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Antibacterial and Antioxidant Activities of *Dacryodes edulis* Methanolic Leaf Extract

Olasunkanmi Oluwaseun Oyetunji[1*] and Adeniyi Paul Opeyemi[1]

[1]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author OOO designed and carried out the study and performed the statistical analysis of the manuscript. Author APO carried out the study, managed the analyses of the study, managed the literature searches and wrote the first draft and the protocol. Both authors read and approved the final manuscript.

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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 activity 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 12.0±0.0 mm and 23.3±1.2 mm while conventional streptomycin and ampicillin diameter of zones of inhibition ranged from 13.0±0.0 mm

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

– 31.0±1.0 mm and 18.3±0.6 mm - 29.3±1.5 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, *D. edulis* methanolic leaf extract consisted of various phytochemical compounds which accounted for the appreciable antioxidant activity as well as the antibacterial activity exhibited against some of the test organisms used for this study and this supports its usefulness in folklore remedies.

Keywords: Antibacterial activity; antioxidant 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. 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 down, with a deep, dense crown. The trunk is rather short, slightly fluted, 50-170 cm in diameter and more or less sinuous [6].

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

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

2. MATERIALS AND METHODS

2.1 Microorganisms

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

2.2 Preparation of Microorganisms Used

Firstly, five colonies of each test bacteria used for this experiment were picked into nutrient broth (Rapid Labs Ltd) and incubated at 37℃ for 18-24 h in an incubator. After 18 h of incubation, the test organisms were standardized to 0.5 McFarland standard [11].

2.3 Plant Specimen

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

2.4 Drying and Extraction of Leaf of *Dacryodes edulis*

Dacryodes edulis leaf was dried in hot-air oven at 40℃ until a constant weight of the sample was obtained and later ground into fine powder. Exactly 1000 g of the powered sample was soaked in methanol and this was left on the laboratory bench for 96 h with occasional agitation during this period. The mixture was later filtered into a sterile flask using glass wool and funnel. The filtrate collected was concentrated *in vacuo* in a rotary evaporator and then lyophilized. The resultant powder was kept in air-tight bottle and stored at 4℃ in the refrigerator for further use [12].

2.5 Antibacterial Susceptibility Testing of the Crude Extract of Leaf of *Dacryodes edulis* against Some Selected Bacterial Isolates

The crude extract was screened for antibacterial activity using agar-well diffusion method on Muller-Hinton agar medium (Rapid Labs Ltd) as described by Akinpelu [13]. With the aid of an inoculating loop, a loop full of the standardized broth culture of the test organism was added to 18 mL of sterile molten Muller-Hinton agar medium which had already been cooled down to 45℃. This was well mixed and poured into a sterile Petri dish and allowed to set. The required numbers of wells were bored into the medium using 6 mm sterile cork borer. The wells were made about 5 mm to the edge of the plate and labelled accordingly. The extract was reconstituted into solution (35 mg/mL concentration) with methanol (95% v/v)/sterile distilled water at 1:1 (v/v) and was filled up into the wells. Streptomycin and ampicillin were used as positive controls at a concentration of 1 mg/mL. The plates were allowed to stand for one hour and thereafter, the plates were incubated upright at 37℃ for 24 h. The relative susceptibility of the test organisms to the extract as indicated by clear zones of growth inhibition around the wells was measured and recorded in millimetres.

2.6 Determination of Minimum Inhibitory Concentrations (MICs)

The MICs of the extract was done using method described by Akinpelu and Kolawole [14]. Two-fold dilutions of the plant extract were prepared and 2 mL of different concentration of the solution were added to 18 mL of pre-sterilized molten nutrient agar (Rapid Labs Ltd) in McCartney bottle at a temperature of 45℃ to a series of concentrations between 0.137 mg/mL and 35 mg/mL. The media were then poured into sterile Petri dishes and allowed to set. The surface of the nutrient agar plate was allowed to dry before streaking with standardized 18 h old broth culture of the susceptible bacterial strains. All the plates were then labelled accordingly and incubated at 37℃ for 24 h and then examined for the presence or absence of growth of the test organisms. The lowest concentrations preventing the growth of the test organisms were taken as the minimum inhibitory concentrations (MICs) of the extract.

2.7 Determination of Minimum Bactericidal Concentrations (MBCs)

Samples from plates with no apparent growth in the MIC assay plate were sub-cultured onto freshly prepared sterile nutrient agar plates without extract inclusion and then incubated at 37℃ for 48 h. The lowest concentration of the extract that did not show any growth on a new set of nutrient plate was taken as the minimum bactericidal concentration of the extract [15].

2.8 Phytochemical Analysis of the Methanolic Leaf Extract of *Dacryodes edulis*

A small portion of the dried extract was used for phytochemical screening following the methods of Harbourne and Trease and Evans [16-17].

2.8.1 Test for alkaloids

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

2.8.2 Test for tannins

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

2.8.3 Test for flavonoids

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

2.8.4 Test for saponins

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

2.9 Antioxidant Activity of Methanolic 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 [18] as described by Brand-Williams et al*.* [19]. 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:

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

Where;

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

$A_{517\ nm\ control}$ 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 Leaf Extract

The rate of kill was determined using the method described by Odenholt et al*.* [20] with some modifications. Experiment was performed using the extract on the viability of *E. coli* (NCIB 86) representing Gram-negative and *S. 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℃) 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 45ºC and plated out. The plates were allowed to set and incubated upside down at 37ºC for 72 h. Control experiment which was set up without the inclusion of antimicrobial agent (i.e every other things except the extract. The extract was reconstituted with methanol and sterile distilled water at 1:1). 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).

Micrococcus luteus, B. polymxa, E. faecalis, E. coli, K. pneumoniae, S. aureus, P. aeruginosa, Shigella sp*., B. stearothermophilus, C. freundii* and *T. pyogenes* were susceptible to the extract. The zones of inhibition exhibited by the extract against the test organisms ranged between 12 mm and 23 mm. *Bacillus polymyxa, Micrococcus luteus, Pseudomonas aeruginosa* and *Staphylococcus aureus* showed the lowest zone of inhibition of 12 mm while *Klebsiella pneumoniae* showed the highest zone of inhibition with 23 mm. The MIC exhibited by the extract against *Pseudomonas aeruginosa, Shigella* sp*.* and *Klebsiella pneumoniae* was 0.27 mg/mL, *Bacillus stearothermophilus* was 0.55 mg/mL, while that of *Enterococcus faecalis* was 1.09 mg/mL (Table 2). On the other hand, MIC exhibited against *Bacillus polymyxa, Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Citrobacter freundii* was 2.19 mg/mL while *Trueperella pyogenes* exhibited 4.375 mg/mL (Table 2).

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

Microorganisms	Zones of inhibition (mm)*			
	***D. edulis* (35 mg/mL)**	**Strep (1 mg/mL)**	**Amp (1 mg/mL)**	**M/W (1:1) (v/v)**
Enterococcus faecalis (NCIB 775)	14.0±1.0	19.0±0.0	18.3±0.6	0
Escherichia coli (NCIB 86)	16.3±1.5	0	26.0±1.0	0
Bacillus polymyxa (LIO)	12.0±0.0	23.3±0.7	0	0
Micrococcus luteus (NCIB 196)	12.3±0.7	14.0±0.0	28.3±1.2	0
Pseudomonas aeruginosa (NCIB 950)	12.0±0.0	31.0±1.0	0	0
Staphylococcus aureus (NCIB 8588)	12.3±0.7	15.3±0.6	23.3±1.5	0
Klebsiella pneumoniae (NCIB 418)	23.3±1.2	21.0±1.0	0	0
Shigella sp. (LIO)	13.0±1.0	13.0±0.0	20.0±1.0	0
Bacillus stearothermophilus (NCIB 8222)	14.3±0.6	20.0±0.0	28.3±0.7	0
Citrobacter freundii (LIO)	12.7±1.7	0	22.0±1.0	0
Trueperella pyogenes (LIO)	19.3±0.7	21.3±0.6	27.3±0.6	0
Bacillus cereus (NCIB 6344)	0	13.3±0.7	0	0
Clostridium sporogenes (NCIB 582)	0	0	29.3±1.5	0
Bacillus subtilis (NCIB 3610)	0	28.3±0.7	0	0

Keys: mm: Mean of three replicates, D. edulis: Dacryodes edulis, Strep: Streptomycin, Amp: Ampicillin, LIO: Locally Isolated Organism, NCIB: National Collection for Industrial Bacteria, M/W: Methanol/Sterile distilled water*

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

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

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.

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

Phytochemicals	Results
Alkaloids	Positive
Flavonoids	Positive
Tannins	Positive
Saponins	Positive

Fig. 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.

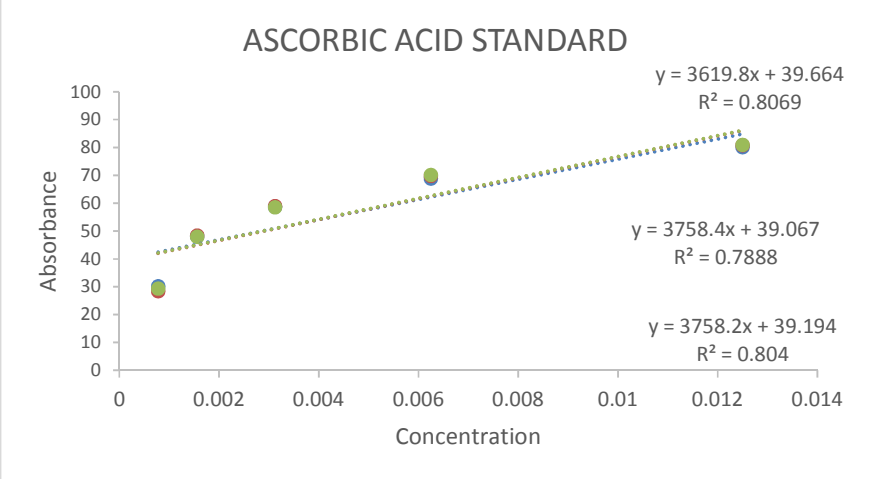

Fig. 1a. The graph of concentration against absorbance for the standard (ascorbic acid)

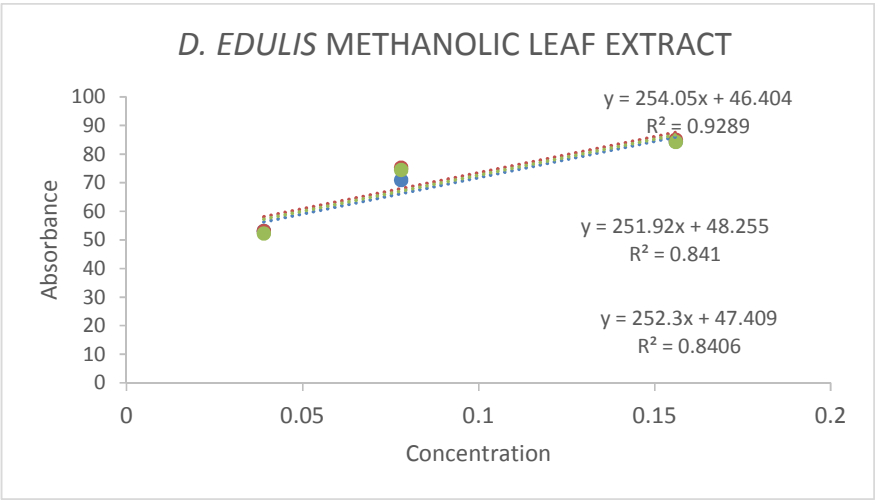

Fig. 1b. The graph of concentration against absorbance for *D. edulis* methanolic leaf extract

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 Fig. 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.

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 Fig. 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.

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

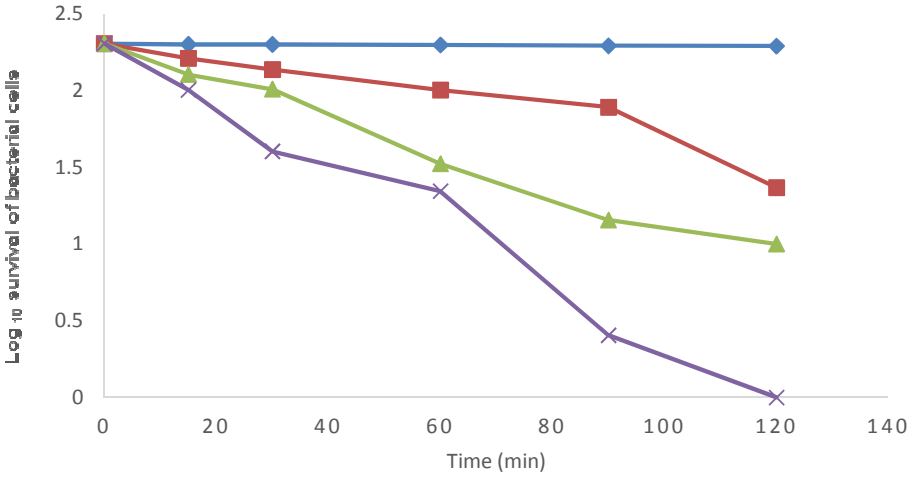

Fig. 2. The extent and rate of killing of *E. coli* cells by methanolic crude extract at 1 x MIC (—■—), 2 x MIC (—▲—), 3 x MIC (—×—) and control (—◆—)

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

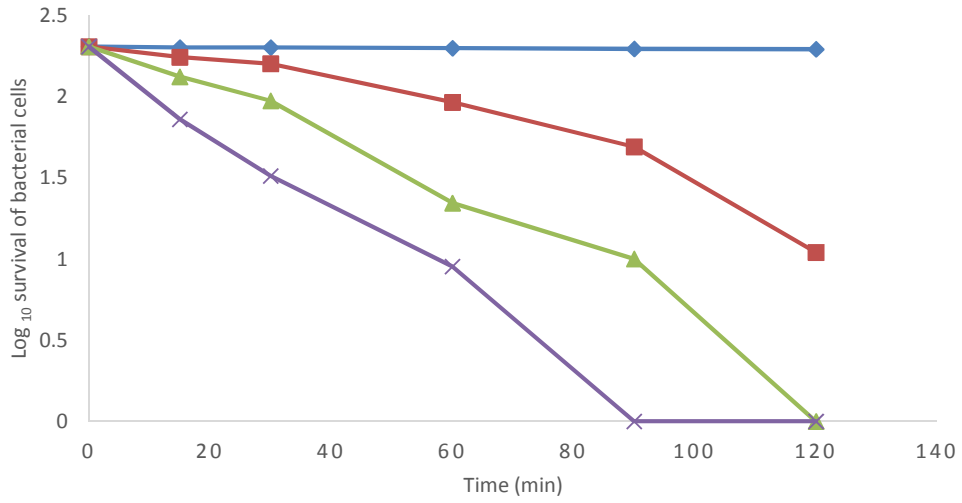

Fig. 3. The extent and rate of killing of *S. aureus* cells by methanolic crude extract at 1 x MIC (—■—), 2 x MIC (—▲—), 3 x MIC (—×—) and control (—◆—)

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

susceptible bacteria ranged between 12.0±0.0 mm and 23.3±1.2 mm while the zone of inhibition exhibited by streptomycin against the bacterial isolates ranged between 13.0±0.0 mm – 31.0±1.0 mm and that of ampicillin was between 18.3±0.6 mm - 29.3±1.5 mm (Table 1).

The value of the minimum inhibitory concentrations is usually and adequate guide for the treatment of most infections [15]. 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 Riwom et al. [21] 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 Riwon et al. [21] 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 [22]. 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 [23]. Pure isolate alkaloids and their synthetic derivatives have been used as analgesic, anti-spasmodic and bactericidal agent [24]. 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 [25]. 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 [26]. Flavonoids in human diet may reduce risk of various cancer as well as preventing menopause symptoms [25]. 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 [27]. Tannins have been implicated with various pharmaco-therapeutic effects [28]. 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 [29]. Tannins-rich remedies are used as antihelmintics, antioxidants, antimicrobials, antivirals, in cancer chemotherapy and to chelate dietary iron [30-33]. 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 (Fig. 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. And it has been reported that *D. edulis* is rich in vitamins A, B and E which are useful against skin wrinkling [34]. The results of the findings support the usefulness of *D. edulis* in folklore remedies.

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

The leaf extract of *Dacryodes edulis,* compared favourably with streptomycin and/or ampicillin used as standard control in this work. For example, the zone of inhibition exhibited against *Enterococcus faecalis* was 14.0±1.0 mm for the extract while 19.0±0.0 mm and 18.3±0.6 mm for streptomycin and ampicillin respectively. Also, *Micrococcus luteus* showed a zone of 12.3±0.7 mm relative to the streptomycin standard, which showed a zone of 14.0±0.0 mm and lastly, *Trueperella pyogenes* with a zone of 19.3±0.7 mm relative to both streptomycin and ampicillin standard which showed zones of 21.3±0.6 mm and 27.3±0.6 mm respectively. In some cases where the streptomycin and ampicillin standard did not show any activity, the extract did. For example, where the streptomycin showed no inhibition against *E. coli* but the extract inhibited the growth of this organism. Again, ampicillin was not able to inhibit the growth of *Pseudomonas aeruginosa* but the extract did*.* 18.3±0.6 mm - 29.3±1.5 mm.

The test organisms comprised of both Gram positive and Gram negative and the extract was sensitivity against both groups which indicates that *D. edulis* leaf extract has a broad spectrum activity. Drug formulated from this plant extract may go a long way in combating 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 (Figs. 2 - 3). This is an indication of monophasic effect exhibited by the extract which was similar to the effect reported by Akinpelu et al*.* [35]. 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. 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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