ - = Trace; - = Absent.

antioxidant properties [22-24]. The ability of flavonoids to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxyradicals highlights many of its health promoting functions in organisms which are important for the prevention of diseases associated with oxidative damage of membranes, proteins and DNA [25-27]. These conditions can be seen in dental caries, gingivitis, and oral candidiasis to mention a few. Tannins act via a different mechanism to flavonoids. Tannins act by iron deprivation or specific interactions with vital proteins such as enzymes in microbial cells [28]. Motal *et al*., [29] reviewed the importance of tannins for the treatment of inflamed or ulcerated tissues as seen in gingivitis, caries and plaque. Saponins are considered a key ingredient in traditional Chinese medicine [30]. Saponins produce inhibitory effect on inflammation (Just *et al*., [31]. Phenolic glycosides are an important class of naturally occurring drugs whose actions help in the treatment of congestive heart failure. Plants containing phenolic glycosides are used to treat cardiac infections like endocarditis. Some of the causative organisms of endocarditis e.g. *S. aureus* have their origin in the oral cavity. Plants containing phenolic glycosides are also useful in the treatment of chest pains, tooth ache and cough among the "Yoruba" tribe of south western Nigeria [32]. All these observations cited on the action of phytochemicals support the use of *C. benthamiana**M. benthamianum*** root as a traditional remedy for oral diseases as its therapeutic effects can be attributed to the actions of its phytochemical constituents.

Formatted: Font: Not Bold

Formatted: Font: Not Bold

Formatted: Font: Not Bold

5. CONCLUSION

The result of this work showed that ethanol root extract of *C. benthamiana**M. benthamianum*** demonstrated appreciably high activities on the oral isolates (*S. mutans*, *S. pyogenes*, *S. salivarius*, *S. aureus* and *C. albicans*) and the type organism (*S. aureus* NCIB 8588)

employed in this study, with the Ethyl acetate fraction being the most active. This provided a scientific basis for the acclaimed traditional use of its root as chewing stick for the maintenance of oral hygiene, prevention of dental caries and the treatment of tooth pain resulting from oral infections. It is interesting to note also, that the ethanol root extract is highly effective against bacteria and fungi (*C. albicans)* alike. This is an added advantage in the activity of this plant. It is recommended that further work on the ethyl acetate fraction of the ethanol root extract of *C. benthamiana**M. benthamianum*** be carried out with the hope of developing an effective antimicrobial oral rinse from the plant.

REFERENCES

1. Loesche W. Dental caries and periodontitis: contrasting two infections that have mdical implications. *Infectious Disease Clinics of North America*. 2007; 21(2): 471–502.
2. Nishimura J. Sait T. Yoneyama H. Sai L. Okimura K. and Isogai E. Biofilm formation by *Streptococcus mutans* and related bacteria. *Advances in Microbiology*. 2012; 2: 208-215
3. Carroll KC. Bacteriology. In Jawetz, Melnick and Adelberg's Medical Microbiology, 26th ed. USA: The McGraw-Hill Companies, Inc.; 2013
4. Zadik Y Burnstein S Derazne E Sandler V Ianculovici C Halperin T. Colonization of Candida: prevalence among tongue-pierced and non-pierced immunocompetent adults. Oral Dis. 2010; 16 (2): 172–5.
5. Cannon RD and Chaffin WL. Oral colonization by *Candida albicans*. Crit. Rev. Oral Biol. Med. 1999; 10: 359 – 383.
6. Badria FA and Zidan OA. Natural products for dental caries prevention. *Journal of Medicinal Food*. 2004; 7(3):381–384.
7. Prabu GR Gnanamani A and Sadulla S. Guaijaverin-a plant flavonoid as potential antiplaque agent against *Streptococcus mutans Journal of Applied Microbiology*. 2006; 101(2): 487–495.
8. Cox PA. The ethno-botanical Approach to Drug Discovery: Strength and Limitation. 1994; ed. Prance GT. 1994; 25-40.
9. African Flowering Plants Database (AFPD). 2008)- Base de Donnees des Plantes a Fleurs D'Afrique.
10. Verger P. Awon ewe Osanyin (Yoruba Medicinal Leaves). University of Ife. (1997; 1-55.
11. Mbagwu HOC Anene RA Adeyemi OO. The analgesic, antipyretic and anti-inflammatory properties of *Mezoneuron benthamianum*. *Nigerian Ot J. Hosp. Med.* 2007; 17(1): 35-41.
12. Dickson RA Houghton PJ Hylands PJ. Gibbons S. Antimicrobial, resistance-modifying effects, antioxidant and free radical scavenging activities of *Mezoneuron benthamianum* Baill., *Securinega virosa* Roxb. &Wlld. and *Microglossa pyrifolia* Lam. Phytotherapy Research. 2006;20(1): 41-5.
13. Fayemi SO, Osho A. Comparison of Antimicrobial Effects of *Mezoneuron benthamianum, Heliotropium indicum* and *Flabellaria paniculata* on Candida species. *Journal of Microbiology Research*. 2012; 2(1): 18-23.

14. Binutu OA. and Cordell GA. Gallic acid derivatives from *Mezoneuron benthamianum* leaves Pharmaceutical Biology. 2000; 38(4): 284–286
15. Trease GE and Evans WC. Test Book of Pharmacognosy. 15th ed. London; Saunders Publishers; Saunders, London. 2002;2(4):214-393.
16. Harbone JB. Phytochemical Methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall London. 1998; 60-6.
17. Russell AD Furr JR. . Microbial susceptibility and resistance to biocides. ASM News 1997;63: 481–487.
18. Irobi ON Moo-Young M and Anderson WA. Antimicrobial activity of Annatto (*Bixa orellana*) extract. *International Journal of Pharmacy* 1996;34: 87 – 90.
19. Balows A Hausler WJ Hermann KL Isenberg HD Shadamytt J. Manual of clinical microbiology. 5th ed. Washington (DC): American Society for Microbiology. 1991.
20. Rogers AH editor. Molecular Oral Microbiology. Caister Academic Press. ISBN 978-1-904455-24-0. 2008
21. Pankey GA and Sabath LD. Clinical relevance of bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. Clin Infect. Dis.; 2004; **38**:864-870.
22. Hodek P. Trefil P. Stiborova M. . Flavonoids- Potent and versatile biologically active compounds interacting with cytochrome P450. *Chemico- Biological Inter.* 2002;139(1): 1-21.
23. Sevindik M. Investigation of Antioxidant/Oxidant Status and Antimicrobial Activities of *Lentinus tigrinus*. Adv Pharmacol Sci. 2018; https://doi.org/10.1155/2018/1718025
24. Sevindik M. The novel biological tests on various extracts of *Cerioporus varius*. Fresen Environ Bull. 2019;28(5):3713-3717.
25. Ferguson LR. Role of plant polyphenols in genomic stability. Mutat. Res. 2001;475: 89 – 111.
26. Sevindik M. Investigation of oxidant and antioxidant status of edible mushroom *Clavariadelphus truncatus*. The Journal of Fungus. 2018; 9(2):165-168.
27. Mohammed FS, Akgul H, Sevindik M, Khaled BMT. Phenolic content and biological activities of *Rhus coriaria* var. *zebaria*. Fresen Environ Bull. 2018;27(8): 5694-5702.
28. Scalbert A. Antimicrobial properties of Tannins. Phytochem. 1991;30:3895-3883.
29. Motal MLR Thomas G and Barbosa-Fillo GM. Effects of *Anacadium occidentale* stem back extract on in vivo inflammatory models. J. Ethnopharmacol. 1985;95(2-3): 139-142.
30. Liu J and Henkel T. Traditional Chinese Medicine (TCM). Are Polyphenols and Saponins the Key ingredients Triggering Biological Activities? Curr. Med. Chem. 2002; 9: 1483-1485.
31. Just MJ Recio MC Giner RM Cueller MJ Manez S Bilia AR and Rios JL. Anti-inflamatory Activity of Unusual Lupine Saponins from *Bupleurium fruitcescens*. Planta Medica 1998;64:404-407.
32. Ikeda Y Fujii Y Nakaya I and Yamazak M. Quantitative HPLC analysis of cardiac glycosides in Digitalis purpurea leaves. J. Nat. Prod. 1995;**58**(60): 897 – 901.