Original Research Article

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

ABSTRACT

This study investigated the activities of ethanol root extract of *Caesalpinia benthamiana* (Baill) Herend. and Zarucchi. (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 ^oC 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. Time-kill-assay of the most active fraction was carried out on each of the organisms namely, *Staphylococcus aureus* (NCIB 8588) clinical isolates of *Streptococcus mutans*, *S. pyogenes*, *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) 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* ethanol root extract was highly active against oral isolates with its ethyl acetate fraction being the most effective.

Keywords: C. benthamiana; 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 organisms happen to be the early colonisers of the oral cavity. These organisms, essentially the *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 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 *C. albicans,* in the oral cavity especially in persons with immune impairment, resulting from organ transplant, HIV, cancer or chemotherapy [4]. *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].

Caesalpinia benthamiana 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 *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 *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 *C. 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* 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

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The root of the plant was washed clean, air dried at room temperature and subsequently activated in the oven, regulated at 45 °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 °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 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 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 °C, while the *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 for *C. albicans* respectively. The cell suspensions (200 µL) were seeded into previously sterilized molten (45 °C) nutrient agar (Fluka Biochemical, England), gently mixed and poured into a sterile Petri dish and left to solidify. The *C. 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 concentration of the extract and 1mg/mL of the standard antibiotics respectively and allowed to diffuse for 45 minutes at room temperature. The plates were then incubated at 37 °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 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 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 °C, mixed gently and poured into a sterile

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129 Petri dish and allowed to solidify. Approximately 1 x 10^6 cfu/mL of each organism was then
130 streaked on the pre-dried surface of the nutrient agar and later incubated at 37 °C for 24 h.
131 The *C. albicans* was streaked on the pre-dried surface of SDA and incubated at 25 °C. The
132 least concentration inhibiting growth of the organisms was taken as the MIC.
133

134 **2.8 Time-kill assay for the test organisms**

135
136 The rate of kill experiment was carried out on the most active fraction (ethyl acetate) as
137 described by Balows *et al*., [19] with modifications. A 5 mL overnight broth culture of the test
138 organism was centrifuged at 2000 rpm for 10 minutes. The broth supernatant was carefully
139 decanted out and the organism washed twice with 5 mL normal saline for 10 minutes, at 2000
140 rpm respectively. The washed cells of each bacterial strain and *C. albicans* were first
141 standardized to approximately 1x10^6 cfu/mL and 1x10^5 cfu/mL respectively. A 0.5 mL aliquot
142 of standardized cells suspension was introduced into 4.5 mL of the ethyl acetate fraction
143 solution at the test concentrations of 0.16 mg/mL, 0.32 mg/mL and 0.48 mg/mL respectively.
144 Exactly 0.5 mL aliquot was introduced first into a recovery broth medium containing 3 %
145 "Tween 80" in order to wash off the residual effect of the agent on the cells. A 0.5 mL volume
146 was serially diluted and plated out at intervals of 5, 10, 15, 20, 30, 40, 50 and 60 minutes and
147 incubated for 24 hours at 37 °C. Controls of untreated cells were also set up alongside the
148 experimental. Colony count was done after the incubation period to determine the viable count
149 at the different time intervals and compared with the control. Decrease in population of growth
150 with time indicated killing by the fraction.
151

152 **2.9 Statistical analysis**

153
154 All experiments were carried out in triplicates and the mean of the values was compared using
155 the Student t-test at significant ($p < 0.05$) level. Data was analysed graphically using
156 GraphPad PRISM.
157
158

159 **3. RESULTS**

160
161 The yield obtained from the powdered sample of the plant was 16.84 g (1.75 %). The extract
162 was dark in colour. Partitioning of 10 g of the ethanol crude extract yielded 0.953 g aqueous,
163 1.11 g ethyl acetate, 0.94 g petroleum spirit and 0.31 g chloroform fractions. The result
164 presented in Table 1 shows the activities of the ethanolic root extract at 25 mg/mL
165 concentrations. The zones of inhibition exhibited by the crude extract against the test bacterial
166 strains ranged between 16.3 mm and 20.4 mm, while for the different fractions, the activities
167 ranged between 20.6 – 23.7mm, 14.7 – 18.7 mm, 13.7 – 18.3 and 11.3 – 15.7 for ethyl
168 acetate, petroleum spirit, aqueous and chloroform fractions respectively at a concentration of
169 10 mg/mL. Tetracycline and ampicillin gave zones of inhibition range of 21.3+0.33 –
170 24.0+0.58 mm, 22.3+0.89 – 24.0+0.33 mm respectively, for all the bacteria while
171 Amphotericin B exhibited a zone of inhibition of 21.3+0.33 mm against the *C. albicans*.
172 The results of the minimum inhibitory concentration showed that the ethyl acetate fraction had
173 an MIC of 0.16 mg/mL for all the organisms while the petroleum spirit fraction had an MIC
174 range of 2.50 mg/mL - 5 mg/mL (Table 2). The aqueous fraction had an MIC range of 1.25
175 mg/mL - 5 mg/mL while chloroform fraction had a range of 0.31 mg/mL – 2.50 mg/mL. Thus,
176 ethyl acetate fraction being the most potent, was used for further test to determine its killing
177 rate on all the organisms.
178
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180 **Table 1: Antimicrobial activities of the partitioned fractions of the ethanolic root extract**
181 **of *C. benthamiana***

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

182
183 *: Values are mean of three readings; EtOH: Ethanolic extract; PSF: Petroleum spirit Fraction;
184 CLF: Chloroform Frac tion; EAF: Ethyl Acetate Fraction; AQF: Aqueous Fraction; TET:
185 Tetracycline; AMP: Ampicillin; APB: Amphotericin B, CI: Clinical isolate, NCIB: National
186 Collection of Industrial Bacteria
187
188
189 **Table 2: Minimum inhibitory concentration (MIC) of ethanolic extract and partitioned**
190 **fractions of *C. benthamiana***
191

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

192
193 EtOH: Ethanolic extract; PSF: Petroleum spirit Fraction; CLF: Chloroform Frac tion; EAF:
194 Ethyl Acetate Fraction; AQF: Aqueous Fraction; TET: Tetracycline; AMP: Ampicillin; APB:
195 Amphotericin B, ND: Not done

196
197
198 The reduction in population of the test organisms by the ethyl acetate fraction with time is as
199 revealed in the graph of the log of viable count of the organisms against time at different test
200 concentrations (Figures 1-6). The log of viable count of *S. aureus* (NCIB 8588) against time
201 (Fig. 1) showed that at 1 x MIC (0.16 mg/mL), 13.55 % of the organisms were killed at 5
202 minutes. At 10 minutes, the percentage of cells killed slightly increased to 19.41 % while no
203 viable count was observed at the end of 15 minutes of exposure time to the ethyl acetate
204 fraction. At 2 x MIC (0.32 mg/mL), 16.36 % of the organism were killed within 5 minutes, while
205 no viable count was observed as time increased to 10 minutes. At concentration of 3 x MIC
206 (0.48 mg/mL) all the organisms were killed within a short period of 5 minutes.
207 Figure 2 shows the graph of the log of viable count of *S. mutans* at different test
208 concentrations and time intervals. At 1 x MIC (0.16 mg/ml) 12.81 % of the organism was killed
209 at the end of 5 minutes. This rose slightly to 15.02 % at 20 minutes period, while no viable
210 count was observed at the end of 30 minutes. At 2 x MIC (0.32 mg/mL) 13.79 % of the
211 organism have been eradicated in 10 minutes, while a total kill was observed at the end of 15

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212 minutes. When the organism was introduced to 3 x MIC (0.48 mg/mL) of the fraction no viable
213 count was observed at the end of 5 minutes contact time.
214 The result also revealed that at 1 x MIC value 11.27 % of *S. pyogenes* was killed within 5
215 minutes of exposure to the ethyl acetate fraction (Figure 3). Not much increase in killing rate
216 was noticed from this time up to the end of 30 minutes when 16.36 % killing was achieved,
217 while at the end of 40 minutes no viable count was observed. When the organism was
218 exposed to 2 x MIC of the fraction, 12.97 % was killed in five minutes, while at 10 minutes
219 18.79 % of the cells were killed. The rate of killing increased to 21.45 % at the end of 15
220 minutes, while 100 % killing was achieved at the end of 20 minutes of contact with the
221 organism. When the concentration was increased to 3 x MIC, total elimination of the organism
222 was achieved at the end of 5 minutes of contact. Figure 4 showed that at 1 x MIC
223 concentration (0.16 mg/mL) 12.12 % and 15.00 % of *S. salivarus* was killed at the end of 5
224 and 10 minutes respectively. At the end of 15 minutes of exposure to the ethyl acetate
225 fraction, the organism was totally eliminated. When the concentration was doubled (2 x MIC =
226 0.32 mg/mL) the time of total death reduced to 10 minutes. At 3 x MIC total elimination of the
227 organism was achieved at 5 minutes of exposure.
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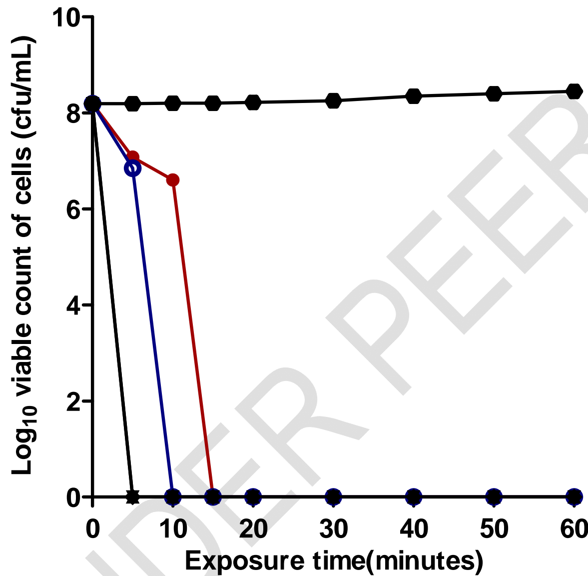

231
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233
234 Fig. 1; The rate and extent of kill of *S. aureus* (NCIB 8588) by ethyl acetate fraction at 1 x MIC
235 (●), 2 x MIC (○), 3 x MIC (▼), Tetracycline (▲), Ampicillin (◆) and Control (⬢) Each
236 point represent the mean log10 survival of bacterial cells at a particular time interval in the
237 presence of the fraction.
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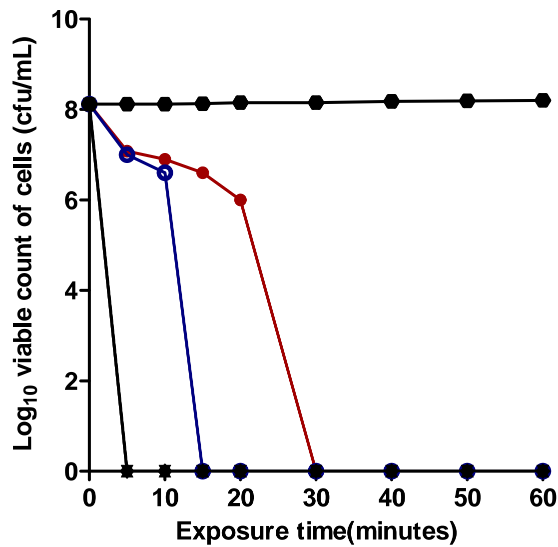

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241
242 Fig. 2: The rate and extent of kill of *S. mutans* (CI) by ethyl acetate fraction at 1 x MIC (), 2
243 x MIC () 3 x MIC (), Tetracycline (), Ampicillin () and Control (). Each point
244 of the represent the mean log10 survival of bacterial cells at a particular time interval in the
245 presence fraction.
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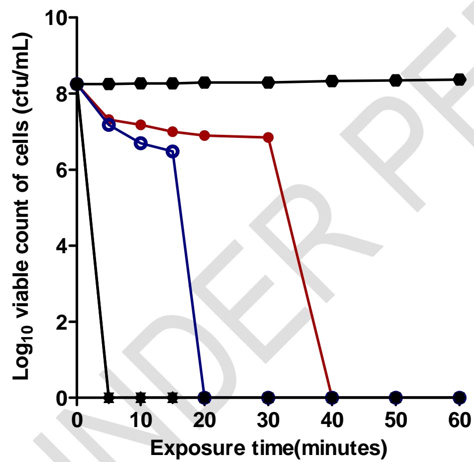

247
248
249 Fig. 3; The rate and extent of kill of *S. pyogenes* (CI) by ethyl acetate fraction at 1 x
250 MIC (), 2 x MIC (), 3 x MIC (), Tetracycline (), Ampicillin () and
251 Control (). Each point represent the mean log10 survival of bacterial cells at a
252 particular time interval in the presence of the fraction.
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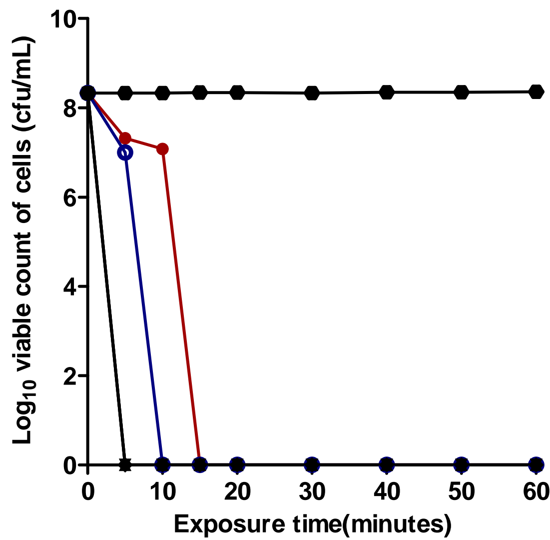

256
257
258 Fig. 4: The rate and extent of kill of *S. salivarus* (CI) by ethyl acetate fraction at 1 x
259 MIC (), 2 x MIC (), 3 x MIC (), Tetracycline ( ), Ampicillin () and
260 Control (). Each point represent the mean $\log_{10}$ survival of bacterial cells at a
261 particular time interval in the presence of the fraction.
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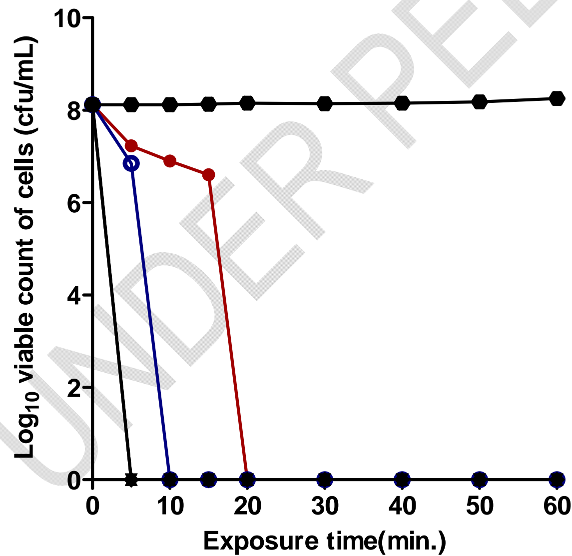

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266 Fig. 5: The rate and extent of kill of *S. aureus* (CI) by ethyl acetate fraction at 1 x MIC (), 2
267 x MIC ( ), 3 x MIC (), Tetracycline ( ), Ampicillin () and Control ( ). Each point
268 represent the mean log10 survival of bacterial cells at a particular time interval in the presence
269 of the fraction.
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272
273 The log of viable count of *S. aureus* (CI) against time (Figure 5) at 1 x MIC (0.16 mg/mL) of
274 the fraction revealed that 10.96 % killing was achieved at 5 minutes, 15.02 % at 10 minutes
275 and 18.72 % at 15 minutes while total elimination was achieved at 20 minutes of exposure, At
276 2 x MIC (0.32 mg/mL) total elimination time dropped to 10 minutes. At 3x MIC (0.48 mg/mL),
277 all the organisms were completely eliminated at the end of 5 minutes. The log of viable count
278 of *C. albicans* against time at different test concentrations (Figure 6) showed that no viable
279 count was observed after exposure to the MIC of the fraction at 15 minutes, while at MIC x 2
280 concentration (0.32 mg/mL.), total elimination time was reduced to 10 minutes. However at a
281 concentration of MIC x 3 (0.48 mg/mL) all the cells were killed at 5 minutes after the exposure
282 of the organism to the fraction. For the standard anti-Candidal agent (Amphotericin B), all the
283 organisms were killed at 5 minutes after exposure to its minimum inhibitory concentration.
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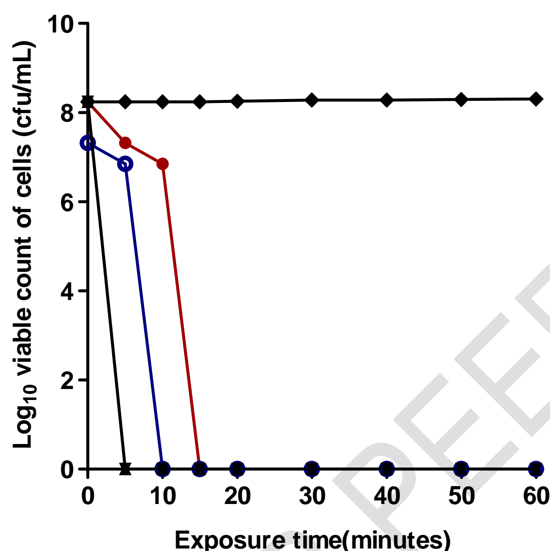

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286
287 Fig. 6: The rate and extent of kill of *C. albicans* (CI) by ethyl acetate fraction at 1 x MIC ( ) 2 x
288 MIC ( ), 3 x MIC (, Amphotericin B ( ) and Control ( ). Each point represent the
289 mean $\log_{10}$ survival of bacterial cells at a particular time interval in the presence of the fraction.
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291
292
293 **4. DISCUSSION**
294
295 The antimicrobial potential of *C. benthamiana* was investigated against some Gram-positive
296 bacteria and *Candida albicans* commonly implicate in human oral infections. The
297 phytochemical property of the plant was also investigated. The root extract of *C. benthamiana*
298 and its chloroform, petroleum spirit, ethyl acetate and aqueous fractions exhibited a high level
299 of activity against the test organisms which include *S. aureus* (NCIB) 8588 and clinical
300 isolates of *S. mutans*, *S. pyogenes*, *S. salivarius, S. aureus and C. albicans* (Table 1) from the
301 human oral cavity. *Streptococcus. mutans*, *S. pyogenes*, *S. salivarius* are found in plaque
302 while *C. albicans* is the causative agent of oral candidiasis. *Streptococcus. salivarius* is also
303 the major causative organism of periodontal disease in children as it is the organism that first
304 colonizes the oral cavity [20]. *Streptococcus. mutans* has also been implicated in gingivitis
305 and dental caries. The action of the partitioned fractions showed a trend of increasing

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activities from petroleum spirit <aqueous <chloroform <ethyl acetate against all the test organisms at the test concentration of 10 mg/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* 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 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 the crude extract and three of the four fractions i.e. chloroform, ethyl acetate and water, showed significantly high activities and that purification with these solvents enhances the activities of the plant extract. It is also 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. The activity of a plant extract is considered significant if the MIC is less than 200mg/ml [21]. In this study, the extract and three of the fractions have exhibited MIC values that are far lower than this value. It is an acceptable fact that a low MIC value of a medicinal plant is an indication of better antimicrobial agent. 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. Majority of the organisms responsible for oral infections originated from the normal flora of the oral cavity. The control of the organisms focuses therefore on either reducing their population or rendering them a virulent. The killing rate therefore becomes necessary in addition to the MIC of the most active fraction in this study.

The bactericidal efficacy of the ethyl acetate fraction as revealed by the killing rate 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), 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 [22]. Thus the bactericidal activity of ethyl acetate fraction obtained from the ethanol root extract of *C. benthamiana* 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* is a good source of

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357 potent antimicrobial agent for the treatment and prevention of oral infections caused by these
358 organisms and can also help in the reduction of dental caries. In addition, it serves as a
359 support for the ethno medical claim of the use of the root as chewing stick for the treatment of
360 tooth pain resulting from oral infections.

361 This study revealed the presence of flavonoids, tannins, terpenes, saponins, phenolics and
362 phenolic glycosides and anthraquinones in the root extract of the plant (Table 3). These
363 phytochemicals are known to have biological activities and hence, might have contributed to
364 the observed activities noted in this study. Flavonoids are known to exhibit a wide range of
365 biological activities including antimicrobial, anti-inflammatory, analgesic, cytostatic and

366

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

368

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

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

371

372 antioxidant properties [23-25]. The ability of flavonoids to scavenge hydroxyl radicals,
373 superoxide anion radicals and lipid peroxyradicals highlights many of its health promoting
374 functions in organisms which are important for the prevention of diseases associated with
375 oxidative damage of membranes, proteins and DNA [26-28]. These conditions can be seen in
376 dental caries, gingivitis, and oral candidiasis to mention a few. Tannins act via a different
377 mechanism to flavonoids. Tannins act by iron deprivation or specific interactions with vital
378 proteins such as enzymes in microbial cells [29]. Motal *et al.*, [30] reviewed the importance of
379 tannins for the treatment of inflamed or ulcerated tissues as seen in gingivitis, caries and
380 plaque. Saponins are considered a key ingredient in traditional Chinese medicine [31].
381 Saponins produce inhibitory effect on inflammation (Just *et al.*, [32]. Phenolic glycosides are
382 an important class of naturally occurring drugs whose actions help in the treatment of
383 congestive heart failure. Plants containing phenolic glycosides are used to treat cardiac
384 infections like endocarditis. Some of the causative organisms of endocarditis e.g. *S. aureus,*
385 *enterococcus spp*. have their origin in the oral cavity. Plants containing phenolic glycosides
386 are also useful in the treatment of chest pains, tooth ache and cough among the "Yoruba"
387 tribe of south western Nigeria [33]. All these observations cited on the action of
388 phytochemicals support the use of *C. benthamiana* root as a traditional remedy for oral
389 diseases as its therapeutic effects can be attributed to the actions of its phytochemical
390 constituents. Such compounds can be exploited for the development of oral antimicrobial drug
391 of natural origin, for the treatment of oral infectious diseases.

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392

393 ## 5. CONCLUSION

394 The result of this work showed that ethanol root extract of *C. benthamiana* demonstrated
395 appreciably high activities on the oral isolates (*S. mutans*, *S. pyogenes*, *S. salivarius*, *S.*
396 *aureus* and *C. albicans*) and the type organism (*S. aureus* NCIB 8588) employed in this study,
397 with the Ethyl acetate fraction being the most active. This provided a scientific basis for the
398 acclaimed traditional use of its root as chewing stick for the maintenance of oral hygiene,
399 prevention of dental caries and the treatment of tooth pain resulting from oral infections. It is
400 interesting to note also, that the ethanol root extract is highly effective against bacteria and
401 fungi (*C. albicans)* alike. This is an added advantage in the activity of this plant. It is
402 recommended that further work on the ethyl acetate fraction of the ethanol root extract of *C.*
403 *benthamiana* be carried out with the hope of developing an effective antimicrobial oral rinse
404 from the plant.

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