Antibacterial constituents of the leaves of *Dacryodes edulis*

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Accepted 5 October, 2011

*Dacryodes edulis* is a dioecious, small to medium-sized tree, reaching 20 to 25 m high. Different parts of the plant are used to treat many diseases including skin infections, digestive tract disorder and dysentery. The leaves were macerated in 50% ethanol and the liquid extract concentrated to dryness. The extract was evaluated for antibacterial activity by using agar diffusion method. The extract was partitioned between water, ethyl acetate and butanol successively and further subjected to antibacterial testing. The most active fraction, ethyl acetate fraction, was purified through various chromatographic methods to obtain pure compounds identified by spectroscopic methods as ethylgallate and quercitrin. These compounds gave good antibacterial effects, while the minimum inhibitory concentrations of the fractions and the pure compounds ranged between 12.5 and 250 µg/ml. These phenolic compounds are reported for the first time in this plant.

**Key word:** *Dacryodes edulis*, antibacterial activity, ethylgallate, quercitrin.

**INTRODUCTION**

*Dacryodes edulis* is a dioecious, small to medium-sized tree reaching 20 to 25 m high, and it is low branching (Hutchinson and Dalziel, 1958). 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 bark are used to cure dysentery and anaemia (Ayuk et al., 1999). The root bark is used for leprosy in Congo Brazaville (Bouquet, 1969), while resin from the bark heals scars and other skin problems in Nigeria (Ekong and Okogun, 1969; Burkill, 1985). 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 wound (Igoli et al., 2005; Ajibesin et al., 2008). The fruit and seed of the plant are rich in oil which contains lipid and fatty acid reported to produce essential oil of the monoterpene, sesquiterpene, diterpene and triterpene types (Ekong and Okogun, 1969; Jirovetz et al., 2003; Onocha et al., 1999). Antibacterial effect of the essential oil has been reported (Obame et al., 2008), but no report on the organic extract of the plant and its chemical constituents is available. Thus, applying activity guided purification, this study aimed at validating the antibacterial effect of the plant and identifying the chemical constituents responsible for such effect.

**MATERIALS AND METHODS**

The nuclear magnetic resonance (NMR) spectra were recorded on a Brucker DR-500 MHz (¹H 1) and 25 MHz (¹H/¹3C HETCOR), in CD3OD using tetramethyilsilane (TMS) as internal standard. Mass spectroscopy was determined using Electro spray ionization (ESI) Full MS and Finnigan LCQ Deca-MS, Agilent series 1100-LC. UV spectroscopy was determined by Dionex, UVD 340 S Dionex. Melting points were determined on a Kofler hot-stage microscope (uncorrected). Thin layer chromatography (TLC) was carried out on silica gel 60 F254 (Merck). Solvent systems, such as EtOAc-CH2OH; 8:2 (A), CH2Cl2-MeOH; 9:1 (B), EtOAc-n-C6H14; 4:1 (C), CH2Cl2-MeOH; 7:3 (D) and CH2Cl2-MeOH-H2O; (7:3:1) were used. UV light (λ max 254 and 366 nm), FeCl3 spray, vanillin/H2SO4 and conc.
Plant

The leaves (6 kg) of _D. edulis_ were collected in June, 2005, at Ikot Ekpene in Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. U. Essiet of the Department of Botany, University of Uyo, Uyo, Nigeria. Voucher specimen (KKA 21) was deposited in the Department of Pharmacognosy and Natural Medicine herbarium, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

**Extraction of the plant**

The dried leaf powder (4 kg) of _D. edulis_ was extracted by maceration using 50% _EtOH_ (10 L). It was filtered and the marc was re-extracted with the fresh solvent mixture for 12 h (x2) and filtered. The filtrates were pooled together and concentrated to dryness _in vacuo_ at 40°C to yield dry ethanolic extract (80 g).

The dry ethanol extract (60 g) was dissolved in water and successively shaken with _EtOAc_ (6 × 300 ml) and _BuOH_ (6 × 300 ml) to afford ethyl acetate (18 g), butanol (20 g) and aqueous fractions (20 g), respectively.

**Antibacterial test**

The bacteria used in this study were _Bacillus cereus_ (NCIB 6349), _Staphylococcus aureus_ (NCIB 8588), _Pseudomonas aeruginosa_ (NCIB 950) and _Escherichia coli_ (NCIB 86). All the organisms were obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. They were maintained on blood agar plates at 4°C prior to use. Each of the organisms was also set up. The MIC was regarded as the lowest concentration of the extract or fraction that did not permit any visible growth when compared with that of the control tubes.

**Isolation and characterization**

The _EtOAc_, butanol and aqueous fractions of the plant species were subjected to TLC analysis, using solvent systems _A_, _B_ and _E_ respectively and visualized under the UV light (λ 254 nm) before using 100% _H₂SO₄_ and _FeCl₃_ solution as detecting spray reagents. The most active _EtOAc_ fraction (18 g) showed phenolic components and was chromatographed on silica (Merck, 0.040 to 0.063 mm particle size) by accelerated gradient chromatography (AGC) column and eluted with _C₆H₂O₆_ containing increasing amount of _EtOAc_ followed by increasing amount of _CH₃OH_ (9:1, 9:1). Six fractions coded _A_, _B_, _C_, _D_, _E_ and _F_ were obtained, two (A and C) of which showed significant antibacterial effects. The more active (A) of the two fractions was further fractionated on silica by repeated AGC, using _C₆H₁₂_ in gradient with _CH₂Cl₂_ and _CH₃OH_ (9:5.5, 9:5.5; 5.5, 9:8; 0.2:1) to yield 1 (5 mg). The less active fraction was purified by vacuum liquid chromatography (VLC) using _CH₂Cl₂_ in gradient with _CH₃OH_ and _H₂O_ (7:2:1). AGC (silica) and on Sephadex LH 20, eluted isocratically with _EtOH_. Final purification was carried out on silica by preparative thin layer chromatography (prep. TLC), using _CH₂Cl₂-CH₃OH_ (4:1) as mobile phase to yield 2 (10 mg). The two compounds were also subjected to antibacterial test.

**Activity-guided fractionation of _D. edulis_**

The antibacterial principles were partitioned mostly into ethyl acetate fraction followed by butanol and aqueous fractions, respectively, and these were subjected to further fractionation using different chromatographic techniques.

**Minimum inhibitory concentration (MIC)**

The MIC was determined by incorporating various amounts (250 to 6.25 µg/ml) of the solution of the extracts and fractions into sets of test tubes containing the culture media. 50 µl of the standard test bacterial broth cultures were added into each of the test tubes. The set of tubes containing a mixture of bacteria and the sample (extracts and fractions) were incubated at 37°C for 24 h (Cos et al., 2006).

A positive control tube containing only the growth medium of each of the organisms was also set up. The MIC was regarded as the lowest concentration of the extract or fraction that did not permit any visible growth when compared with that of the control tubes.

**RESULTS AND DISCUSSION**

Two phenolic compounds were isolated from the leaves of _D. edulis_. Ethylgallate 1, a siloxy, fluffy, white crystal was isolated with the aid of accelerated gradient chromatography (AGC), while quercitrin 2 was separated with vacuum liquid chromatography (VLC), accelerated gradient chromatography (AGC), size exclusion chromatography (Sephadex LH-20) and preparative thin layer chromatography (prep. TLC).

The ¹HNMR spectrum of compound 1 gave a triplet at δ 1.33 representing three methyl protons, and a quartet at δ 4.28 indicating two methylene protons of _O-CH₂CH₃_ group. Presence of a sharp singlet at δ 7.08 corresponds to two aromatic protons, indicating the symmetrical nature of the molecule. It also suggests the attachment of hydroxyl group at 3-, 4- and 5- positions. These data agree with the literature values (Metha and Sharma, 1988; Adesina et al., 2000). On this basis, compound 1 was characterized as ethylgallate (Ethyl 3, 4, 5-trihydroxybenzoate).

Quercitrin 2 exhibited band I and band II with the UV
Table 1. Antibacterial activity of the extracts of *D. edulis* leaf.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition of organisms (mm)</th>
<th>L</th>
<th>E</th>
<th>B</th>
<th>Aq</th>
<th>Gen</th>
<th>MeOH:H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> NCIB 86</td>
<td></td>
<td>8 ± 1.00*</td>
<td>8 ± 1.41*</td>
<td>3 ± 1.00</td>
<td>4 ± 1.41</td>
<td>12 ± 1.58*</td>
<td>0</td>
</tr>
<tr>
<td><em>B. cereus</em> NCIB 6349</td>
<td></td>
<td>13 ± 1.00*</td>
<td>12 ± 1.73*</td>
<td>5 ± 2.12</td>
<td>5 ± 2.20</td>
<td>13 ± 1.41*</td>
<td>0</td>
</tr>
<tr>
<td><em>S. aureus</em> NCIB 8588</td>
<td></td>
<td>10 ± 1.41*</td>
<td>11 ± 1.58*</td>
<td>6 ± 1.00*</td>
<td>4 ± 0.00</td>
<td>13 ± 1.00*</td>
<td>0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> NCIB 950</td>
<td></td>
<td>9 ± 1.00*</td>
<td>8 ± 0.00*</td>
<td>4 ± 1.00</td>
<td>3 ± 1.00</td>
<td>15 ± 2.12*</td>
<td>0</td>
</tr>
</tbody>
</table>

L, Leaf ethanol extract; E, ethyl acetate extract; B, butanol extract; Aq, aqueous extract, Gen, gentamicin. Values are mean ± SD (n = 4), *: P < 0.01 with respect to control.

Table 2. Antimicrobial activity of the fractions of *D. edulis* leaf.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>1</th>
<th>2</th>
<th>Gen</th>
<th>MeOH:H₂O</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> NCIB 86</td>
<td>12 ± 1.00*</td>
<td>5 ± 0.00</td>
<td>8 ± 1.00*</td>
<td>3 ± 1.00</td>
<td>3 ± 2.00</td>
<td>2 ± 0.00</td>
<td>17 ± 1.00*</td>
<td>14 ± 2.12*</td>
<td>15 ± 1.58*</td>
<td>0</td>
</tr>
<tr>
<td><em>B. cereus</em> NCIB 6349</td>
<td>16 ± 1.00*</td>
<td>5 ± 2.12</td>
<td>10 ± 1.00*</td>
<td>3 ± 0.00</td>
<td>1 ± 0.00</td>
<td>2 ± 0.00</td>
<td>26 ± 2.00*</td>
<td>15 ± 2.35*</td>
<td>19 ± 1.41*</td>
<td>0</td>
</tr>
<tr>
<td><em>S. aureus</em> NCIB 8588</td>
<td>14 ± 1.00*</td>
<td>3 ± 1.73</td>
<td>8 ± 1.00*</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21 ± 1.73*</td>
<td>15 ± 1.00*</td>
<td>20 ± 1.00*</td>
<td>0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> NCIB 950</td>
<td>9 ± 1.73*</td>
<td>3 ± 2.00</td>
<td>6 ± 1.73</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>15 ± 1.00*</td>
<td>10 ± 1.00</td>
<td>15 ± 2.12*</td>
<td>0</td>
</tr>
</tbody>
</table>

Gen, gentamicin, values are mean ± SD (n = 4); *: P < 0.01 with respect to control.

The extract, fractions and the pure compounds isolated showed varying degrees of antibacterial activities against all the tested microorganisms (Tables 1 and 2). These test bacteria have been implicated in the pathogenesis of human infections (Duguid et al., 1978). The ethanol extract gave the highest activity against *B. cereus* with inhibition zone of 13 mm, followed by *S. aureus* with the inhibition zone of 10 mm. The good activity elicited by ethanol extract was partitioned into ethyl acetate fraction, which also showed significant activity against *B. cereus* with zone of inhibition of 12 mm and *S. aureus* with zone of inhibition of 11 mm. The butanol and aqueous fractions were less active. Previous studies on the antimicrobial activities of plants, such as different species of Acalypha and spectrum at λ max nm: 350.0, 256.0 nm, respectively, indicating it to be 3-O- substituted flavonol (Bilia et al., 1993; Ibewuike, 1997). The ESI full mass spectrum showed a [M+H]^+ peak at m/z 447 (C_{21}H_{20}O_{11}) as the base peak. Retro Diels Alder (RDA) fragment at m/z 301.2 showed the loss of rhamnose.

In the ¹H NMR spectrum, the glycosidic nature of the compound was shown by the presence of a sugar moiety at δ 3.3 to 4.3. In the sugar portion, a doublet at δ 3.75 (H-3") coupled with a doublet each at δ 3.3 (H-4") and δ 3.75 (H-2"). The sugar moiety was inferred to be rhamnose from the methyl proton signal at δ 0.94 (3H, d, H-6") which was split into a doublet by a proton at δ 3.4 (1H, m, H-5"). The aromatic doublets at δ 6.20 and 6.40 (J = 2.5 Hz, H-6, H-8) were caused by m-coupled protons of an AB system characteristic of a 5, 7-substituted ring A. The signals at δ 6.90 (1H, d, J = 8.0 Hz, H-5") and δ 7.30 (1H, dd, J = 1.6 Hz, H-6") showed o-coupled protons on ring B, while the signal at δ 7.35 (1H, d, J = 1.6 Hz, H-2") exhibited m-coupled, all of an ABC system, characteristic of 3', 4'-substituted ring B.

The ¹H/¹³C COSY (HECTOR) spectrum showed the characteristic rhamnose methyl signal at δ 17.2. It also indicated the other sugar carbon signals at δ 71.5 to 72.7, with the anomeric carbon evident at δ 103.4. Thus, compound 2 was identified as quercitrin (quercitrin-3-O-rhamnoside). This was confirmed by HPLC-UV (Chromelone Dionex) of the authentic sample as well as by comparison with literature values (Bombardelli et al., 1973; Lin et al., 2002).
different varieties of *Lasianthera africana* gave similar activity guided purification pattern (Adesina et al., 2000; Ajibesin and Bassey, 2011). Out of the six bulked fractions obtained from further purification of ethyl acetate fraction by column chromatography, bulked fraction A gave the best activity against all the test bacteria, followed by bulked fraction C (Table 2). These active bulked fractions A and C showed low minimum inhibitory concentration (MIC) values ranging from 100 to 250 µg/ml (Table 3). The most active bulked fraction A yielded ethylgallate which gave the highest antibacterial activity against the test microorganisms with the lowest MIC (12.5 µg/ml). This activity was higher than that of Gentamicin, the standard drug. Thus, its presence accounted for the antibacterial activity of the leaves of the plant. The antimicrobial properties of ethylgallate have been established in literature (Adesina et al., 2000; Lamikanra et al., 1990; Burapadaja and Bunchoo, 1995). Bulked fraction C gave quercitrin which showed less antibacterial activity with the MIC value of 50 µg/ml.

Quercitrin has been isolated from plants, such as *Piliostigma thonningii* (Ibewuike, 1997; Bombardelli et al., 1973), *Koelreuteria paniculata* (Lin et al., 2002) and *Hypericum caprifoliatum* (Dall et al., 2003), and its antimicrobial activity widely reported in such plants. The antibacterial effects of *D. edulis* leaf have been determined to be mainly due to ethylgallate, while quercitrin was also isolated as antibacterial principle showing less inhibitory activity.

**Conclusion**

The two compounds, ethylgallate and quercitrin have been identified from the leaves of *D. edulis*, and were found to be responsible for the antibacterial effects of the plant. This validates its use in traditional medicine for treating infections.

**REFERENCES**


Lin W, Deng Z, Lei H, Fu H, Li J (2002). Polyphenolic compounds from

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**Table 3. MIC of the fractions and phenolics isolated from *D. edulis* (µg/ml).**

<table>
<thead>
<tr>
<th>Microorganism Gen</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> NCIB 86100</td>
<td>A 200</td>
<td>&gt;250</td>
<td>200</td>
<td>&gt;250</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td><em>B. cereus</em> NCIB 634950</td>
<td>100</td>
<td>&gt;250</td>
<td>250</td>
<td>&gt;250</td>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td><em>S. aureus</em> NCIB 858850</td>
<td>100</td>
<td>&gt;250</td>
<td>250</td>
<td>&gt;250</td>
<td>12.5</td>
<td>50</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> NCIB 95050</td>
<td>200</td>
<td>&gt;250</td>
<td>200</td>
<td>&gt;250</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

Gen: gentamicin