INTRODUCTION
The African black pear (Dacryodes edulis G. Don Lam) Burseraceae also called African plum or bush butter is an indigenous fruit tree of tropical Africa. The fruit pulp constitutes an important and much cherished local delicacy when in season. The tree grows up to 18m in height and exudes an odoriferous gummy substance from injured or excised portion of the stem. It is cultivated in most rural communities by the peasant farmers for its fruits. The fruit is red, turning blue-black when ripe with unpleasant turpentine smell. The fruit is oval in shape and matures within the months of May and June. The fruit consists of large seeds, surrounded by the mesocarp. The pulp is boiled, roasted or eaten raw as a dessert fruit. The pulp may also be boiled or roasted to form a kind of butter. The leaves are pinnate with leaflets measuring 3 to 4cm by 2–3cm. The leaflets are glabrous narrowly oblong and elliptic. Resins or exudates occur in the genus and the resins from some species is used in African medicine. The stem bark yields a resin or exudate which is also primitive oil. The resin is medicinal and is applied to cure skin diseases such as ringworms, craw-craw and wounds. They are also used to treat parasitic organisms such as ticks and jiggers. The exudates are used in food and cosmetic industry as

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ABSTRACT
The ethanolic extract of the stem barks of Dacryodes edulis yielded yellow oil (2.68g). The oil was subjected to GC-MS studies. Thirteen components were identified with ascorbic acid (21.02%) constituting the bulk of the oil, followed by 6-Octadecenoic acid (Z) (20.06%) and Octadecanoic acid contained (13.70%) of the oil. Other monoterpenoids, oxygenated components and fatty acids identified include isooctanol (1.60%), 8-methyl-1-decene (3.18%), 2-Butenedioic acid (Z) monododecyl ester (6.37%), Hexadecanoic acid (6.05%), 9-Tricosene (Z) (7%), 2,3-Diazabicyclo [2.2.1] hept-2-ene (7.96%), 1-Hexadecanol (6.37%) 1-Isopropyl-1-methyl-2-nonylcyclopropane (4.78%) while 2-(2H-1,2,3-Benzotriazol-2-yl)-4-methylphenyl-3-benzoate and 3-methyl-4-heptanone constitutes (0.96%) respectively of the oil. The volatile oils displayed antibacterial activity against Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae and Proteus mirabilis. This result lend credence for the use of Dacryodes edulis stem bark extract in phytomedicine for the treatment of cough, ringworms, craw-craw and wounds in Nigeria.

KEY WORDS: Dacryodes edulis, GC-MS analysis, Bioactive compounds, Pharmaceutical agents.
thickeners, flavors, stabilizers and as emulsifying agents in drugs and cosmetics\textsuperscript{2}. Exudates from \textit{D. edulis} when applied in lotions and creams stabilize emulsion, add smooth to the skin and form protective coating to the skin. The exudates are used in traditional medicine as antibacterial agent and as incense\textsuperscript{3}. It is believed that the smoke and sweet smell from the exudates when burning wades off evil spirit\textsuperscript{3}. Recently exudates from \textit{D. edulis} have been used in cosmetics\textsuperscript{2}. In Nigeria, research has not been extensively conducted for the improvement and refining technology in upgrading the quality of \textit{D. edulis} exudates into high-grade oils that can be harnessed for pharmaceuticals. This paper furnished the chemical constituents of the oil fraction of ethanol extract of the stem bark of \textit{D. edulis} and consequently evaluates the antibacterial activity against some pathogenic bacteria for possible development of new drugs for the prevention and treatment of infections.

**MATERIALS AND METHOD**

**General Experimental Procedure**

GC analyses were carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25nm x 50m) and the conditions were as follows: Temp programming from 80–200°C held at 80°C for 1 min, rate 5°C/min and at 200°C for 20min. FID temp 300°C, injection temp 250°C, carrier gas nitrogen at a flow rate of 1 ml/min, split ratio 1:75. GC–MS [Gas Chromatography Mass Spectrum] analysis was conducted using GCMS – QP 2010 PLUS SHIMADZU JAPAN with injector temperature of 230°C and carrier gas pressure of 100 kpa. The column length was 30 m with a diameter of 0.25 mm and the flow rate of 50 ml/min. The elutes were automatically passed into a mass spectrometer with a dictator voltage set at 1.5kv and sampling rate of 0.2 sec. The mass spectrum was also equipped with a computer fed mass spectra data bank. HERMLE Z 233 M-Z centrifuge Germany was used. Reagents and solvents like ethanol, chloroform, diethyl ether, hexane, were all of analytical grade and were procured from Merck, Germany. The nutrient agar was purchased from Scharian chemie (APHA) Spain.

**Plant Materials**

Fresh leaves and stem barks of \textit{D. edulis} were collected on 15\textsuperscript{th} January 2008, from Umudike, Abia State, Nigeria. Plant materials (stems, fruits and leaves) were identified by Dr. A Nmereghin of Taxonomy Section, Forestry Department, Michael Okpara University of Agriculture, Umudike, Nigeria. Voucher Specimen No DE/311 has been deposited at the Forestry Department of the University.

**Extraction of Plant Materials**

The stem barks (1kg) were cleaned with water and dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (850 g) using a Thomas Wiley Machine (Model 5 USA). The powdered plant sample (300 g) was successively extracted with 2 L of benzene (8 hours/3 times/80°C) followed by 2 L of ethanol (8 hours/3 times/65°C). The extracts were concentrated under reduced pressure and the supernatant yellow oil was decanted (2.68 g) after complete removal of the solvent. The oil was centrifuged at 10,000 rpm for 20 minutes and the clear supernatant oil (1 µl) was subjected to systematic GC and GC-MS analysis.

**Component Identification**

Oil components were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature\textsuperscript{6-8}.

**Bioassay**

The \textit{in vitro} antibacterial activity of the oil was carried out for 24h culture of four selected bacteria. The bacteria organisms used were \textit{Escherichia coli}, \textit{Staphylococcus aureus}, \textit{Streptococcus pneumoniae} and \textit{Proteus mirabilis}. All the test organisms are clinical isolates of human pathogens obtained from the Federal Medical Centre (FMC) Umuahia, Nigeria. Cultures were brought to laboratory conditions by resuscitating the organism in buffered peptone broth (Scharian chemie) and thereafter nutrient agar (peptone 5 g/l and meat extract 3
g/l) and incubated at 37°C for 24 hours. The antibacterial activity was performed by filter paper disc diffusion technique. The medium (7 g nutrient agar in 250 ml distilled water, autoclave at 115°C for 15 mins) was cooled to 50°C. 20 ml of the medium was poured into a sterile Petri-dish and allowed to solidify. It was allowed to stay for 8 hours and observed for contamination. The oil (1 g) was dissolved in 1 ml of absolute ethanol and made up to 10 ml with distilled water to a concentration of 100 mg/ml (10% dilution), 50mg/ml, 25mg/ml, 12.5mg/ml 6.5mg/ml respectively. A colony of each test organism was subcultured on nutrient broth which contained peptone (5g/l) and meat extract (3 g/l) and incubated at 37°C for 8 hours. 30 ml of the nutrient broth was used to flood the agar plates. A sterilized Whatman No. 1 filter paper disc soaked in the oil (0.02 ml) was used to test for the sensitivity or antimicrobial effect of the oil. The plates were incubated at 37°C for 24 hours. After incubation plates were observed for zones of inhibition (in mm diameter). The minimum inhibitory concentration was determined by comparing the different concentrations of the oil.

RESULTS AND DISCUSSION

The oils (2.68 g) obtained from ethanol extract of the stem barks of *D. edulis* on GC-MS analysis showed thirteen peaks (Figure 1) indicating the presence of thirteen
compounds (1-13) in the oil (Table 1). The first compound is a hydrocarbon 1-Isopropyl-1-methyl-2-nonylcyclopropane 1 with molecular formula C_{16}H_{32} (m/z 224). Detachment of pentenyl fragment from compound 1 produces the base peak m/z 70 calculated for C_{5}H_{11} (m/z 71). The composition of compound 1 in the oil was 4.78%. Compound 2 contained 6.37% of the oil with molecular formula C_{13}H_{26}O (m/z 198) and base peak occurred at m/z 55 due to the detachment of a butyl fragment C_{6}H_{13} (m/z 57 calculated). The third compound is a carboxylic acid octadecanoic acid 3. The constituent in the oil was 13.70% with molecular formula of C_{18}H_{34}O_{3} (m/z 282) and base peak of m/z 73 which resulted due to the cleavage of C_{6}H_{13}O (m/z 73) fragment from the compound. Compound 4 is also a carboxylic acid 6-Octadecenoic acid. The spectra 6-Octadecenoic acid has molecular formula C_{18}H_{34}O_{3} (m/z 282) with base peak of m/z 55. The constituent of compound 4 in the oil was found to be 20.06%. Compound 5 has molecular formula of C_{38}H_{66}O_{5} (m/z 652). The exceptional high quantity of compound 5 (21.02%) is noteworthy. The base peak occurred at C_{6}H_{13}O (m/z 73). This peak occurred due to McLafferty re-arrangement. Other prominent peaks observed occurred at m/z 43 (C_{3}H_{7}^{+}), m/z 55 (C_{6}H_{11}^{+}) and m/z 41 (C_{7}H_{8}^{+}). Compound 5 was identified as L-(-)-Ascorbic acid 2,6-dihexadecanolate.

The other constituents of the oil comprises alkaloids, carboxylic acids, ketones and alcohols. Compound 6 which is an alkaloid has molecular formula C_{11}H_{18}N_{2} (m/z 178) and contained 7.96% of the oil. The base peak occurred at m/z 81 calculated for C_{6}H_{13}N_{2} (m/z 80). The compound was identified as 2,3-Diazabicyclo [2.2.1] hept-2-ene. Compound 7 occurred as a hydrocarbon with molecular formula C_{23}H_{46} (m/z 322) and base peak occurred at m/z 83 (C_{6}H_{13}^{+}). The compound was identified as 9-Tricosene (Z) commonly known as muscalure. It is this compound that may be responsible for the turpentine smell of D. edulis and the constituent was 7% of the essential oil. Compound 8 was obtained as carboxylic acid with molecular formula of C_{10}H_{19}O_{2} (m/z 256) and base peak at C_{3}H_{7}O_{2} (m/z 73). The compound constitutes 6.05% of the oil. It is identified as n-Hexadecanoic acid also known as palmitic acid. Compound 9 is a carboxylic acid ester with molecular formula C_{16}H_{30}O_{4} (m/z 284) and base peak at m/z 83 (C_{6}H_{11}). The compound constitutes 6.37% of the oil. It was identified as 2-Butenedioic acid (Z). Compound 10 is a hydrocarbon with molecular formula C_{11}H_{22} (m/z 154) and base peak at m/z 55 (C_{4}H_{7}^{+}). It comprises 3.18% of the oil. It was identified as 8-methyl-1-decene. Compound 11 is a ketone with molecular formula C_{6}H_{10}O (m/z 128) and base peak C_{4}H_{7} (m/z 43). The base peak occurred due to alpha cleavage at C_{3} of the compound. The compound which comprises 0.96% of the oil was identified as 3-methyl heptan-4-one. Compound 12 is identified as an alcohol 6-methyl heptan-1-ol with molecular formula of C_{6}H_{15}O (m/z 130) while the base peak occurred at m/z 43 (C_{4}H_{7}). It comprises 1.60% of the oil. Compound 13 is an alkaloid obtained as benzoic acid ester with molecular formula C_{28}H_{27}N_{2}O_{2} (m/z 343) and base peak at m/z 119. The compound was identified as 2-[2H-1,2,3-Benzotriazo-2-yl]-4-methylphenyl-3-benzoate and comprises 0.96% of the oil.

Benzoic acids and its esters are employed externally as antiseptics, lotions, ointments, creams and mouth washes. It is more effective as a preservative in foods and pharmaceutical products. Benzoic acid is an antiseptic but irritating, so used only externally. It is used in the treatment of burns, frostbite, chaps, cracks, erythema, pruritus, ulcers, infected dermatitis and other minor wounds. The occurrence of 2-(2H-1,2,3-Benzotriazo-2-yl)-4-methyl phenyl-3-benzoate and L-(+) Ascorbic acid. 2,6-dihexadecanolate in D. edulis stem barks may be the reason for the use of the resins or exudates from D. edulis in the treatment of skin diseases such as ringworms, craw-craw and wounds. Natural ascorbic acid is crucial for the body performance. It possesses anti-scorbutic activity. Ascorbic acid in the body helps in absorption from the intestine. It is required for connective metabolism especially the scar tissue, bones and teeth. It is necessary as an anti-stress and protects against colds.
DONATUS EBERE OKWU, et al. ANTIBACTERIAL ACTIVITY OF THE STEM BARKS OF DACRYODES EDULIS G. DON LAM

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### Table 1: GC-MS Analysis of the various fractions from the stem barks of Dacryodes edulis

<table>
<thead>
<tr>
<th>Chromatogram Peak</th>
<th>Compound Name</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Retention Time (Mins)</th>
<th>Percentage Content</th>
<th>Fragment Peaks (m/z) and % Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cyclopropane 1-methyl-1-(1-methylethyl)-2-nonyl</td>
<td>C₁₉H₃₂</td>
<td>224</td>
<td>31.9</td>
<td>4.78</td>
<td>41(40%), 55(80%), 69(70%), 70(100%), 97(50%), 111(30%), 125(20%), 181(10%), 224(20%).</td>
</tr>
<tr>
<td></td>
<td>(1-Isopropyl-1-methyl-2-nonyl-cyclopropane)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1-Hexadecanol, n-Hexadecan-1-ol, n-Hexdecanol, n-1-Hexdecanol</td>
<td>C₁₆H₃₄O</td>
<td>242</td>
<td>30.6</td>
<td>6.37</td>
<td>27(20%), 41(70%), 59(100%), 69(80%), 83(60%), 97(60%), 111(30%)</td>
</tr>
<tr>
<td>3</td>
<td>Octadecanoic acid stearic acid n-Octadecanoic acid Humko Industreme</td>
<td>C₁₈H₃₆O₂</td>
<td>284</td>
<td>29.6</td>
<td>13.70</td>
<td>27(20%), 41(70%), 43(90%), 60(80%), 73(100%), 85(30%), 98(30%), 115(20%), 129(50%), 143(20%), 171(20%), 185(30%), 199(10%), 227(10%), 241(20%), 284(30%).</td>
</tr>
<tr>
<td>4</td>
<td>6-Octadecenoic acid (Z) (6Z)-6-Octadecenoic acid</td>
<td>C₁₈H₃₄O₂</td>
<td>282</td>
<td>29.5</td>
<td>20.06</td>
<td>27(20%), 41(90%), 55(100%), 69(80%), 83(70%), 97(60%), 98(60%), 114(20%), 137(10%), 222(10%), 264(30%), 282(10%)</td>
</tr>
<tr>
<td>5</td>
<td>L(+-)-Ascorbic acid, 2,6-dihexadecanoate</td>
<td>C₁₉H₃₆O₃</td>
<td>652</td>
<td>28.3</td>
<td>21.02</td>
<td>27(20%), 41(40%), 43(90%), 57(96%), 73(100%), 85(40%), 98(60%), 115(40%), 129(50%), 143(20%), 157(30%), 171(30%), 185(30%), 199(10%), 213(20%), 227(10%), 239(30%), 256(30%)</td>
</tr>
</tbody>
</table>
chills and dumps. It prevents muscle fatigue and scurvy which is characterized by the hemorrhages, bleeding gums, fragile bones, anemia and pains in the joints and defects in skeletal calcification. This function of ascorbic acid also accounts for its requirement for normal wound healing\textsuperscript{10}. This also supported the use of \textit{D. edulis} in treating wounds by the natives in Nigeria. Ascorbic acid acts as antioxidant in the skin by scavenging and quenching free radicals generated by ultraviolet (UV) radiation stabilization. Ascorbic acid is an important antioxidant. It acts as an electron donor for eight important enzymes in humans\textsuperscript{11,12}. Ascorbic acid may protect against the oxidative damage of light in the eye\textsuperscript{13} and may also play an important role in sperm maturation\textsuperscript{14}. It helps in stabilizing various plasma components and has been shown to be an effective scavenger of superoxide radical anion (H$_2$O$_2$), the hydroxyl radical (OH$^-$), singlet oxygen (O$_2^-$) and reactive

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Concentration of essential oil 1 mg/ml</th>
<th>Mic mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus}</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>\textit{Escherichia coli}</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>\textit{Streptococcus pneumoniae}</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>\textit{Proteus mirabilis}</td>
<td>8</td>
<td>3</td>
</tr>
</tbody>
</table>

Data are means of triplicate determinations
- No inhibition

Table 2: Inhibitory effects of the essential oils from the stem bark of \textit{Dacryodes edulis}
nitrogen oxide (NO)\textsuperscript{15}. Fatty acids always occur in plants. The presence of fatty acids, aromatics and alkaloids in \textit{D. edulis} shows the pharmacological properties of the plant. Fatty acids and alcohols in the plant undergo esterification reaction to form esters which frequently exudes out of the plant as resins/exudates and is used in African medicine in treating wounds, craw-craw and ringworms\textsuperscript{3,4}. Insecticidal and germicidal activities of the stem barks of \textit{D. edulis} may be due to the synergistic effect of these chemical constituents or any single chemical compound may have the toxic effect. The dominant component of the essential oil of this plant \textit{L-(-)-Ascorbic acid 2,6-dihexadecanoate has been reported to have antioxidant, anti-inflammatory and anti-nociceptive properties}\textsuperscript{10}

The oil exhibited antibacterial activity \textit{in vitro} against some pathogenic microorganisms (Table 2). The oil successfully inhibited \textit{S. aureus}, \textit{E. coli}, \textit{S. pneumoniae} and \textit{P. mirabilis}. The observed inhibiting role on these pathogens explains the reason behind the utilization of \textit{D. edulis} in traditional medicine as cough suppressant and wound healing activity\textsuperscript{9}. This however, supported the findings of Igoli \textit{et al}\textsuperscript{16} who reported that \textit{D. edulis} stem barks extract is used in treating cough in Igede traditional medicine in Nigeria. The stem barks of \textit{D. edulis} possess phyto-constituents capable of inhibiting the growth of microbial wound contaminants; accelerate wound healing and consequently resulting to cell proliferation. \textit{D. edulis} apart from being important food crop also produces other non-timber natural products. It is an economic tree which can greatly improve the income of the peasant farmers and provide raw materials for the pharmaceutical industries. If judiciously extracted and processed, the stem barks of \textit{D. edulis} could provide raw materials for the pharmaceutical industries in the country.

Acknowledgments

The authors are grateful to Dr. N.O. Eddy of Ahamadu Bello University Zaria and Mr. Ime Ekanem of the Akwa Ibom State Polytechnic for their help in GC-MS analysis.

Our gratitude goes to Dr. A. Nmeregini of the Forestry Department, Michael Okpa ra University of Agriculture Umudike, Nigeria who has been kind in identifying our plant samples.

References

17. DE Okwu and IN Emenike (2006) Evaluation of the

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Source of Support: NIL