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Phytochemical Analysis, *In vitro* Antibacterial Activity and Rate of Kill of Different Fractions of *Dacryodes edulis* Leaf

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Authors' contributions

This work was carried out in collaboration among all authors. Authors REHO and AMDA designed the study, did the literature search and wrote the first draft of the manuscript. Authors OO and OMA performed the statistical analysis. Author OOO wrote the protocol. Authors TAB and AEA managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Dacryodes edulis is a fruit tree attaining a height of 18–40 meters mostly grown around the house and rarely in the forest. It has a relatively short trunk and a deep, dense crown and it is native to Africa hence often called African pear. The leaves are a compound with 5-8 pairs of leaflets. Acetone extract/solvent fractions of the leaves of *Dacryodes edulis* were used. The *in vitro* antibacterial activities and rate of kill of different fractions were investigated. The phytochemical screening was done by some chemical tests, antibacterial activity by agar well diffusion method and rate of kill was carried out on *S. aureus* and *E. coli* organisms. The fractions exhibited antibacterial activities with zones of inhibition ranged between 20 and 30 mm by Aqueous (AQU)

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fraction while Dichloromethane (DCM) fraction ranged between 22 and 32 mm. The standard antibiotics, streptomycin ranged between 10 and 20 mm and ampicillin between 11 and 27 mm. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of both fractions had range values between 0.78 and 6.25 mg/mL. The phytochemical screening revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoid and glycoside. Overall, the two fractions had better activities than the standard antibiotics used. The time kill assay showed that the percentage of the cells killed increased with increasing concentrations of the fractions, as well as, contact time intervals. The AQU fraction killed 100% of *Escherichia coli* and *Staphylococcus aureus* cells at concentration of 4.68 mg/mL (3 X MIC) within 120 min while DCM fraction killed 100% of *Escherichia coli* cells within 90 min at a concentration of 4.68 mg/mL and killed 100% of *S. aureus* cells within 120 min at a concentration of 3.12 mg/mL (2X MIC). In conclusion, *D. edulis* leaf fraction has a broad spectrum antibacterial activity, with the AQU and DCM fractions being bactericidal as exemplified by the killing rate and MIC index of 2 (ratio of MBC/MIC) for both fractions.

Keywords: Dacryodes edulis; phytochemicals; antibacterial activity; fractions; rate of kill.

1. INTRODUCTION

There is a global trend of antimicrobial resistance (AMR) culminating into major threats facing mankind and this has led to the renewed interest in the search of antibacterial in medicinal plants. The researchers have taken advantage of the rich and huge heritage of herbal plants in Nigeria to screen for antimicrobials and phytochemical components these plants [1]. The importance of medicinal plants cannot be over emphasized as they are beneficial to health especially to individuals, communities and globally in general. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body [2].

Dacryodes edulis is a dioecious shade loving species of non-flooded forests in the humid tropical zone [3,4,5] where its seed is widely cultivated for the production of its fruits which has vast economic and health-related benefits [6]. It consists of a seed surrounded by a pulpy butyraceous pericarp, which is the edible portion consumed either raw or cooked. The decoction of the leaves of the plant is employed in traditional medicine in the treatment of certain disorders of the digestive tract, toothache and earache. The leaf and stem or stem backs are used to cure dysentery and anemia [7]. The root bark is used for leprosy in Congo Brazzaville [8] while resin from the bark has been reported to heal scars and other skin problems in Nigeria [9,10]. In Nigeria, the stem and root are also used as chewing sticks for oral hygiene while the leaves are employed to cure skin diseases, such as rashes, scabies, ringworm and wounds [11,12]. The fruits and seeds of the plant are rich in oil which contains lipid and fatty acid reported to exhibit considerable nutritional value [13].

Nwokonkwo [14] in her study reported that the seed extracts of *Dacryodes edulis* and its secondary metabolites possessed potential antibacterial activity against some human pathogens in varying degrees. Antibacterial effect of the essential oil as well as the organic extracts of *D. edulis* plant has been reported [15,16]. It was indicated that the leaf extract of *Dacryodes edulis* possess broad spectrum antibacterial activity in a recent study by Olasunkanmi and Adeniyi, [17]. The phytochemical components reported in this *D. edulis* include tannins, saponins, glycosides, terpenoid, flavonoid and alkaloids. Ajibesin et al. [16] had identified phenolics such as ethylgallate and quercitrin in the plant leaves while Okwu and Nnamdi, [18] reported that the stem exudates of the plant contain tannin, saponins, and alkaloids. The presence of bioactive compounds such as saponins, tannins, alkaloids and flavonoids identified in the plant has been suggested to be responsible for the various uses of *D. edulis* in traditional medicine to cure ringworm, wound, scabies, skin diseases and inflammation [18]. There is dearth of information on microbial killing potential of the fractions of *D. edulis* leaf in terms of the kinetics of bacterial death. Evaluating the time course killing of bacterial cells by antimicrobials is essential to speculate whether the antimicrobial action of a compound is instantaneous (affecting the membrane integrity) or time dependent (affecting the cellular processes) [19]. In order to determine the mode of action of *D. edulis* leaf fractions used in this study, the rate of bacterial killing at different concentrations was evaluated. This study

therefore evaluated the antibacterial activity, phytochemical components and time-kill study of *D. edulis* leaf fractions in order to contribute to the ongoing exploration of indigenous plants for potential antibiotics.

2. MATERIALS AND METHODS

2.1 Plant Sample

The leaves of *D. edulis* were collected from Opa area, Ile-Ife, Osun State, Nigeria between January and February 2018, which is outside the fruiting season. The plant was identified and authenticated by Mr. G. A. Ademoriyo of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria and Voucher specimen (IFE-17653) was deposited. The sample was oven-dried at 40°C until constant weight was observed. The dried leaf was powdered and stored in an air-tight container for further use.

2.2 Preparation of Extract

About 1 kg of the powdered leaf of *D. edulis* was extracted at room temperature using 75% acetone for 3 days. The mixture was then filtered and the filtrate was dried in vacuo using a rotary evaporator. A glossy black yield of 122 g was collected. Preparation of bacterial isolates for experiment.

The following organisms were used for the experiment:

Bacillus subtilis (NCIB 3610), *E. coli* (NCIB 86), *S. aureus* (NCIB 8588), *Micrococcus luteus* (NCIB 196)*, Klebsiella pneumoniae* (NCIB 418), *Pseudomonas aeruginosa* (NCIB 950), *Pseudomonas fluorescens* (NCIB 3756) and locally isolated organisms (LIO): *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Corynebacterium pyogenes, Proteus vulgaris.* The locally isolated organisms (LIO) were obtained from the Department of Medical Microbiology and parasitology laboratory of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Nigeria.

2.2.1 Fractionation of crude acetone extract

The crude extract was successfully partitioned using organic solvents in order of their polarity index n-Hexane (0.1), Dichloromethane (3.1), n-Butanol (4.0), Ethylacetate (4.4). Exactly 90 g of the crude extract was resolved in 100 mL of sterile distilled water in a 500 mL separatory funnel, extracted with n-hexane until clear and colourless layer of n-hexane was obtained. The resulting n-hexane fraction was concentrated to dryness *in vacuo* and the fraction residue was kept in a freezer in an air-tight container. The resultant aqueous phase was re-concentrated *in vacuo* to remove traces of n-hexane. The residue was further extracted with dichloromethane until clear and colourless layer of DCM was obtained. The DCM fraction obtained was also concentrated *in vacuo* to dryness and the powder collected was kept in freezer for further use. N-butanol and Ethylacetate fractions were also obtained using similar procedure. The aqueous fraction was freeze-dried and the resultant powder was kept in the freezer for further use.

2.3 Antibacterial Activity of Fractions Obtained from *D. edulis* Leaf Extract on Bacterial Strains

The sensitivity testing of the extract was determined using agar-well diffusion method as described by Irobi et al. [20]. The bacterial strains were first grown in nutrient broth for 18 hours before use. For standardization the broth culture was diluted until the bacterial suspension matched with the turbidity of 0.5 McFarland turbidity standards. Exactly 0.1 mL of the standardized test isolates was evenly spread on agar medium using a sterile glass spreader. Wells were bored into the agar medium using a sterile 6 mm cork borer and carefully filled up with 50 mg/mL concentration of the extract solution without spilling. One out of the wells in each plate was filled with Acetone, Dichloromethane and Distilled sterile water for controls. The plates were allowed to stand on laboratory bench for 1 h to allow for proper diffusion of the extract into the medium before incubation at 37°C for 24 h. The zones of inhibition were observed and recorded. Standard Streptomycin and Ampicillin antibiotics were tested alongside.

2.4 Determination of the Minimum Inhibitory Concentrations (MICs)

The MICs of the fractions were determined using the method described by Irobi et al. [20]. A stock solution of plant aqueous extract was then made by dissolving 100 mg of extract in 1 ml of sterile Distilled Water (D/W) giving a concentration of 100 mg/mL stock solution. Two-fold dilution was made from the stock by adding 2 mL of stock in 2 mL of Nutrient broth (50 mg/mL) and this was repeated eight times to obtain: 25 mg/mL, 12.5

mg/mL, 6.25 mg/mL 3.125 mg/mL, 1.15 mg/mL, 0.78 mg/mL, 0.39 mg/mL, 0.2 mg/mL and 0.1 mg/mL. Sterile tubes (12) were arranged in a rack and 1 mL sterile peptone water was put in tubes 2 to 10. Then 1 mL of the stock solution of the extract was added to tubes 1 and 11 while sterile D/W was added to tube 12. Tube 2 was mixed and 1 mL was transferred to tube 3. This was repeated upto tube 11 and 1 mL was discarded from there. From the standardized (0.5 McFarland) bacterial broth culture, 1 mL was added to all the tubes. Tube one contained extract stock solution plus bacterial suspension (should show no growth) and tube 12 contained sterile DW and bacterial suspensions (should show turbidity for growth). All tubes were incubated overnight at 37°C. MIC was observed as the lowest concentration showing no turbidity indicating no growth according to CLSI guidelines [21].

2.5 Determination of Minimum Bactericidal Concentrations (MBCs) of the Crude Extract and Fractions of the Leaves of *D. edulis* on Bacterial Strains

The minimum bactericidal concentration of the extract was determined in accordance with the method of Olorundare et al. [22] and Irobi et al. [20]. To determine the MBC, from the last tube showing no growth to the previous ones, Nutrient and Chocolate agar plates (labelled according to the different concentrations) were inoculated. The plates were incubated overnight at 37°C. The MBC was taken to be the lowest concentration of the extract that did not allow any bacterial growth on the surface of the agar plates.

2.6 Qualitative Analysis of Phytochemical Constituents of *D. edulis*

A small portion of the dry extract was screened for phytochemical components using Trease and Evans [23], Harborne [24], Edoga et al. [25]; Egwaikhide, [26] methods for alkaloids, tannins, flavonoids, saponins, terpenoid and glycoside.

2.6.1 Test for tannins

Tannin testing was carried out according to method described by Ng et al. [2]. Half gram of powdered extract was boiled in 20 mL of distilled water in a test tube and then filtered using filter paper, 0.1% $FeCl_2$ was added to the filtrate and was observed for brownish green or a blue black coloration, which indicates the presence of tannins.

2.6.2 Tests for saponins

Freshly prepared 7% blood agar medium was used and wells were made in it. The extract in acetone was applied with distilled water and acetone used separately as negative controls, while commercial saponin (BDH) solution was used as positive control. The plates were incubated at 35°C for 6 h. Presence of complete hemolysis of the blood around the extract was indicative of saponins.

2.6.3 Test for alkaloids

Exactly 0.2 g of aqueous extract was warmed with 2% H_2SO_4 for two minutes, filtered and three drops of Dragendorff's reagent was added. Formation of orange-red precipitate indicates the presence of alkaloids.

2.6.4 Test for terpenoids

Five ml of aqueous extract was mixed with 2 mL of $CHCl_3$ in a test tube. 3 mL of conc. H_2SO_4 was carefully added to the mixture to form a layer. Appearance of a reddish brown coloration interface indicates the presence of terpenoid constituents.

2.6.5 Test for flavonoids

About 0.2 g of the extract was dissolved in 2 mL of methanol and heated. A chip of magnesium metal was added to the mixture, followed by the addition of a few drops of concentrated HCl. The occurrence of a red or orange coloration was indicative of the flavonoids [27].

2.6.6 Test for glycosides

One mL of concentrated H_2SO_4 was prepared in a test tube, 5 mL of aqueous extract was mixed with 2 mL of glacial CH_3CO_2H containing one drop of $FeCl_3$. The mixture was carefully added to the 1 mL of concentrated H_2SO_4 so that the concentrated H_2SO_4 was underneath the mixture. The appearance of a brown ring indicates the presence of the cardiac constituent.

2.7 Determination of Extent and Rate of Kill (ROK)

The rate of kill of the susceptible bacterial strains was determined using Aqueous (AQU) and Dichloromethane (DCM) fractions on the viability

of *S. aureus* representing Gram positive organisms and *E. coli* representing Gram negative [28]. Cultures of these bacterial strains were first standardized to approximately 10^6 cfu/mL before use.

2.7.1 Preparation and standardization of inoculum suspension

Direct colony suspension technique was used for the standardization and preparation of inoculum. Pure cultures of the test organisms were transferred into sterile screwcapped McCartney bottles containing normal saline (0.90% w/w) using a flamed inoculating loop. A suspension with a turbidity equivalent to 0.5 McFarland standards was also prepared at the same time to serve as a reference for turbidity. To achieve equal turbidity, both the reference and inoculum suspensions were placed against a white card with black stripes. Turbidity was observed with the unaided eye. Standardized inoculums were refrigerated [29].

Exactly 0.5 mL of the standardized suspension of the culture was added to 4.5 mL of different concentrations of the fraction relative to MIC. The experiment was held at room temperature over a period of 2 h to determine the killing rate of the organisms. A volume of 0.5 mL of each of the suspension was withdrawn at time interval and transferred to 4.5 mL of recovery medium containing 3% "Tween 80" to remove the effect of the fraction carry over from the bacterial cells. The suspension was then serially diluted and plated out for viable counts. These plates were then incubated at 37°C for 48 h before determining the survival cells. Control plates containing the test cells without the inclusion of antibacterial agents were set up along with the experimental. Viable counts were made in triplicates for each sample and compared with the counts of the control. Decrease in viable counts indicated killing by the fractions.

2.8 Statistical Analysis

Statistical analyses used were Paired Sample and Correlation tests.

3. RESULTS

3.1 Extent and Rate of Killings

The extent and rate of kill of the two bacterial representatives of Gram negative and Gram positive, *Escherichia coli* and *Staphylococcus aureus* respectively is shown in Figs. 3 - 6 The control tests which contained only Sterile distilled water and the bacterial organism showed the survival of the bacteria throughout the test period (♦) whereas, as the concentrations of the extracts increased [1 x MIC (■), 2 x MIC (▲) and 3 x MIC (**×**)] with increase in contact time, there is notable increase in the number of bacteria cells killed.

Table 1. The minimum inhibitory concentration and minimum bactericidal concentration of Aqueous (AQU) and Dichloromethane (DCM) fractions

Test Organisms	AQU fraction		DCM fraction		MIC index
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	
Staphylococcus aureus (NCIB 8588)	1.56	3.1	0.78	1.56	2.0
Streptococcus pneumoniae (LIO)	3.12	6.25	3.12	6.25	2.0
Micrococcus luteus (NCIB 196)	1.15	3.12	0.78	1.56	2.0
Escherichia coli (NCIB 86)	1.56	3.12	1.56	3.12	2.0
Klebsiella pneumonia (NCIB 418)	1.56	3.12	1.56	3.12	2.0
Pseudomonas aeruginosa (NCIB 950)	3.12	6.25	1.56	6.25	2.0
Pseudomonas fluorescens (NCIB 3756)	0.78	1.56	0.78	1.56	2.0
Corynebacterium pyogenes (LIO)	1.56	3.12	1.56	3.12	2.0
Bacillus subtilis (NCIB 3610)	1.56	3.12	0.78	1.56	2.0
Proteus vulgaris (LIO)	0.78	1.56	1.56	3.12	2.0

Key: AQU = Aqueous fraction; DCM = Dichloromethane fraction. MIC_{index} is 2 for both fractions, LIO=Locally Isolated Organisms, NCIB= National Collection for Industrial Bacteria, MIC index =Ratio of MBC to MIC, The P-value at 0.05 = 0.02 under Paired Sample Test shows that there is significant difference between DCM and AQU at 95 % confidence interval (CI) (P<0.05)

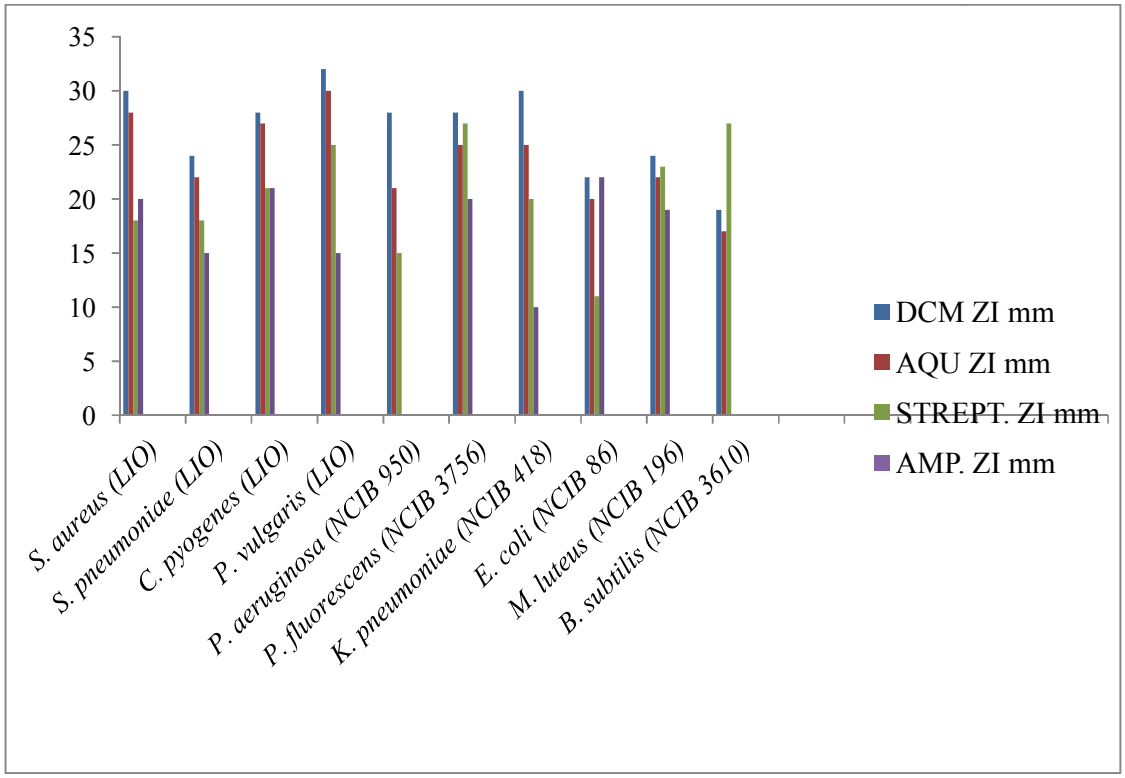

Fig. 1. Sensitivity result of Dichloromethane (DCM) and Aqueous (AQU) funnel fractions at 35 mg/mL

Key: ZI-Zone of Inhibition, mm=Millimeter, LIO=Locally Isolated Organisms, NCIB=National Collection for Industrial Bacteria, AQU=Aqueous, DCM=Dichloromethane STREPT.= Streptomycin AMP. =Ampicillin, The P-value at 0.05 = 0.02 under Paired Sample Test shows that there is significant difference between DCM and AQU at 95 % confidence interval (CI) (P<0.05). This indicates that DCM is a better solvent than water

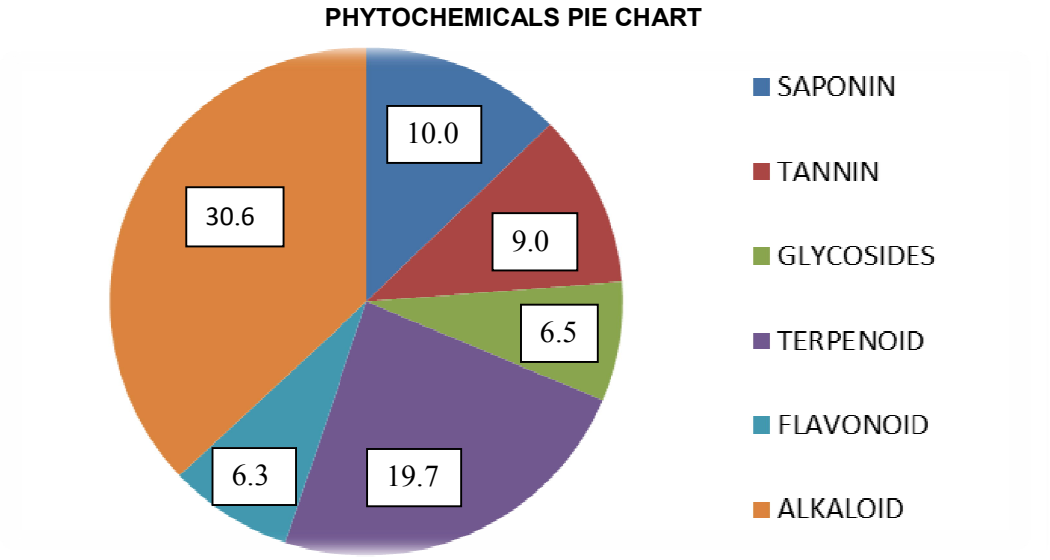

Fig. 2. Mean estimated phytochemicals of *D. edulis* leaf (mg/g)

The extent and rate of killing of *E. coli* by aqueous fraction at 1 x MIC (■), 2 x MIC (▲), 3 x MIC **(×)** and control (♦) is shown in Fig. 3. Each point represents the $\log_{10}$ survival of bacterial cells at a particular time interval in the presence of the added extract. The amount of *E. coli* cells killed by aqueous fraction at 1 x MIC, 2 x MIC and 3 x MIC after 120 minutes were 1.83 $\log_{10}$ (21.79%), 1.51 $\log_{10}$ (35.47%), and 0.00 $\log_{10}$ (100%) respectively. The control test showed evidence of the bacterial survival and no killing recorded.

There was no correlation between the mean colony count for control and time. However there was a negative correlation between the mean colony counts at 1 X, 2 X and 3 X MIC and time. This means, as time increased, the mean colony counts at the three MIC decreased and the colony counts reduced with increasing strength of the fractions at different time intervals. There was statistically significant reduction in the mean colony count at 1 X MIC with time (r=-0.9953; P=0.00015), at 2 X MIC with time (r=-0.9601; p=.002368) as well as at 3 X MIC with time (r=-0.8674; p=.025357).

Presented on Fig. 4 is the amount of *E. coli* cells killed by DCM fraction at 1 x MIC and 2 x MIC after 120 minutes were 1.86 $\log_{10}$ (20.51%), 1.18 $\log_{10}$ (49.57%) respectively, while at 3 x MIC, all the bacterial cells were totally killed within 90 minutes (0.00 $\log_{10,}$ 100%).

There was no correlation between the mean colony count for control and time. However there was a negative correlation between the mean colony counts at 1 X, 2 X, and 3 X MIC and time. Interpretation indicates that as time increased, the mean colony counts at the three MICs decreased. This also means that, the colony counts reduced with increasing strength of the fractions at different time intervals ($p<0.05$).

The amount of *S. aureus* cells killed by aqueous fraction at 1 x MIC, 2 x MIC and 3 x MIC after 120 minutes were 1.83 $\log_{10}$ (22.78%), 1.28 $\log_{10}$ (45.99%), and 0.00 $\log_{10}$ (100%) respectively (Fig. 5). There was no correlation between the mean colony count for control and time. However there was a negative correlation between the mean colony counts at 1 X, 2 X, and 3 X MIC and time. This means, as time increased, the mean colony counts at the three MICs decreased. This also means that, the colony counts reduced with increasing strength of the fractions at different time intervals ($p<0.05$).

As shown on Fig. 6, the amount of *S. aureus* cells killed by DCM fraction at 1 x MIC and

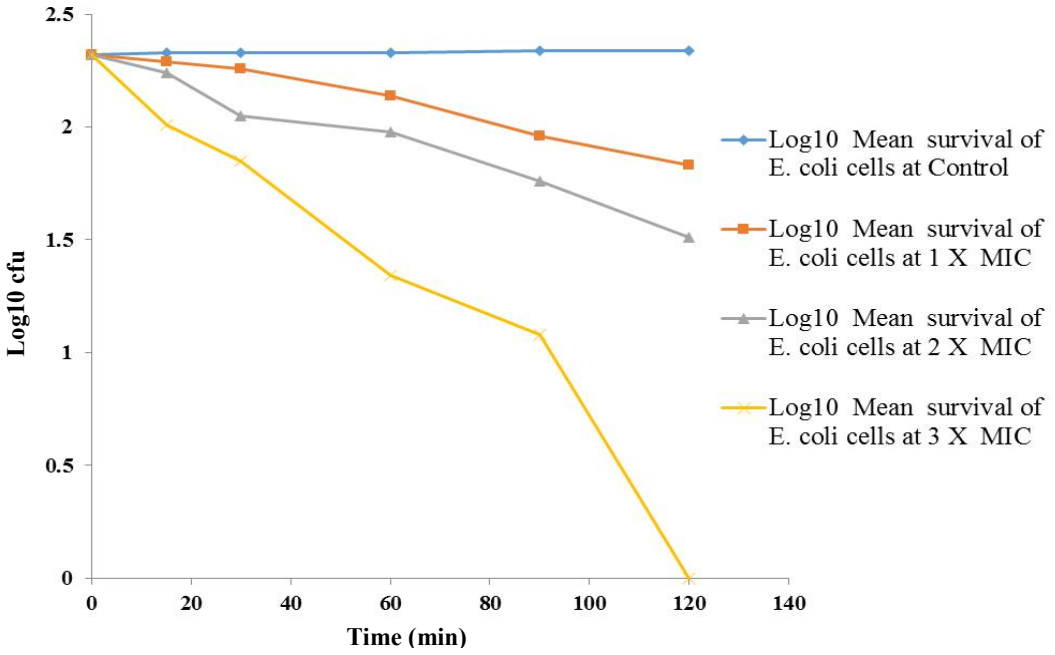

Fig. 3. Graphical representation showing survivility of *E. coli* cells
KEY: cfu = colony forming unit

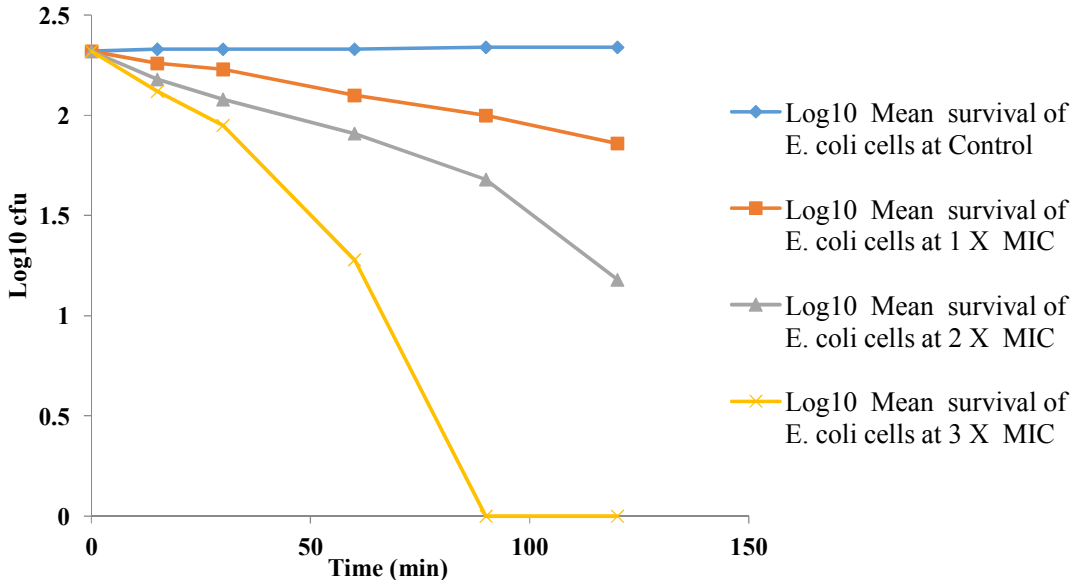

Fig. 4. Time-kill rate assay of Dichloromethane fraction with *E. coli*

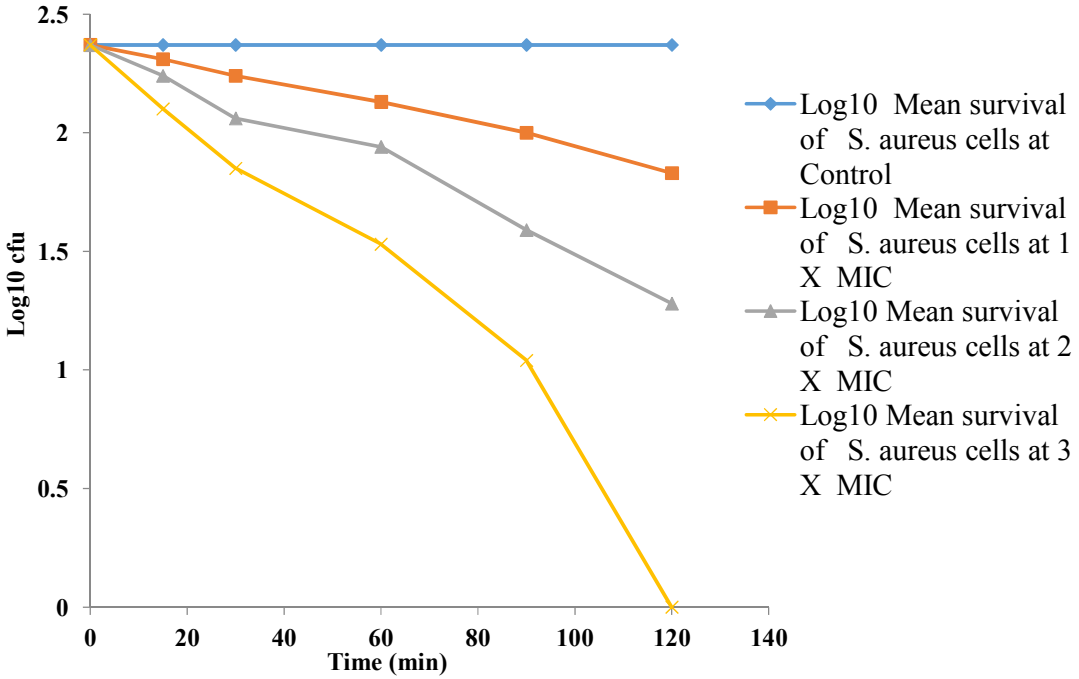

Fig. 5. Time-kill rate assay of aqueous fraction with *S. aureus*

2 x MIC after 120 minutes were 1.865 $\log_{10}$ (30.38%) and 0.00 $\log_{10}$ (100%) respectively but at 3 x MIC all the bacterial cells were totally killed within 90 minutes (0.00 $\log_{10}$, 100%).

There was no correlation between the mean colony count for control and time. However there was a negative correlation between the mean colony counts at 1 X, 2 X, and 3 X MIC and time. This is interpreted to mean that as time increased, the mean colony counts at the three MIC decreased. This also means that, the colony counts reduced with increasing strength of the fractions at different time intervals ($p<0.05$).

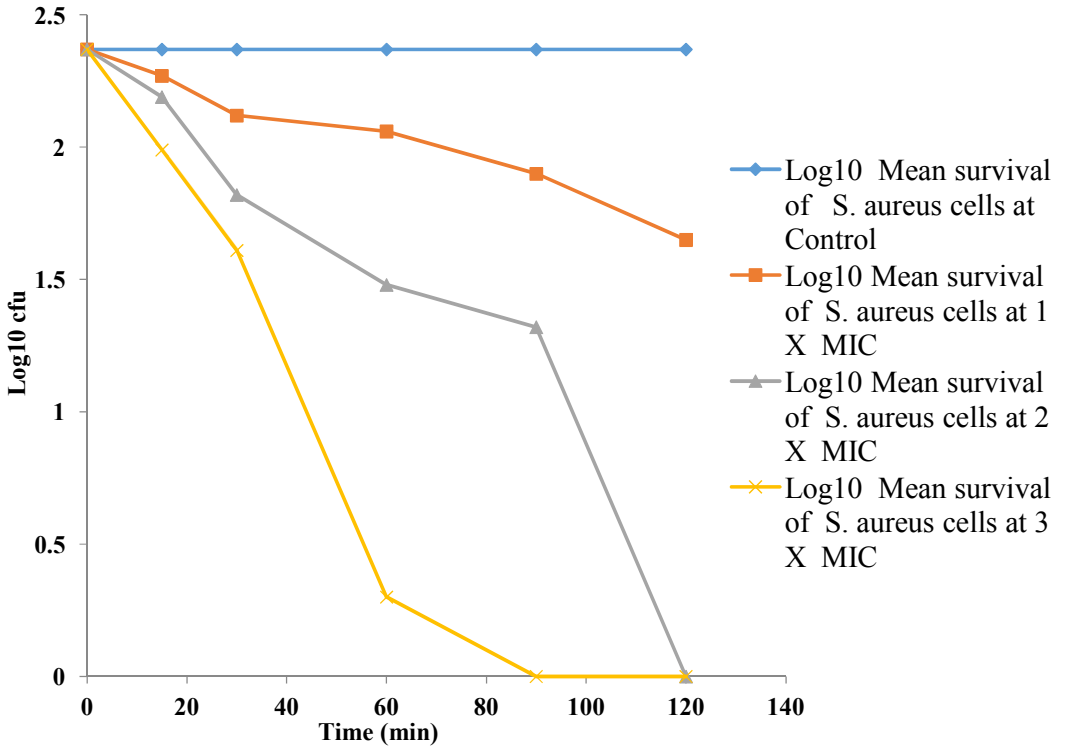

Fig. 6. Time-kill rate assay of Dichloromethane fraction with *S. aureus*

4. DISCUSSION

Medicinal plants and medicinal herbs have been identified and used from prehistoric times. The need to study the mechanisms of actions of promising medicinal plants is paramount to producing drugs against pathogens that are multi drug resistant to conventionally used antibiotics. The antibacterial activities of AQU and DCM fractions against ten bacterial organisms comprising of Gram positive and Gram negative, revealed that the fractionated extracts exhibited significant *in vitro* antibacterial activities against all the ten bacteria employed in this work indicating broad spectrum antibacterial activities. However, the results of both fractions were DCM (22-32 mm) and AQU (20-30 mm) are presented in Fig. 1. The P-value at 0.05 = 0.02 under Paired Sample Test shows that there is significant difference between DCM and AQU at 95% confidence interval (CI) ($P<0.05$). This indicates that DCM is a better solvent than water.

However both fractions had significantly higher antibacterial activity than the standard (control) antibiotics, Streptomycin (15 to 27 mm) and Ampicillin (15 to 22 mm) as presented in Fig. 1 ($P < 0.05$). The DCM fraction had highest inhibitory activity against *P. aeruginosa* and *P. fluorescens* (28 mm) and higher activity than the standard control antibiotics indicating a very promising source of antibiotics against these highly resistant bacteria. It is also noteworthy that the MIC of DCM fraction against well-known resistant bacteria, *P. aeruginosa,* is 1.56 mg/mL and it is a proof of the high efficiency of this plant being used for wound treatment as earlier reported by Okunomo and Egho [30]. For *K. pneuomoniae, P. pyogenes, P. aeruginosa, S. aureus, S. pneumoniae* and *B. subtilis* the fraction was more effective than the standard control antibiotics.

The MIC and MBC of the fractions are presented in Table 1, the MIC of AQU and DCM fractions exhibited against the susceptible bacteria ranges between 0.78 mg/mL and 3.12 mg/mL while the MBC ranges between 1.56 mg/mL and 6.25 mg/mL and agrees Achinto and Munirrudin, [31] who reported that low MIC value of medicinal plant extract indicate a better antimicrobial agent. Both fractions (AQU and DCM) show same values of MIC and MBC *for E. coli, P. fluorescens, C. pyogenes, K. pneumoniae and S. pneumoniae.* The MIC index of plant extract ≤ 2

mg/mL is considered as bactericidal while > 2 mg/mL but < 16 mg/mL is bacteriostatic [32]. This bactericidal activity of *D. edulis* leaf agrees with past studies of Olasunkanmi and Adenyi, [17]; Ajibesin, [16] who at various times recorded low MICs and MBCs of the fractions against some bacterial organisms.

The antimicrobial activities of Nigerian plants have been attributed to interaction of phytochemicals (such as alkaloids, tannins, phenols, saponins, flavonoids, and essential oils) and bioactive compounds contained in their tissues [33,34]. These active components in the crude extract may be acting synergistically to produce good antimicrobial effects [35]. The enumerated amount of phytochemicals present in *D. edulis* leaf asserts its usefulness in traditional medicines and its relevance on folklore medicine in the management of various infectious diseases. The leaf fractions demonstrated credible antibacterial potentials with broad spectrum activities and this cannot be unrelated to the presence of secondary metabolites detected in the plant. The P-value at 0.05 = 0.02 under Paired Sample Test shows that there is significant difference between DCM and AQU at 95% confidence interval (CI) ($P<0.05$). This indicates that DCM is a better solvent than water.

Time kill assay, as a mechanism of action of extract was performed and the results for the bacteria tested against both aqueous and dichloromethane fractions indicate that the extract is concentration–dependent and time-dependent. Similar reports have been recorded by previous researchers including Olasunkanmi and Adenyi, [17] who used a different solvent for *D. edulis;* Shami and Almasri [36] and Akinpelu et al. [37] who worked on deer musk and *C. nucifera* respectively. The time kill course show the bactericidal activity and the duration of a bacteriostatic effect of a fixed concentration of the antibacterial agent, thereby providing a clear analysis of the relationship between the extent of microbial population mortality and the antimicrobial agent concentration [38]. The time dependent killing of bacteria representatives by extract of *D. edulis* leaf suggests that the antibacterial action observed could be due to the effect on a variety of physiological factors inside the cell as reported by Kotzekidou and colleagues [39].

The rate of kill activity of the fractions in this study as seen in Figs. 3-6 showed bacteriostatic effect at 1 x MIC and 2 x MIC but bactericidal at 3 x MIC for *Escherichia coli* within the 2 hours duration of the test. The aqueous fraction on *Staphylococcus aureus*, was bacteriostatic at 1 x MIC and 2 x MIC but bactericidal at 3 x MIC as well as bactericidal at 2 x and 3 x MIC in dichloromethane fraction also within the 2 hours duration. Bactericidal action or activity was shown at 3 x MIC (*E. coli*) and 2 x MIC (*S. aureus* in DCM) within 2 hours exposure time, indicating a reduction of the viable bacterial density of ≥ 99.9% or ≥3 Log10 in cfu/mL being the standard of measurement for bactericidal efficacy [36,40,41,42]. The MIC_{index} values, which is less than or equal 2, indicate the bactericidal attribute of an extract and suggests that bactericidal effects of the extract could be expected on most of the test organisms [36] in a disease state. This is corroborated by the significant reduction in the cell counts between 90 and 120 min of incubation period in this study, therefore acknowledged the fact that the fraction was highly bactericidal seeing that the bacterial colonies were totally wiped out after incubating for 2 h. This is suggesting *Dacryodes edulis* leaf to be a potential source of active compounds of significant relevance as template for antibiotic preparation and therapeutic applications.

The observations revealed that the rate at which these fractions kill the bacteria increases with the concentration and contact time intervals. This supported results obtained by Rhodes [43] on antilisterial properties of red grape juice and red wine. The ability of an antibacterial agent to inhibit and kill bacteria is a measure of its effectiveness [44]. It also confirms the use of the plant in traditional folklore medicine and in herbal combination with other plants like *Lanata camara* and *Persea Americana* [45] and with *Aframomum melegueta* [8]. Where MIC equals MBC, the bactericidal potential with a broad spectrum and great therapeutic potential of the plant is indicated [46], this was however not the findings in this project.

5. CONCLUSION

In conclusion, from this study, we assert that the bactericidal and bacteriostatic abilities of this plant fraction at minimum contact time has established that broad spectrum antibacterial drugs of natural origin can be developed from *D. edulis* leaf. Consequently, this plant can provide useful substrate for the production of new broad spectrum antibiotics for the treatment of infections caused by the tested organisms and

different formulations can also be prepared for clinical trials.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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