

**ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF
DACRYODES EDULIS METHANOLIC LEAF EXTRACT**

Abstract

Dacryodes edulis is used for the treatment of several ailments such as malaria, oral and ear infection. This study was designed to determine the antibacterial and antioxidant potentials of *Dacryodes edulis* methanolic leaf extract as well as its rate of killing on some selected bacterial strains.

The antimicrobial activity of the crude extract obtained was determined against panel of bacteria using agar-well diffusion method. The minimum inhibitory concentrations (MIC), minimum bactericidal concentrations (MBC), phytochemical and antioxidant properties of the crude extract were also studied.

The extract showed appreciable antibacterial activities against *Bacillus polymyxa* (LIO), *Bacillus stearothermophilus* (NCIB 8222), *Citrobacter freundii* (LIO), *Trueperella pyogenes* (LIO), *Enterococcus faecalis* (NCIB 775), *Escherichia coli* (NCIB 86), *Klebsiella pneumoniae* (NCIB 418), *Micrococcus luteus* (NCIB 196), *Pseudomonas aeruginosa* (NCIB 950), *Shigella sp.* (LIO) and *Staphylococcus aureus* (NCIB 8588) at a final concentration of 35 mg/mL. The mean diameter of zones of inhibition exhibited by the extract ranged between 12 mm and 23 mm while conventional Streptomycin and ampicillin diameter of zones of inhibition ranged from 13 mm to 28 mm and 18 mm to 29 mm respectively. The MIC exhibited by the extract against susceptible test organisms ranged between 0.27 mg/mL and 4.375 mg/mL while MBC ranged between 0.55 mg/mL and 17.5 mg/mL. The phytochemical analysis revealed the presence of tannins, flavonoids, saponins and alkaloids. The antioxidant

property result revealed 50% inhibition of the extract at 0.01045 mg/mL while that of the standard (ascorbic acid) was 0.00288 mg/mL. The time kill assay showed that the percentage of the cells killed increased with increasing concentrations of the extract, as well as, contact time intervals.

In conclusion, *Dacryodes edulis* leaf extract exhibited appreciable antibacterial activity against some of the test organisms used for this study and this supports its usefulness in folklore remedies.

Keywords: Antibacterial activity, *Dacryodes edulis*, MIC, MBC, Phytochemical

1. Introduction

Herbal and traditional medicine has been a major aspect of the socio-cultural heritage in Africa for hundreds of years even before the advent of conventional medicine. It was once believed to be primitive and wrongly challenged by foreign religions dating back during the colonial rule in Africa subsequently by the conventional or orthodox medical practitioners [1]. Plant-derived medicines have been part of traditional health care in most parts of the world for thousands of years and there is increasing interest in them as sources in the treatment of diseases [2-4]. Therefore, plants used as medicine is known as medicinal plants [5].

Dacryodes edulis known as “Native Pear”, “African Pear” or “Bush Butter Tree” in English belong to the family of Burseraceae (G. Don). *Dacryodes edulis* is a dioecious plant and it is found natively in Angola, Benin, Cameroon, Central African Republic, Congo, Cote d’Ivoire, Democratic Republic of Congo, Equatorial Guinea, Gabon, Ghana, Liberia, Nigeria, Sierra Leone, Togo and Uganda [6]. It is a medium-sized, evergreen tree attaining a height of 18-40 m in the forest but not exceeding 12 m in plantations. It is generally branched from low

48 down, with a deep, dense crown. The trunk is rather short, slightly fluted, 50-170 cm in
49 diameter and more or less sinuous [6].

50 The extracts from *Dacryodes edulis* have been found to show various biological activities
51 which include antimicrobial, antioxidant and anti-sickling [7]. The leaf sap is instilled into
52 the ear for ear problems and a decoction of the leaves is prepared as a vapour for feverish
53 stiffness with headache [8]. It was reported that the leaves were made into plaster to treat
54 snakebite in Southwest Cameroon [9]. *Dacryodes edulis* is used traditionally medicine to
55 treat ringworm, wound, scabies, skin diseases and inflammation [10].

56 The aim of this study was to screen qualitatively for the chemical groups and also to evaluate
57 the *in vitro* antibacterial and antioxidant properties of the **methanolic** leaf extract of
58 *Dacryodes edulis* from Nigeria.

59 **2. Materials and Methods**

60 **2.1 Microorganisms**

61 Microorganisms used for this study were obtained from Department of Microbiology,
62 Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. These organisms include typed
63 cultures of **National Collection of Industrial Bacteria (NCIB) and Locally Isolated Organisms**
64 **(LIO), some of which were Gram-positive bacteria (*Bacillus subtilis* (NCIB 3610), *Bacillus***
65 ***stearothermophilus* (NCIB 8222), *Bacillus cereus* (NCIB 6344), *Enterococcus faecalis***
66 **(NCIB 775), *Micrococcus luteus* (NCIB 196), *Clostridium sporogenes* (NCIB 582),**
67 ***Staphylococcus aureus* (NCIB 8588), *Bacillus polymyxa* (LIO) and *Trueperella pyogenes***
68 **(LIO)) and some Gram-negative bacteria (*Escherichia coli* (NCIB 86), *Klebsiella***
69 ***pneumoniae* (NCIB 418), *Pseudomonas aeruginosa* (NCIB 950), *Citrobacter freundii* (LIO)**
70 **and *Shigella* sp. (LIO)).**

71 2.2 Preparation of microorganisms used

72 Firstly, the test bacteria used for this experiment were reactivated in nutrient broth (Rapid
73 Labs Ltd) and incubated at 37 °C for 18-24 h in an incubator. After 18 h of incubation, the
74 test organisms were standardized to 0.5 McFarland standard.

75 2.3 Plant specimen

76 The leaf of *Dacryodes edulis* used for this experiment was collected from Opa Area of Ile-Ife,
77 Osun State and identified at Ife Herbarium, with specimen voucher number IFE 17621.

78 2.4 Drying and extraction of leaf of *Dacryodes edulis*

79 *Dacryodes edulis* leaf was dried in hot-air oven at 40 °C until a constant weight of the sample
80 was obtained and later ground into fine powder. Exactly 1000 g of the powdered sample was
81 soaked in methanol and this was left on the laboratory bench for 96 h with occasional
82 agitation during this period. The mixture was later filtered into a sterile flask using glass wool
83 and funnel. The filtrate collected was concentrated *in vacuo* in a rotary evaporator and then
84 lyophilized. The resultant powder was kept in air-tight bottle and stored at 4 °C in the
85 refrigerator for further use [11].

86 2.5 Antibacterial susceptibility testing of the crude extract of leaf of *Dacryodes edulis* 87 against some selected bacterial isolates

88 The crude extract was screened for antibacterial activity using agar-well diffusion method on
89 Muller-Hinton agar medium (Rapid Labs Ltd) as described by [12]. With the aid of an
90 inoculating loop, a loop full of the standardized broth culture of the test organism was added
91 to 18 mL of sterile molten Muller-Hinton agar medium which had already been cooled down
92 to 45 °C. This was well mixed and poured into a sterile Petri dish and allowed to set. The
93 required numbers of wells were bored into the medium using 6 mm sterile cork borer. The

94 wells were made about 5 mm to the edge of the plate and labelled accordingly. The extract
95 was reconstituted into solution (35 mg/mL concentration) with methanol/sterile distilled
96 water at 1:1 (v/v) and was filled up into the wells. Streptomycin and ampicillin were used as
97 positive controls at a concentration of 1 mg/mL. The plates were allowed to stand for one
98 hour and thereafter, the plates were incubated upright at 37 °C for 24 h. The relative
99 susceptibility of the test organisms to the extract as indicated by clear zones of growth
100 inhibition around the wells was measured and recorded in millimetres.

101 **2.6 Determination of minimum inhibitory concentrations (MICs)**

102 The MICs of the extract was done using method described by Akinpelu and Kolawole [13].
103 Two-fold dilutions of the plant extract were prepared and 2 mL of different concentration of
104 the solution were added to 18 mL of pre-sterilized molten nutrient agar (Rapid Labs Ltd) in
105 McCartney bottle at a temperature of 45 °C to a series of concentrations between 0.137
106 mg/mL and 35 mg/mL. The media were then poured into sterile Petri dishes and allowed to
107 set. The surface of the nutrient agar plate was allowed to dry before streaking with
108 standardized 18 h old broth culture of the susceptible bacterial strains. All the plates were
109 then labelled accordingly and incubated at 37 °C for 24 h and then examined for the presence
110 or absence of growth of the test organisms. The lowest concentrations preventing the growth
111 of the test organisms were taken as the minimum inhibitory concentrations (MICs) of the
112 extract.

113 **2.7 Determination of minimum bactericidal concentrations (MBCs)**

114 Samples from plates with no apparent growth in the MIC assay plate were sub-cultured onto
115 freshly prepared sterile nutrient agar plates without extract inclusion and then incubated at 37
116 °C for 48 h. The lowest concentration of the extract that did not show any growth on a new
117 set of nutrient plate was taken as the minimum bactericidal concentration of the extract [14].

118 **2.8 Phytochemical analysis of the leaf extract of *Dacryodes edulis***

119 A small portion of the dried extract was used for phytochemical screening following the
120 methods of Harbourne and Trease and Evans [15-16].

121 **2.8.1 Test for alkaloids**

122 Exactly 0.5 g of the plant extract was dissolved in 5 mL of 1% HCl separately on a steam
123 bath. One millilitre of the filtrate was treated with a few drops of Meyer's reagent. Also,
124 another 1 mL of filtrate was treated again with a few drops of Wagner's reagent. Turbidity or
125 precipitation with these reagents was taken as an evidence for the presence of alkaloids.

126 **2.8.2 Test for tannins**

127 Exactly 0.5 g of the plant extract was stirred with 10 mL sterile distilled water. This was
128 filtered using a sterile filter paper and ferric chloride reagent was added to the filtrate. The
129 presence of blue-black or green precipitate indicates the presence of tannin in the extract.

130 **2.8.3 Test for flavonoids**

131 Exactly 0.1 g of the extract was dissolved in 2 mL of ethylacetate in a test tube. The tube was
132 vigorously shaken to ensure proper dissolution. The solution was decanted into another test
133 tube in order to remove the particles. Few drops of dilute ammonia were then added to the
134 filtrate. Since the liquids were immiscible, there was a formation of an alkaline layer. The test
135 was observed for a light brown colour change of the alkaline layer below the aqueous layer,
136 which indicated the presence of flavonoids.

137 **2.8.4 Test for saponins**

138 Exactly 1.0 g of the extract was dissolved in 5 mL of distilled water. Few drops of Fehling's
139 solution were added to the extract solution. Presence of green colour indicated the presence
140 of saponins.

141 **2.9 Antioxidant activity of leaf extract of *D. edulis***

2.9.1 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The hydrogen radical scavenging properties of the extract from the plant sample was determined by the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) method proposed by Blois [17] as described by Brand-Williams *et al.* [18]. The reaction of DPPH with an antioxidant compound which can donate hydrogen leads to its reduction and there is a colour change from deep violet to light yellow, this change in colour is measured spectrophotometrically at a wavelength of 517 nm.

To 1 mL of varying concentration of the extract sample and ascorbic acid serving as standard was added to 1 mL 0.3 mM DPPH in methanol respectively and allowed to react. The mixture was vortexed and incubated in the dark for 30 min and the absorbance was measured at 517 nm against a DPPH negative control containing 1 mL of methanol in place of the extract sample. The percentage inhibition of the DPPH scavenging activity was calculated using the equation below:

$$\text{DPPH \% Inhibition} = \{1 - (A_{517 \text{ nm sample}} / A_{517 \text{ nm control}})\} \times 100$$

Where;

$A_{517 \text{ nm sample}}$ is the absorbance of the sample (extract/standard) at 517 nm.

$A_{517 \text{ nm control}}$ is absorbance of the negative control at 517 nm.

Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph by plotting inhibition percentage against extract concentration.

2.10 Rate of killing of *D. edulis* methanolic extract

The rate of kill was determined using the method described by Odenholt *et al.* [19] with some modifications. Experiment was performed using the extract on the viability of *Escherichia coli* (NCIB 86) representing Gram-negative and *Staphylococcus aureus* (NCIB 8588) representing Gram-positive organisms. Viable count of the test organisms was initially determined. A 0.5 mL volume of known cell density (by viable counts of 10^6 cfu/mL) from each test organism suspension was added to 4.5 mL of different concentration of the extract. The suspension was thoroughly mixed and held at room temperature (28-30 °C) and the killing rate was determined over a period of 2 h. Exactly 0.5 mL of each suspension was withdrawn at the appropriate time intervals and transferred to 4.5 mL nutrient broth recovery medium containing 3% “Tween 80” to neutralize the carry-over effects of the antimicrobial compounds from the test suspensions. The suspension was shaken properly then serially diluted up to 10^{-5} in sterile physiological saline. Exactly 0.5 mL of the final dilution of the test organism was transfer into pre-sterile nutrient agar at 45 °C and plated out. The plates were allowed to set and incubated upside down at 37 °C for 72 h. Control experiment which was set up without the inclusion of antimicrobial agent (i.e extract). Viable counts were made in triplicates for each sample. Depression in the viable counts indicated killing by the antimicrobial agent.

3. Results and Discussion

3.1 Results

The extract obtained from *D. edulis* leaves was brownish yellow in colour with a yield of 62 g of extract from 1000 g of the powdered leaves of *D. edulis* that was soaked.

The extract of *D. edulis* at a concentration of 35 mg/mL was found to be active against eleven out of fourteen of the test bacterial isolates used for this study with varying level of activity (Table 1).

186 *Micrococcus luteus*, *Bacillus polymyxa*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella*
187 *pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella* sp., *Bacillus*
188 *stearothermophilus*, *Citrobacter freundii* and *Trueperella pyogenes* were susceptible to the
189 extract. The zones of inhibition exhibited by the extract against the test organisms ranged
190 between 12 mm and 23 mm. *Bacillus polymyxa*, *Micrococcus luteus*, *Pseudomonas*
191 *aeruginosa* and *Staphylococcus aureus* showed the lowest zone of inhibition of 12 mm while
192 *Klebsiella pneumoniae* showed the highest zone of inhibition with 23 mm. The MIC
193 exhibited by the extract against *Pseudomonas aeruginosa*, *Shigella* sp. and *Klebsiella*
194 *pneumoniae* was 0.27 mg/mL, *Bacillus stearothermophilus* was 0.55 mg/mL, while that of
195 *Enterococcus faecalis* was 1.09 mg/mL (Table 2). On the other hand, MIC exhibited against
196 *Bacillus polymyxa*, *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli*, *Citrobacter*
197 *freundii* was 2.19 mg/mL while *Trueperella pyogenes* exhibited 4.375 mg/mL (Table 2).

198 **Table 1: Antibacterial activity of *D. edulis* leaf extract against some selected bacterial**
199 **isolates**

Microorganisms	Zones of Inhibition (mm)*			
	<i>D. edulis</i> (35 mg/mL)	Strep (1 mg/mL)	Amp (1 mg/mL)	M/W (1:1) (v/v)
<i>Enterococcus faecalis</i> (NCIB 775)	14	19	18	0
<i>Escherichia coli</i> (NCIB 86)	16	0	26	0
<i>Bacillus polymyxa</i> (LIO)	12	23	0	0
<i>Micrococcus luteus</i> (NCIB 196)	12	14	28	0
<i>Pseudomonas aeruginosa</i> (NCIB 950)	12	31	0	0
<i>Staphylococcus aureus</i> (NCIB 8588)	12	15	23	0
<i>Klebsiella pneumoniae</i> (NCIB 418)	23	21	0	0
<i>Shigella</i> sp. (LIO)	13	13	20	0
<i>Bacillus stearothermophilus</i> (NCIB 8222)	14	20	28	0
<i>Citrobacter freundii</i> (LIO)	13	0	22	0
<i>Trueperella pyogenes</i> (LIO)	19	21	27	0

<i>Bacillus cereus</i> (NCIB 6344)	0	13	0	0
<i>Clostridium sporogenes</i> (NCIB 582)	0	0	29	0
<i>Bacillus subtilis</i> (NCIB 3610)	0	28	0	0

200 Keys:

201 mm*: Mean of three replicates.

202 *D. edulis*: *Dacryodes edulis*

203 Strep: Streptomycin

204 Amp: Ampicillin

205 LIO: Locally Isolated Organism.

206 NCIB: National Collection for Industrial Bacteria.

207 M/W: Methanol/Sterile distilled water.

208 **Table 2: Minimum inhibitory concentration (MIC) and Minimum bactericidal**
 209 **concentration (MBC) exhibited by *D. edulis* methanolic leaf extract against**
 210 **susceptible bacterial test organisms**

Microorganisms	MIC (mg/mL)	MBC (mg/mL)
<i>Enterococcus faecalis</i> (NCIB 775)	1.09	2.19
<i>Escherichia coli</i> (NCIB 86)	2.19	4.375
<i>Bacillus polymyxa</i> (LIO)	2.19	8.75
<i>Micrococcus luteus</i> (NCIB 196)	2.19	8.75
<i>Pseudomonas aeruginosa</i> (NCIB 950)	0.27	0.55
<i>Staphylococcus aureus</i> (NCIB 8588)	2.19	4.375
<i>Klebsiella pneumoniae</i> (NCIB 418)	0.27	0.55
<i>Shigella</i> sp. (LIO)	0.27	0.55
<i>Bacillus stearothermophilus</i> (NCIB 8222)	0.55	2.19
<i>Citrobacter freundii</i> (LIO)	2.19	4.375
<i>Trueperella pyogenes</i> (LIO)	4.375	17.5

211

212

Table 2 shows the minimum bactericidal concentration of the leaf of *Dacryodes edulis* against bacterial isolates. The MBC exhibited by the extract against *Pseudomonas aeruginosa*, *Shigella* sp. and *Klebsiella pneumoniae* was 0.55 mg/mL, *Bacillus stearothermophilus* and *Enterococcus faecalis* was 2.19 mg/mL, while that of *Staphylococcus aureus* and *Citrobacter freundii* was 4.375 mg/mL. On the other hand, MBC exhibited against *Bacillus polymyxa* and *Micrococcus luteus* was 8.75 mg/mL. Finally, the MBC exhibited against *Escherichia coli* and *Trueperella pyogenes* was 17.5 mg/mL.

The phytochemical analysis of the leaf extract of *D. edulis* revealed the presence of alkaloids, saponins, flavonoids and tannins as presented in Table 3 below.

Table 3: Phytochemical components revealed by *D. edulis* leaf extract

Phytochemicals	Results
Alkaloids	Positive
Flavonoids	Positive
Tannins	Positive
Saponins	Positive

Figures 1a and 1b show result of the antioxidant property of the extract at different concentrations in addition to comparison with the standard (ascorbic acid) at initial concentration of 5 mg/mL. The 50% inhibition concentration for the extract was observed at 0.01045 mg/mL while that of the standard (ascorbic acid) was at 0.00288 mg/mL.

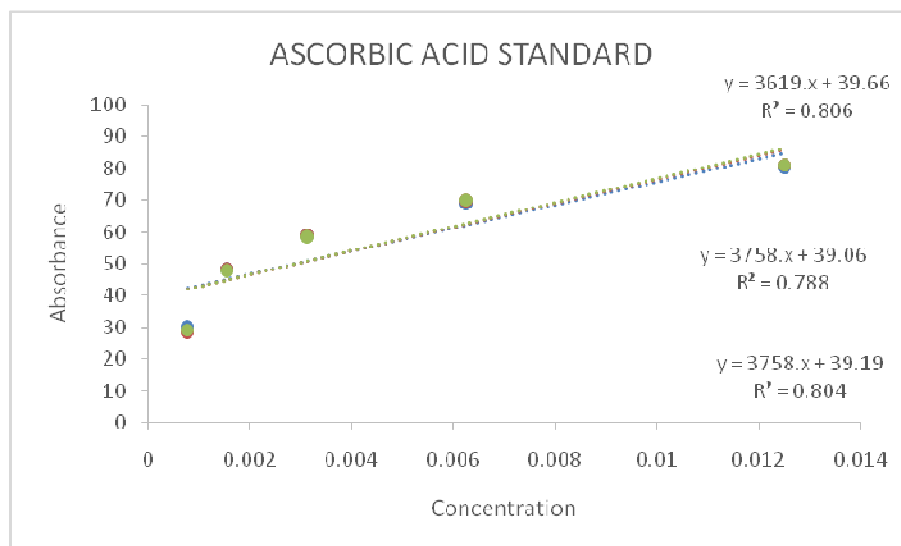


Figure 1a: The graph of concentration against absorbance for the standard (ascorbic acid).

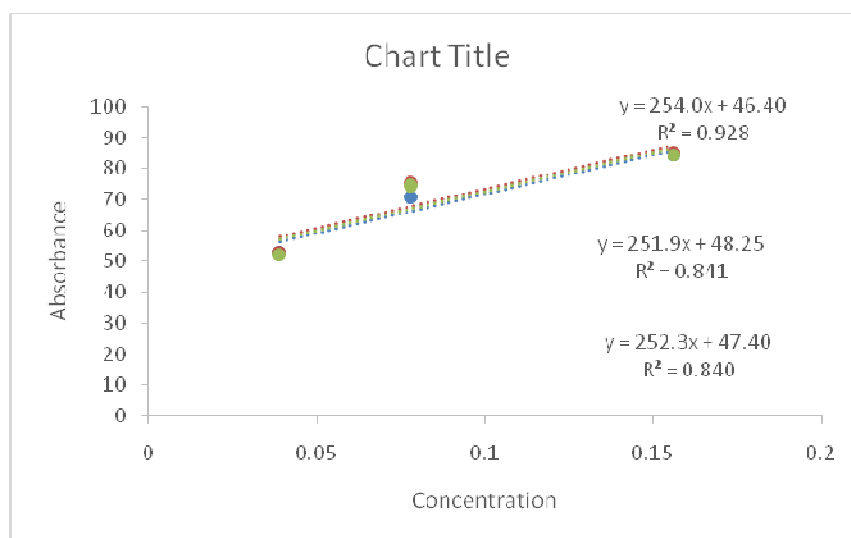


Figure 1b: The graph of concentration against absorbance for *D. edulis* extract.

The extent and rate of killing of *E. coli* by methanolic extract at 1 x MIC, 2 x MIC and 3 x MIC concentrations are as shown in Figure 2. The percentage of the organisms killed by the extract at 1 x MIC in 15 minutes was 6.2% while the percentage of the cells killed at 30 minutes rose to 10.7%. After 60 minutes of contact time with this extract, the percentage of the organisms killed was 21.9%. When the contact time was later increased to 90 minutes,

34.7% of the test organisms were killed and this rose to 48.3% after 120 minutes of contact time.

When the MIC of the extract was doubled, the percentage of the cells killed was 20.3% at 15 minutes of contact time. At 30 minutes of contact time, 39.2% of the test organisms were killed and when the contact time was increased to 60 minutes, the percentage of the cells killed was 45.8%. When the contact was increased to 90 minutes, the percentage of the organisms killed has increased to 59.6%, while it rose to 67.9% after 120 minutes of contact time. The extent and rate of killing of the organism by the extract at 3 x MIC followed the same trend as the concentrations of the extract increased with increase in time, the percentage of the organisms killed also increased.

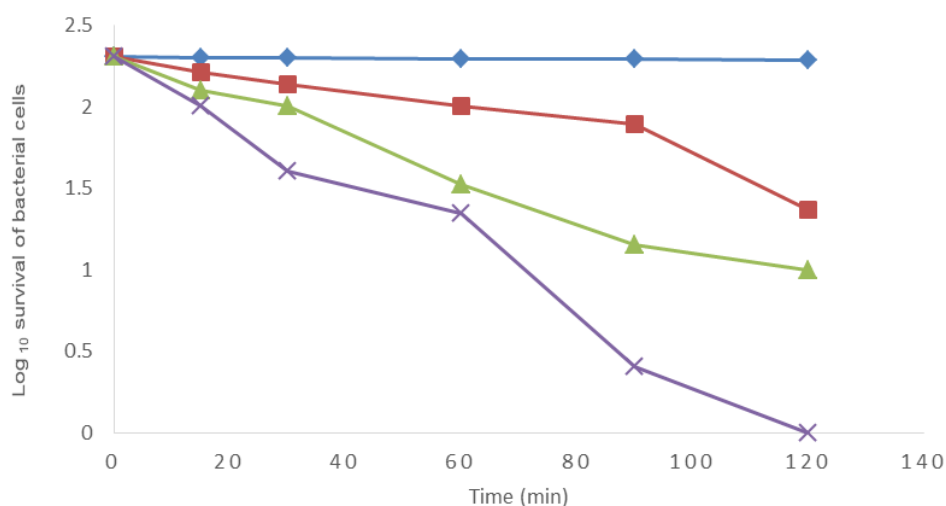


Figure 2: The extent and rate of killing of *E. coli* cells by methanolic crude extract at 1 x MIC (—■—), 2 x MIC (—▲—), 3 x MIC (—×—) and control (—◆—).

Each point represents the $\log_{10}$ survival of bacterial cells at a particular time interval in the presence of the extract.

The extent and rate of killing of *S. aureus* by methanolic extract at 1 x MIC, 2 x MIC and 3 x MIC concentrations are as shown in Figure 3. The percentage of the organisms killed by the

extract at 1 x MIC in 15 minutes was 9.1% while the percentage of the cells killed at 30 minutes rose to 17.5%. After 60 minutes of contact time with this extract, the percentage of the organisms killed was 29.3%. When the contact time was later increased to 90 minutes, the percentage of the cells killed was 35.7% and this rose to 53.4% after 120 minutes of contact time. When the MIC of the extract was doubled, the percentage of cells killed was 21.4% at 15 minutes of contact time. At 30 minutes of contact time, the percentage of cells killed increased to 35.3%; and when the contact increased to 60 minutes, the cells killed was 52.8%. At 90 minutes contact time, the percentage of the organisms killed has increased to 74.7% while it rose to 100.0% after 120 minutes of contact time. The extent and rate of killing of the extract at 3 x MIC followed the same trend with the previous tests.

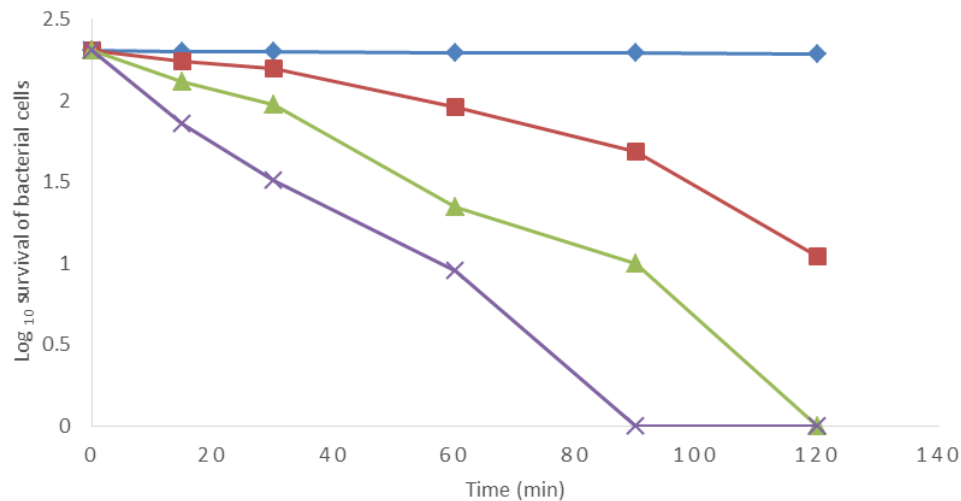


Figure 3: The extent and rate of killing of *S. aureus* cells by methanolic crude extract at 1 x MIC (—■—), 2 x MIC (—▲—), 3 x MIC (—×—) and control (—◆—).

Each point represents the log₁₀ survival of bacterial cells at a particular time interval in the presence of the extract.

3.2 Discussion

The antibacterial activity of the leaf extract of *Dacryodes edulis* was determined against fourteen microorganism comprising of both Gram positive and Gram negative bacteria. The result of this investigations shows that the extract exhibited *in vitro* antibacterial activity against eleven out of fourteen of the tested bacterial isolates at a final concentration of 35 mg/mL (Table 1). The test organisms inhibited by the extract were *E. coli*, *B. polymyxa*, *E. faecalis*, *P. aeruginosa*, *S. aureus*, *Shigella* sp., *B. stearothermophilus*, *K. pneumoniae*, *C. freundii*, *M. luteus* and *T. pyogenes* (Table 1). The zones of inhibitions exhibited by the extract against the susceptible bacteria ranged between 12 mm and 23 mm while the zone of inhibition exhibited by streptomycin against the bacterial isolates ranged between 13 mm and 28 mm and that of ampicillin was between 18 mm and 29 mm (Table 1).

The value of the minimum inhibitory concentrations is usually and adequate guide for the treatment of most infections [14]. Methanolic leaf extract showed higher activity against *S. aureus* with MIC value of 2.19 mg/mL as compared to 18.75 mg/mL reported by Riwoom *et al.* [20] for the same microorganism. The highest activity as determined by MIC was observed against *Shigella* sp., *P. aeruginosa* and *K. pneumoniae*. *Shigella* sp. had MIC of 0.27 mg/ml which was far lower than the value obtained by Riwoon *et al.* [20] for this microorganism.

The phytochemical analysis of the leaf extracts of *D. edulis* revealed the presence of alkaloids, flavonoids, tannins and saponins (Table 3). These phytochemical compounds are known to be biologically active and thus contributed to the antibacterial and antioxidant properties of *D. edulis*. Studies have shown that saponins although non-toxic can generate adverse physiological responses in animal that consume them. They exhibit cytotoxic effect and growth inhibition against a variety of cell which make them have anti-inflammatory and anti-cancer properties. They also show tumour inhibiting activity in animals [21]. Alkaloid

which was revealed in the extract of *Dacryodes edulis* have been widely studied for their potential use in elimination of human cancer cell lines [22]. Pure isolate alkaloids and their synthetic derivatives have been used as analgesic, anti-spasmodic and bactericidal agent [23]. Flavonoid also detected in the leaf of *Dacryodes edulis* are known to possess antibacterial, anti-inflammatory, anti-allergic, anti-mutagenic, antiviral, anti-thrombotic, vasodilatory and anti-oxidant properties [24]. Flavonoids ability of scavenging hydroxyl radicals, super oxide anions and lipid peroxyradicals highlight many of the flavonoids health promoting functions in organisms, which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA [25]. Flavonoids in human diet may reduce risk of various cancer as well as preventing menopause symptoms [24]. Tannins present in the *Dacryodes edulis* have been studied to be one of the major phytochemical found in many higher plants. Tannins have a characteristic strange smell and bitter taste. Tannins could bind to proteins through the effective formation of strong complexes with proteins and other macromolecules. Thus, they could have a major impact on animal nutrition, including inhibition of growth rate digestive enzymes [26]. Tannins have been implicated with various pharmaco-therapeutic effects [27]. Tannins are useful as an anti-inflammatory agent and in the treatment of burns and other wounds based on their anti-hemorrhagic and antiseptic potentials [28]. Tannins-rich remedies are used as antihelminthics, antioxidants, antimicrobials, antivirals, in cancer chemotherapy and to chelate dietary iron [29-32]. Results observed in this study support the usefulness of *Dacryodes edulis* in folklore remedies.

The result of the antioxidant property test of the leaf of *Dacryodes edulis* showed that the inhibition of DPPH scavenging radicals increases with increasing concentrations of the extract (Figure 2). This is an indication that this extract is rich in flavonoids which is an anti-stress and anti-ageing. *Dacryodes edulis* may be useful in production of anti-ageing drugs.

320 And it has been reported that *D. edulis* is rich in vitamins A, B and E which are useful against
321 skin wrinkling [33]. The results of the findings support the usefulness of *D. edulis* in folklore
322 remedies.

323 *Klebsiella pneumoniae*, *Citrobacter freundii*, *Shigella* sp., *Enterococcus faecalis* are
324 pathogens that are responsible for various ailment in man. These pathogens were susceptible
325 to the leaf of *Dacryodes edulis*. This is an indication that drugs formulated from this plant
326 may be useful in eradicating diseases caused by these pathogens.

327 The leaf extract of *Dacryodes edulis*, compared favourably with streptomycin and/or
328 ampicillin used as standard control in this work. For example, the zone of inhibition exhibited
329 against *Enterococcus faecalis* was 14 mm for the extract and 19 mm, 18 mm for streptomycin
330 and ampicillin respectively. Also, *Micrococcus luteus* showed a zone of 12 mm relative to the
331 streptomycin standard, which showed a zone of 14mm and lastly, *Trueperella pyogenes* with
332 a zone of 19 mm relative to both streptomycin and ampicillin standard which showed zones
333 of 21 mm and 27 mm respectively. In some cases where the streptomycin and ampicillin
334 standard did not show any activity, the extract did. For example, where the streptomycin
335 showed no inhibition against *E. coli* but the extract inhibited the growth of this organism.
336 Again, ampicillin was not able to inhibit the growth of *Pseudomonas aeruginosa* but the
337 extract did.

338 The test organisms comprised of both Gram positive and Gram negative and the extract was
339 sensitivity against both groups which indicates that *D. edulis* leaf extract has a broad
340 spectrum activity. Drug formulated from this plant extract may go a long way in combating
341 wide range of these pathogenic bacteria.

The extract exhibited appreciable kill rate against *S. aureus* and *E. coli*. As the concentrations of the extract increased with increase in contact time, there appears to be an increase in populations of test bacteria killed (Figures 2 - 3). This is an indication of monophasic effect exhibited by the extract which was similar to the effect reported by Akinpelu *et al.* (2008)[34]. The ability of this extract to eliminate the test organisms within the shortest time is an indication that such extract can be used for the production of antimicrobial compound for the treatment of infections caused by these pathogens.

4.0 Conclusions

The leaf extract of *Dacryodes edulis* was found to possess broad spectrum antibacterial activity. The leaf extract of *Dacryodes edulis* found to contain flavonoids, alkaloids, saponins and tannins which have been confirmed to possess antimicrobial activities, has hence supported the reason for the antibacterial and antioxidant efficacies of this plant.

References

1. Okigbo RN, Mmeka EC. Antimicrobial Effects of Three Tropical Plant Extracts on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. *Afr. J. Tradit. Complement Altern. Med.* 2008;5(3):226-9.
2. Ajayi AO, Akintola TA. Evaluation of antimicrobial activity of some medicinal plants on common enteric food-borne pathogens. *Afr. J. Microbiol. Res.* 2010;4(4):314-6.
3. Mohana DC, Satish S, Raveesha KA. Antibacterial evaluation of some plant extracts against some human pathogenic bacteria. *Adv Biol Res.* 2008;2(3-4):49-55.
4. Ghaleb MA, Bassam AA, Kamel MA. *In vitro* activity of certain drugs in combination with plant extracts against *Staphylococcus aureus* infections. *Afr. J. Biotechnol.* 2009;8(17):4239-41.

- 365 5. Ojokuku SA, Okunowo WO, Apena A. Evaluation of chemical composition of *Khaya*
366 *grandifoliola* and *Ficus capensis*. *J. Med. Plants Res.* 2010;4(12):1126-29.
- 367 6. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. Agroforestry Database: a tree
368 reference and selection guide version. 2009;4:1-7.
- 369 7. Okunomo K, Egbo EO. Economic importance of some underexploited tree species in
370 Nigeria: Urgent need for separate research centres. *CJBiolSci.* 2010;3:16-32.
- 371 8. Nwokonkwo DC. The phytochemicals and antibacterial activities of the seed extract of
372 *Dacryodes edulis*. *Am J Sci Ind Res.* 2014;5(1):7-12.
- 373 9. Jiofack T, Fokunang C, Guedje N, Kumeuze V, Fongnzossie E, Nkongmeneck BA,
374 Mapongmetsem PM, Tsabang N. Ethnobotanical uses of medicinal plants of two
375 ethnoecological regions of Cameroon. *Int. J. Med. Med. Sci.* 2010;2(3):60-79.
- 376 10. Okwu DE, Nnamdi FU. Evaluation of the chemical composition of *Dacryodes edulis* and
377 *Raphia hookeri* mann Exudates used in herbal medicine in South Eastern Nigeria. *Afr.*
378 *J. Tradit. Complement. Altern. Med.* 2008;5(2):194-200.
- 379 11. Abioye OE, Akinpelu DA, Okoh AI. Synergistic Effects of n-Hexane Fraction of *Parkia*
380 *biglobosa* (Jacq.) bark extract and selected antibiotics on bacterial isolates.
381 *Sustainability.* 2017;7:228-43.
- 382 12. Akinpelu DA. Antimicrobial activities of *Vernonia amygdalina* leaves. *Fitoterapia.*
383 1999;70(4):32-434.
- 384 13. Akinpelu DA, Kolawole DO. Phytochemical and Antimicrobial activity of leaf extract of
385 *Piliostigma thonningii* (Schum). *Sci. Focus J.* 2004;7:64-70.

- 386 14. Olorundare EE, Emudianughe TS, Khaar GS, Kuteyi SA, Irobi DN. Antibacterial
387 properties of leaf extract of *Cassia alata*. Bio. Res. Com. 1992;4:113-117.
- 388 15. Trease GE, Evans WC. Pharmacognosy. 15th ed. Saunders; 2002.
- 389 16. Harbourne JB. Phytochemical methods: A guide to modern techniques of plant analysis.
390 Chapman and Hall: London; 1998.
- 391 17. Blois MS. Antioxidant determinations by the use of a stable free radical. **Nature**.
392 1958;181:1199-1200.
- 393 18. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate
394 antioxidant activity. Food Sci. Tech. 1995;28(1):25-30.
- 395 19. Odenholt I, Lowdin E, Cars O. Pharmacodynamics of telithromycin *in vitro* against
396 respiratory tract pathogens. **Antimicrob. Agents Chemother.** 2001;45(1):23-29.
- 397 20. Riwoom SH, M-C Ndoye Foe F, Nyegue MA, Ambewanki R, Voundi SO, Etoa F.
398 Chemical Composition and in vitro Antibacterial Activity of the Essential Oils of the
399 Leaves, Resin and Stem-Barks of *Dacryodes edulis* (G. Don) H. J Lam growing in
400 Cameroon on Diarrhoea Associated Strains. J. Appl. Pharma. Sci. 2015;5(10):6-11.
- 401 21. Just MJ, Recsio MC, Giner RM, Cuellar MJ, Marez S, Bilia AR, Rios J. Anti-
402 inflammatory activity of usual Lupane saponins from *Bulerum fruticescens*. **Plant**
403 **Med.** 2008;64(5):404-7.
- 404 22. Nobori T, Miurak K, Wu DJ, Takabayashik LA, Carson DA. Deletion of the cyclin-
405 dependent kinase-4 inhibitor gene in multiple human cancers. **Nature**.
406 1994;368(6473):753-6.

- 407 23. Okwu DE, M. E. Okwu ME. Chemical composition of *Spondias mombin* linn plant parts.
408 *J. Sustain. Agric Environ*. 2004;6(2):140-7.
- 409 24. Hodek P, Trefil P, Stiborova M. Flavonoids – Potent and versatile biologically active
410 compounds interacting with cytochrome P450. *Chem. Biol. Interact*. 2002;139(1):1-
411 21.
- 412 25. Ferguson LR. Role of plant polyphenols in genomic stability. *Mutat. Res*. 2001;475:89-
413 111.
- 414 26. Bennick A. Interaction of plant polyphenols with salivary proteins. *Crit Rev Oral Biol*
415 *Med*. 2002;13(2):184-196.
- 416 27. Ferreira D, Gross GG, Hangerman AE, Kolodziej H, Yoshida T. Tannins and related
417 polyphenols: Perspectives on their chemistry, biology, ecological effects and human
418 health protection. *Phytochemistry*. 2008;69:3006-8.
- 419 28. Pius OU, Egbuonu ACC, Obasi LN, Ejikeme PM. Tannins and other phytochemical of
420 the *Samanea saman* pods and their antimicrobial activities. *Afr. J. Pure Appl. Chem*.
421 2011;5(8):237-44.
- 422 29. Ketzis JK, Vercruysse J, Stromberg BE, Larsen M, Athanasiadou S, Houdijk JG.
423 Evaluation of efficacy expectations for novel and non-chemical helminth control
424 strategies in ruminants. *Vet. Parasitol*. 2006;139:321-35.
- 425 30. Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review.
426 *Crit. Rev. Food Sci. Nutr*. 1998;38(6):421-64.

- 427 31. Buzzini P, Arapitsas P, Goretti M, Branda E, Turchetti B, Pinelli P, Leri F, Romani A.
428 Antimicrobial and antiviral activity of hydrolysable tannins. *Mini. Rev. Med. Chem.*
429 2008;8:1179-87.
- 430 32. Kolečkar V, Kubikova K, Rehakova Z, Kuca K, Jun D, Jahodar L, Opletal L. Condensed
431 and hydrolysable tannins as antioxidants influencing the health. *Mini. Rev. Med.*
432 *Chem.* 2008;8:436-47.
- 433 33. Ogunwusi AA, Ibrahim HD. Economic significance of Avocado Pear in Nigeria. *J. Dev.*
434 *Country Studies.* 2016;6(3):13-22.
- 435 34. Akinpelu DA, Aiyegoro OA, Okoh A. *In vitro* antimicrobial and phytochemical
436 properties of crude extract of stem bark of *Azizelia africana* (Smith). *Afr. J.*
437 *Biotechnol.* 2008;7:3665-3670.