Antimicrobial Activity of Fractioned Components from *Dacryodes edulis*: Invitro Study

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Authors’ contributions

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

Article Information

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ABSTRACT

Introduction: The history of the relationship between man and plants is as old as creation and man has used plants for different purposes including food and medicines. In this dispensation, man has been able to classify plants into food and medicinal plants and has also discovered that solution to his health problem comes from nature.

Objectives: The antibacterial activities of the dichloromethane (DCM) and aqueous (AQU) fractions of acetone extract of *Dacryodes edulis* leaf were investigated against both Gram positive and Gram negative bacterial organisms made up of locally isolated and standard strains.

Materials and Methods: About 1 kg of the powdered leaves of *D. edulis* was extracted at room temperature (25-27°C) using 75% acetone for 3 days. The mixture was then filtered and the filtrate was dried in vacuo using a rotary evaporator (insert the make and model). The crude extract was
successfully partitioned using organic solvents in order of their polarity, that is, n-Hexane, DCM, n-Butanol, Ethylacetate. The sensitivity testing of the extracts against the organisms was determined using agar-well diffusion method as described by Irob i et al. [1].

Results: DCM and aqueous (AQU) fractions possessed closely related broad spectrum antibacterial activities and greater activity than the control standard antibiotics (Streptomycin and Ampicillin). The zones of inhibition exhibited by AQU fraction ranged between 20 and 30 mm while that of DCM fraction ranged between 22 and 32 mm. On the other hand, the zones of inhibition observed for the standard antibiotics, streptomycin ranged between 10 and 20 mm and ampicillin between 11 and 27 mm. The MIC and MBC of both fractions had range values between 0.78 and 6.25 mg/mL, evaluation of phytochemical composition of *D. edulis* revealed alkaloid (30.63 mg/g), terpenoid (19.72 mg/g), saponins (10.54 mg/g), tannin (9.14 mg/g), flavonoid (6.51 mg/g) and glycoside (6.29 mg/g) which are very significant antibacterial agents in medicinal plants.

Conclusion: This work indicates that the two fractions had better antibacterial potentials than the standard antibiotics used.

Keywords: Dacryodes edulis; antibacterial; phytochemical; industrial bacteria; isolated organism.

1. INTRODUCTION

Since the discovery of antibiotics and their uses as chemotherapeutic agents, there has been a relief and belief in the medical practice that this would lead to the eradication of infectious diseases. But this was short-lived as antimicrobial resistant agents emerged. Medicinal plants had to be resorted to and with time have proved to be of great importance to the health of individuals and communities. This study is therefore one of the approaches to further prove the importance of medicinal plants. *Dacryodes edulis* (African pear) is a dioeciously, small to medium-sized tree reaching 20 to 25 m high and it is low branching [2]. It is characterized with fruits which contain seeds surrounded by the edible fleshy pericarp.

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 [3]. The leaf and stem or stem backs are used to cure dysentery and anemia [4]. The root bark is used for leprosy in Congo Brazzaville [5], while resin from the bark has been reported to heal scars and other skin problems in Nigeria [6,7]. 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 [8,9]. The fruits and seeds of the plant are rich in oil which contains lipid and fatty acid reported to exhibit considerable nutritional value [10]. Nwokonkwo [11] in her study reported that the seed extracts of *D. 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 [3,12]. Recent study by Olasunkanmi and Adeniyi, [13] indicated that the leaf extract of *D. edulis* possess broad spectrum antibacterial activity. The phytochemical compounds present in this extract include tannins, saponins, glycosides, terpenoid, flavonoid and alkaloids.

The objective of this work is to elucidate the antibacterial activities of different fractions of *D. edulis* against some bacterial isolates and to estimate the phytochemical compounds of the plant leaf.

2. MATERIALS AND METHODS

2.1 Plant Sample

The leave samples of *D. edulis* were collected from Opa area, Ile-Ife, Osun State, Nigeria (7°31’ 14.7612” N and 4°31’ 49.1340” E). 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 till constant weight was recorded. The dried leaves were powdered and stored in an air-tight container for further use.

2.2 Preparation of Extract

About 1 kg of the powdered leaves of *D. edulis* was extracted at room temperature using 75% acetone for 3 days. The mixture was then filtered and then dried in vacuo using a rotary evaporator.
(Heldoph, Germany). The yield collected was 122 g.

2.3 Organisms Used for the Experiment

Bacillus subtilis (NCIB 3610), Escherichia coli (NCIB 86), Staphylococcus 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 obtained from the Department of Medical Microbiology and Parasitology of the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife.

2.4 Fractionation of Crude Acetone Extract

The crude extract was successfully partitioned using organic solvents in order of their polarity, that is, n-Hexane, Dichloromethane (DCM), n-Butanol, Ethylacetate. Exactly 90 g of the crude extract was resolved in 100 mL of sterile distilled water in a 500 mL Separatory funnel and first extraction was with n-hexane until clear and colourless layer of n-hexane was obtained. The resulting n-hexane fraction was concentrated 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 [13].

2.5 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. [1]. Freshly grown bacterial culture in nutrient broth for 18 hrs was used for this experiment. 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 100 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 and zones of inhibition were recorded after 24 hr. Standard Streptomycin and Ampicillin antibiotics were tested alongside as controls.

2.6 Determination of the Minimum Inhibitory Concentrations (MICs)

The MICs of the fractions were determined using the method described by Irobi et al. [1]. 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 up to 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 Clinical & Laboratory Standards Institute (CLSI) guidelines [14].

2.7 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. [15] and Irobi et al. [1]. 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.

2.8 Qualitative and Estimation of Phytochemical Constituents of D. edulis

A small portion of the dry extract was subjected to qualitative analysis in order to detect the phytochemicals present and followed by the quantification tests using Trease and Evans [16], Harborne [17], Edoga et al. [18]; Egwaikhide, [19]; Krishnaiah et al. [20] methods for alkaloids, tannins, flavonoids, saponins, terpenoid and glycoside respectively.

2.9 Screening and Estimation of Tannins

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.

About 0.2 g of finely ground extract sample was weighed into a 50 mL sample bottle. Exactly 10 mL of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 hours at 30°C. The solution was then centrifuged and the supernatant stored in ice. 0.2 mL of solution was pipetted into the test tube and 0.8 mL of distilled water was added. Standard tannin acid solutions were prepared from a 0.5 mg/mL solution and the solution made up to 1 mL with distilled water. Exactly 0.5 mL of Folin ciocateau reagent was added to both sample and standard followed by 2.5 mL of 20% Na$_2$CO$_3$. The solutions were then vortexed and incubated in water bath for 40 min at room temperature; its absorbance was read at 725 nm against the reagent blank concentration of the same solution from a standard tannic acid curve prepared [21].

2.10 Screening and Estimation of Saponins

Two grams of powdered extract of the plant was boiled together with 20 mL of distilled water in a water bath and filtered. Exactly 10 mL of the filtered sample was mixed with 5 mL of distilled water in a test tube and shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with three drops of olive oil and observed for the formation of emulsion, which indicates the presence of Saponins.

The spectrophotometric method of Brunner [22] was used for Saponin determination. 2 g of the finely grinded sample (extract) was weighed into a 250 mL beaker and 100 mL of Isobutyl alcohol (But-2-ol) was added. The mixture was shaken for 5 hours to ensure uniform mixing. The mixture was then filtered with No 1 Whatman filter paper into 100 ml beaker containing 20 mL of 40 % saturated solution of magnesium carbonate (MgCO$_3$). The mixture obtained was again filtered through No 1 Whatman filter paper to obtain a clean colourless solution. 1 mL of the colourless solution was transferred into 50 mL volumetric flask using pipette, 2 mL of 5% iron (iii) chloride (FeCl$_3$) solution was added and made up to the mark with distill water. This was allowed to stand for 30 minutes for the colour to develop. The absorbance 380 nm was read against the blank with the reagents using UV/VIS spectrophotometer (Systronics UV-VIS Spectrophotometer; 108) for saponins estimation.

2.11 Screening and Estimation of Flavonoids

Aluminium chloride colorimetric method was used with some modifications to determine flavonoid content [23]. 1 mL of sample of plant extract was mixed with 3 mL of methanol, 0.2 mL of 10% aluminium chloride, 0.2 mL of 1 M potassium acetate and 5.6 mL of distilled water and allowed to stand at room temperature for 30 min. The absorbance was measured at 420 nm. Quercetin was used as standard (1 mg/ml). All the tests were performed in triplicates. Flavonoid contents were determined from the standard curve and were expressed as quercetin equivalent (mg/g of extracted compound) [24].

2.12 Screening and Estimation for Cardiac Glycosides

One mL of concentrated H$_2$SO$_4$ was prepared in a test tube. Exactly 5 mL of aqueous extract was mixed with 2 mL of glacial containing one drop of FeCl$_3$. The mixture was carefully added to the 1 mL of concentrated H$_2$SO$_4$ so that the H$_2$SO$_4$
underneath the mixture. The appearance of a brown ring indicates the presence of the cardiac constituent.

Exactly 10 mL of the extract was pipetted into a 250 mL conical flask. 50 mL chloroform was added and shaken on vortex mixer for 1 hour. The mixture was filtered into 100 mL conical flask. Exactly 10 mL of pyridine and 2 mL of 29% of sodium nitroprusside was added and shaken thoroughly for 10 minutes. 3 mL of 20% NaOH was added to develop a brownish yellow colour. Glycosides standard (Digitoxin) of concentrations ranged from 0 – 50 mg/ml were prepared from stock solution and the absorbance was read at 510 nm. [25].

2.13 Screening and Estimation for Terpenoids

Five ml of aqueous extract was mixed with 2 mL of CHCl₃ in a test tube. 3 mL of conc. H₂SO₄ was carefully added to the mixture to form a layer. Formation of interface with a reddish brown coloration indicates the presence of terpenoid constituents.

The procedure described by Sofowora [25] was used. 0.5 g of finely grounded sample was weighed into a 50 mL conical flask 20 mL of chloroform: methanol 2:1 was added the mixture was shaken thoroughly and allowed to stand for 15 minutes at room temperature. The suspension was centrifuge at 3000 rpm the supernatant was discarded and the precipitate was re-washed with 20 ml chloroform: methanol 2:1 and then re-centrifuge again the precipitate was dissolve in 40ml of 10% Sodium Dodecyl sulphate solution. 1 mL of 0.01M ferric chloride was added and allowed to stand for 30 minutes before taking the absorbance at 510 nm. Comparison with the STD Terpenoid (alpha terpineol) of concentration ranging from 0-5 mg/mL from the stock solution was used for total Terpenoid estimation.

2.14 Screening and Estimation for Alkaloids

Exactly 0.2 g of aqueous extract was warmed with 2% H₂SO₄ for two minutes, filtered and three drops of Dragendorff’s reagent was added. Formation of orange-red precipitate indicates the presence of alkaloids.

Exactly 5 g of the sample was weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol was added and allowed to stand for 4 minutes then filtered. The extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue which is alkaloid was dried and weighed [26].

Mayer’s was also used in alkaloid detection. Mayer’s reagent was freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 ml). To about 3 ml of extract, a few drops of Mayer’s reagent are added and a cream coloured precipitate indicated presence of alkaloid.

3. RESULTS AND DISCUSSION

Shown in Fig. 1 is a flowchart representing the partitioning of acetone extract of Dacryodes edulis leaves. Ethylacetate and Butanol solvents extracted insufficient fractions but dichloromethane (DCM) and aqueous (AQU) fractions were larger in quantities. The antibacterial activity of D. edulis leaf fractions successfully inhibited the growth of both Gram-positive and Gram-negative bacterial isolates used in this study at a final concentration of 35 mg/mL, thus, exhibiting a broad spectrum of activity (Fig. 2). For controls, sterile distil water and dichloromethane liquid were used and both did not show any antibacterial activity. DCM fraction exhibited the highest zone of inhibition of 28 mm against P. aeruginosa (NCIB 950) and P. fluorescens (NCIB 3756). The highest zone of inhibition exhibited by fraction AQU was 25 mm against P. fluorescens (NCIB 3756), thus indicating DCM fraction having higher antibacterial activity than the AQU. Fractions DCM and AQU exhibited lowest zones of inhibition were 19 mm and 17 mm respectively and shown against E. coli (NCIB 86) and B. subtilis (NCIB 3610). The two standard antibiotics used in this work exhibited zones of inhibition: streptomycin (18 mm to 30 mm) and tetracycline (10 mm to 28 mm). No zone of inhibition was recorded against N-Hexane fraction. The standard antibiotics streptomycin and ampicillin used as positive control exhibited zones of inhibition ranging from 19 to 27 and 0 to 22 mm against the bacterial isolates respectively. For K. pneumoniae, P. pyogenes, P. aeruginosa, S. aureus, S. pneumoniae and B. subtilis the
fractions were more effective than the standard control antibiotics. This study showed that the two fractions compared favourably with the standard antibiotics used in this study. It is noteworthy from this study that the fractions inhibited growth of some known pathogens tested and these include *P. aeruginosa*, known to be resistant to many antibiotics and causative agent of Surgical site infections, Otitis media Respiratory and Ocular infections; *S. pneumoniae* (LIO)causes of pneumonia, meningitis, bacteremia, otitis media and sinus infections; as well *S. aureus* (LIO) known to cause a range of diseases from minor skin infections like pimples, impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome and abscesses to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis. The abilities of the plant extract to inhibit or kill these pathogens supports the importance of *D. edulis* in folklore remedies for the treatment of disease caused by these pathogens. The two fractions exhibited very low MICs of 0.78-1.56 mg/mL against most of the test organisms as well as lowest MBCs of 1.56 mg/mL (Table 1). The MIC index of plant extract which is equal or less than 2 mg/mL is considered as bactericidal while those above 2 mg/mL but less than 16 mg/mL are bacteriostatic, [27]. The findings in this research agree with the works of Ajibesin [3], Agbo [28] and Olasunkanmi and Adeniyi [13], who reported low MICs and MBCs of this plant using different solvents for extraction and therefore confirmed that *D. edulis* is a very promising source of potent antibacterial agents for the treatment of infections caused by multi-resistant bacteria. While the MICs exhibited by AQU fraction against *Proteus vulgaris*, *Micrococcus luteus*, *Escherichia coli*, *Corynebacterium pyogenes*, *S. aureus*, *P. vulgaris* and *Klebsiella pneumoniae* were less than 2 mg/mL, that of DCM fraction against *Micrococcus luteus*, *E. coli*, *Staphylococcus aureus*, *P. vulgaris*, *C. pyogenes* and *P. aeruginosa* were also less than 2 mg/mL. This is indicative of *D. edulis* leaf extract exhibiting appreciable bactericidal properties. There was bacteriostatic activity against all other bacterial organisms with MIC of 3.12 mg/mL which is less than 16 mg/mL but greater than 2 mg/mL. The value of the Minimum Inhibitory Concentration is usually an adequate guide for the treatment of most infections [15], however, low MIC value of medicinal plant extract have been reported to indicate a better antimicrobial agent [29]. It is also noteworthy that the MIC of DCM fraction against a 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]. The Broad spectrum antibacterial activities of the fractions 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 showed 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.

### Table 1. The minimum inhibitory concentrations and minimum bactericidal concentrations of aqueous (AQU) and dichloromethane (DCM) fractions

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

**KEY:** AQU = Aqueous fraction; DCM = Dichloromethane fraction. MIC\text{index} is 2 for both fractions, LIO=Locally Isolated Organisms, NCIB= National Collection for Industrial Bacteria, MIC index =Ratio of MBC to MIC

MIC\text{index} of plant extract equal or less than 2 mg/mL is considered as bactericidal while those above 2 mg/mL but less than 16 mg/mL are bacteriostatic [27].
**Fig. 1. Flow chart of fractions partitioning of acetone extract**

- **PLANT** (*D. edulis*)
  - Hexane
    - N-Hexane fraction
      - DCM fraction
        - Residue
          - Ethylacetate
            - Insignificant fraction
              - Butanol
                - Insignificant fraction
                  - Water
                    - Aqueous fraction

- **Fig. 2. Sensitivity result of dichloromethane (DCM) and aqueous (AQU) fractions (5 mg/mL) and control (1 mg/mL)**

  Key: ZI=Zone of Inhibition, mm= millimeter, LIO=Locally Isolated Organisms, NCIB=National Collection for Industrial Bacteria, AQU=Aqueous, DCM=Dichloromethane
The result of the quantitative determination of the phytochemical contents of the leaf of *D. edulis* is recorded in Fig. 3 and reveals various medically important phytochemicals such as alkaloid (30.64 mg/g) followed by terpenoid (19.72 mg/g) while the lowest is glycosides (6.29 mg/g). Others are saponins (10.15 mg/g), tannin (9.14 mg/g) and flavonoid (6.51 mg/g). These phytochemicals are known to be biologically active and thus aid the antibacterial activities of the leaf extract. The antibacterial activity of various compounds such as phenols, flavonoids, terpenoid and alkaloids present in plant extracts is due to several mechanisms, including attacking cell walls and cell membranes, affecting their permeability and releasing intracellular constituents as well as interfering with membrane functions such as electron transport, and enzyme effect or nutrient uptake [31-34]. Saponins are responsible for most of the biological effects in herbal medicines as they are also key ingredients in traditional Chinese medicine [35,36]. Since compounds such as alkaloids and saponins are known to be antimicrobial [37], their presence has been suggested to account for the antimicrobial activity of the plants [38]. Flavonoids and phenols exhibit effective antibacterial activity owing to their ability to form complexes with extra cellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria [39,40,41,42]. More specifically flavonoid have been extensively studied and most of the studies have established their role as potential anti-microbial agents, in addition, it also exhibits anti-trypanosomal and antileishmanial activities [43]. Epidemiological studies suggest that the consumption of flavonoids is effective in lowering the risk of coronary heart disease [32,44,45]. Anti-allergic, anti-mutagenic, antiviral, antioxidant, anti-thrombotic, vasodilator, prevention of menopause, oxidative damage of membrane, proteins and DNA are linked to flavonoids [46,47]. Flavonoids have also been implicated in microbial membrane disruption [48]. Alkaloids mode of action is the ability to intercalate with DNA [49]. Tannins have been implicated with various pharmaco-therapeutic effects as well as used for the treatment of burns and other wounds due to their hemorrhagic and antiseptic potentials [50,51]. Tannins mode of action has been said to be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins and formation of complex with polysaccharide, iron deprivation, hydrogen bounding [39,52] as well as being used as anti helmintics, antioxidants, antiviral, in cancer therapy and chelate dietary iron [53, 54,55,56]. Terpenoids are known to be active against fungi [57,58], bacteria, [59] protozoa [60] and viruses [39,61]. Terpenoids mode of action has been speculated to be membrane disruption [48]. Many of the terpenoids are commercially interesting because of their use as flavours and fragrances in foods and cosmetics examples are menthol and scareol or because they are important for the quality of agricultural products.
such as the flavour of fruits and the fragrance of flowers like linalool [62]. In addition to their antibacterial activities, other important health activities of the phytochemical compounds detected in *D. edulis* include anti-inflammatory, anti-cancer and tumour-inhibition (Saponins) [63]; elimination of human cancer cell line, analgesic and anti-spasmodic (Alkaloids) [64, 65]. The presence of these very important secondary metabolite phytochemicals in *D. edulis* leaf extract confirms and indicates rich bioactive principles in the plant. *D. edulis* leaf can also serve as a source of drugs for treatment and prevention of cancer as well as antioxidant, anti-inflammatory, anti-trypanosomal and anti-leishmanial. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. Phytochemicals analysis of medicinal plants is also important and has commercial interest in both research institutes and pharmaceutical companies for manufacturing of new drugs for treatment of various diseases [66].

The properties of *D. edulis* observed in this study confirm the uses of the plant in the management of wounds, earache, toothache and skin diseases in folklore medicine. The observations enumerated on the properties of phytochemicals from *D. edulis* indicate its importance in traditional medicines. In Nigeria and Cameroon 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.

4. CONCLUSION

Conclusively, isolating and identifying these bioactive compounds can lead to formulation of new drugs to treat or prevent various diseases and infections, therefore improving the health status as well as contributing to the development of new, effective, and safe antimicrobials.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENT

We will like to appreciate Mr. Ademoriyo of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, who authenticated the plant and prepared the voucher sample which is available at the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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