INT.J.DRUG DEV & RES., SEP-DEC 2009;1(1):117-125
©www.ijddr.com, 2009

INTERNATIONAL JOURNAL OF DRUG DEVELOPMENT AND RESEARCH

RESEARCH ARTICLE

GC-MS EVALUATION OF THE BIOACTIVE COMPOUNDS AND ANTIBACTERIAL ACTIVITY OF THE OIL FRACTION FROM THE STEM BARKS OF *DACRYODES EDULIS* G. DON LAM

DONATUS EBERE OKWU AND BENSON UYIOSANO IGHODARO*

Department of Chemistry
Michael Okpara University of Agriculture, Umudike
P.M.B. 7267 Umuahia, Abia State, Nigeria.

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-Butenedioc 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-methylpheny-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.*

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[1]. It grows up to 18m in height and exudes an odoriferous gummy substance from injured or excised portion of the stem[2,3]. 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[3,4]. The leaves are pinnate with leaflets measuring 3 to 4cm by 2–3cm. The leaflets are glabrous narrowly oblong and elliptic[2].

Resins or exudates occur in the genus and the resins from some species is used in African medicine[3,4]. 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[3]. They are also used to treat parasitic organisms such as ticks and jiggars[5]. The exudates are used in food and cosmetic industry as

***Corresponding Author:** *e-mail: : okwudonatus@yahoo.com*
Received on: 30-10-09 ; Accepted on: 28-12-09

thickeners, flavors, stabilizers and as emulsifying agents in drugs and cosmetics[2]. Exudates from *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[3]. It is believed that the smoke and sweet smell from the exudates when burning wades off evil spirit[3].

Recently exudates from *D. edulis* have been used in cosmetics[2]. In Nigeria, research has not been extensively conducted for the improvement and refining technology in upgrading the quality of *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 *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^{o}C held at 80^{o}C for 1 min, rate 5^{o}C/min and at 200^{o}C for 20min. FID temp 300^{o}C, injection temp 250^{o}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^{o}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 *D. edulis* were collected on 15th January 2008, from Umudike, Abia State, Nigeria. Plant materials (stems, fruits and leaves) were identified by Dr. A Nmeregini 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^{o}C) followed by 2 L of ethanol (8 hours/3 times/65^{o}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[6-8].

Bioassay

The *in vitro* antibacterial activity of the oil was carried out for 24h culture of four selected bacteria. The bacteria organisms used were *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *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

*Corresponding Author: e-mail: : okwudonatus@yahoo.com
Received on: 30-10-09 ; Accepted on: 28-12-09

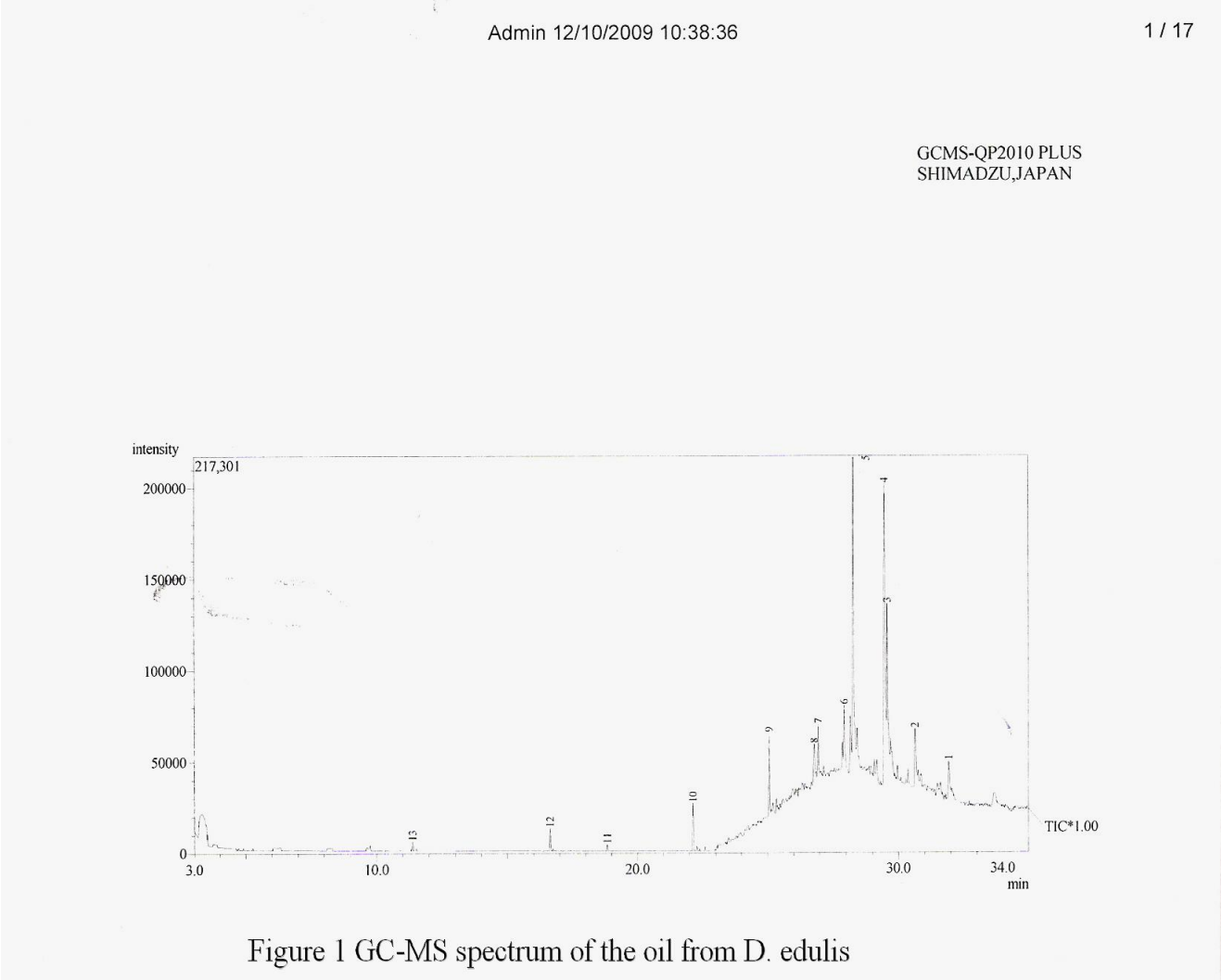


Figure 1 GC-MS spectrum of the oil from D. edulis

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 sub-cultured 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

***Corresponding Author:** *e-mail: : okwudonatus@yahoo.com*
Received on: 30-10-09 ; Accepted on: 28-12-09

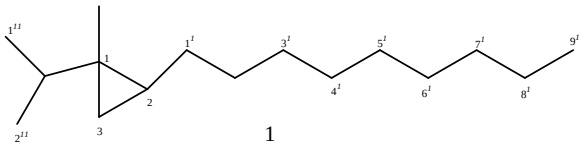
compounds (**1-13**) in the oil (Table **1**). The first compound is a hydrocarbon 1-Isopropyl-1-methyl-2-nonylcyclopropane **1** with molecular formula $C_{16}H_{32}$ (m/z 224). Detachment of pentenyl fragment from compound **1** produce the base peak m/z 70 calculated for C_5H_{11} (m/z 71). The composition 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_4H_9 (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 $C_{18}H_{36}O_2$ (m/z 284) and base peak of m/z 73 which resulted due to the cleavage of $C_3H_5O_2$ (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 $C_{18}H_{34}O_2$ (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 $C_{38}H_{68}O_8$ (m/z 652). The exceptional high quantity of compound **5** (21.02%) is noteworthy. The base peak occurred at $C_3H_5O_2$ (m/z 73). This peak occurred due to McLafferty re-arrangement. Other prominent peaks observed occurred at m/z 43 ($C_3H_7^+$), m/z 55 (C_4H_7) and m/z 41 ($C_3H_5^+$). Compound **5** was identified as L-(+)-Ascorbic acid 2,6-dihexadecanoate.

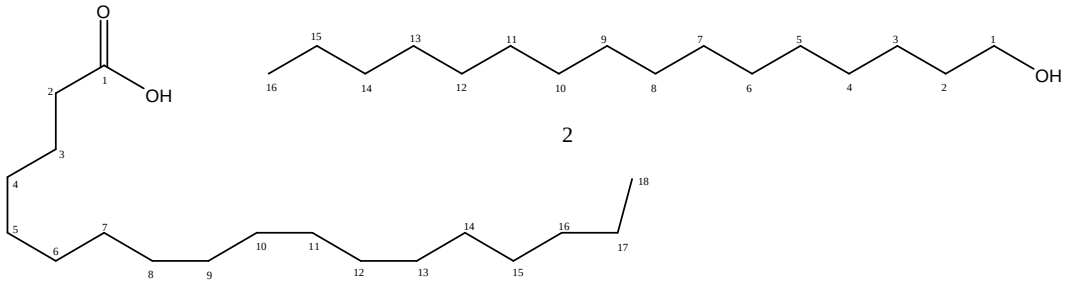
The other constituents of the oil comprises alkaloids, carboxylic acids, ketones and alcohols. Compound **6** which is an alkaloid has molecular formula $C_{11}H_{18}N_2$ (m/z 178) and contained 7.96% of the oil. The base peak occurred at m/z 81 calculated for $C_4H_4N_2$ (m/z 80). The compound was identified 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 ($C_6H_{11}^+$). 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 $C_{16}H_{32}O_2$ (m/z 256) and base peak at $C_3H_5O_2$ (m/z 73). The compound constitutes 6.05% of the oil. It is identified as n-Hexadecaonic acid also known as palmitic acid. Compound **9** is a carboxylic acid ester with molecular formula $C_{16}H_{28}O_4$ (m/z 284) and base peak at m/z 83 (C_6H_{11}). The compound constitutes 6.37% of the oil. It was identified as 2-Butenedioc acid (Z). Compound **10** is a hydrocarbon with molecular formula $C_{11}H_{22}$ (m/z 154) and base peak at m/z 55 ($C_4H_7^+$). It comprises 3.18% of the oil. It was identified as 8-methyl-1-decene. Compound **11** is a ketone with molecular formula $C_8H_{16}O$ (m/z 128) and base peak C_3H_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 $C_8H_{18}O$ (m/z 130) while the base peak occurred at m/z 43 (C_3H_7). It comprises 1.60% of the oil. Compound **13** is an alkaloid obtained as benzoic acid ester with molecular formula $C_{21}H_{17}N_3O_2$ (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[9]. 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[9]. The occurrence of 2-(2H-1,2,3-Benzotriazo-2-yl)-4-methyl phenyl-3-benzoate and L-(+)-Ascorbic acid. 2,6-dihexadecanoate 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[3]. 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[11]. It is required for connective metabolism especially the scar tissue, bones and teeth[11]. It is necessary as an anti-stress and protects against colds,

***Corresponding Author:** *e-mail: : okwudonatus@yahoo.com*
Received on: 30-10-09 ; Accepted on: 28-12-09

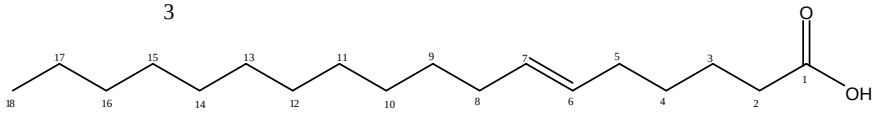


1

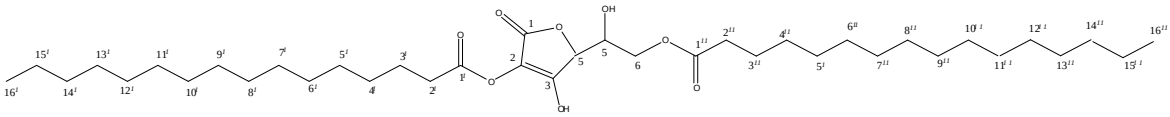


2

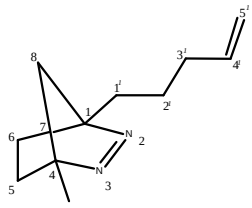
3



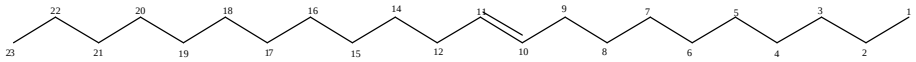
4



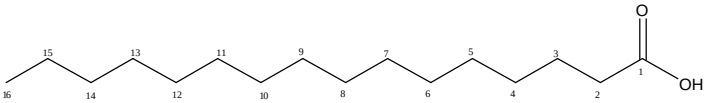
5



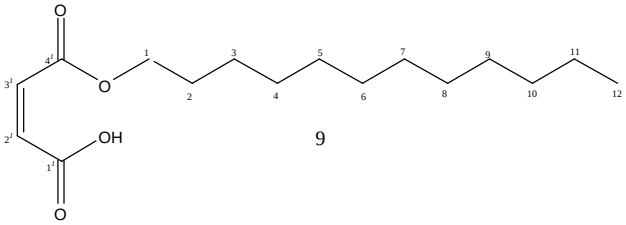
6



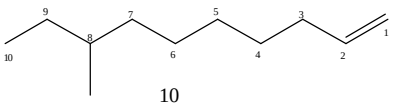
7



8



9



10

***Corresponding Author:** *e-mail: : okwudonatus@yahoo.com*

Received on: 30-10-09 ; Accepted on: 28-12-09

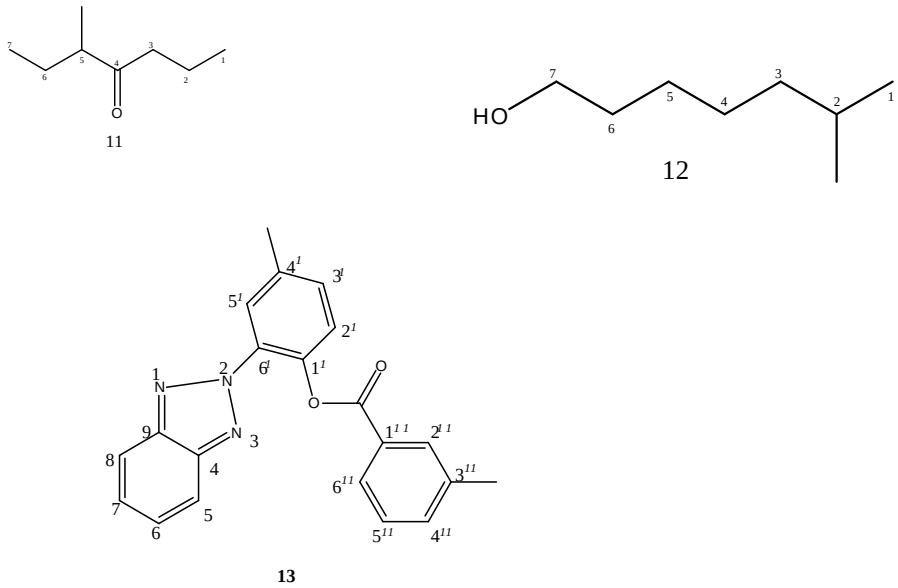


Figure 2: Structures of the compounds from GC-MS Analysis of the oil from stem bark of *Dacryodes edulis*

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

Chromato-gram Peak	Compound Name	Molecular Formula	Molecular Weight	Retention Time (Mins)	Percentage Content	Fragment Peaks (m/z) and % Abundance
1	Cyclopropane 1-methyl-1-(1-methylethyl)-2-nonyl (1-Isopropyl-1-methyl-2-nonyl-cyclopropane)	$C_{16}H_{32}$	224	31.9	4.78	41(40%), 55(80%), 69(70%), 70(100%), 97(50%), 111(30%), 125(20%), 181(10%), 224(20%).
2	1-Hexadecanol, n-Hexadecan-1-ol, n-Hexdecanol, n-1-Hexdecanol	$C_{16}H_{34}O$	242	30.6	6.37	27(20%), 41(70%), 59(100%), 69(80%), 83(60%), 97(60%), 111(30%)
3	Octadecanoic acid stearic acid n-Octadecanoic acid Humko Industreme R	$C_{18}H_{36}O_2$	284	29.6	13.70	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%).
4	6-Octadecenoic acid (Z) (6Z)-6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	29.5	20.06	27(20%), 41(90%), 55(100%), 69(80%), 83(70%), 97(60%), 98(40%), 114(20%), 137(10%), 222(10%), 264(30%), 282(10%)
5	L(+)-Ascorbic acid,2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	28.3	21.02	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%)

***Corresponding Author:** *e-mail: : okwudonatus@yahoo.com*

Received on: 30-10-09 ; Accepted on: 28-12-09

6	2,3-Diazabicyclo[2.2.1] hept-2-ene 4methyl-1-(pent-4-en-1-yl 1-methyl-4-(4-pentenyl)-2,3- diazabicyclo[2.2.1] hept-2-ene	$C_{11}H_{18}N_2$	178	27.93	7.96	38(20%), 41(30%), 55(20%), 67(20%) 81(100%), 93 (20%), 107(30%)
7	9-Tricosene (Z)-9-Tricosene Cis-9-Tricosene Muscalure (9Z)-Tricosene (9Z)-Tricosene	$C_{23}H_{46}$	322	26.94	7.00	27(10%), 41(60%) 55(90%), 83(100%), 97(90%), 111(60%), 125 (30%), 139(20%)
8	n-Hexadecanoic acid Hexadecanoic acid n-Hexadecoic acid Palmitic acid Pentadecanecarboxylic acid 1-Pentadecanecarboxylic	$C_{16}H_{32}O_4$	256	26.90	6.05	27(20%), 41(80%), 43(90%), 73(100%), 85(20%), 98(10%), 157(5%), 213(20%), 256(20%)
9	2-Butenedioic acid (Z) monododecyl ester (2Z)-4-(Dodecyloxy)-4-oxo-2-butenoic	$C_{16}H_{28}O_4$	284	25.06	6.37	26(30%), 41(60%), 55(90%), 69(90%), 83(100%), 97(60%), 112(30%), 126(10%), 140(20%), 168(20%).
10	1-Decene, 8-methyl 8-methyl-1-decene	$C_{11}H_{22}$	154	22.13	31.18	27(20%), 41(80%), 55(100%), 69(90%), 70(60%), 84(50%), 98(10%)
11	4-Heptanone, 3-methyl 3-methyl-4-heptanone	$C_8H_{16}O$	128	18.83	0.96	27(50%), 41(40%), 43(100%), 57(90%) 71(90%), 85(10%)
12	Isooctanol Isooctyl achohol 6 methyl-1-heptanol	$C_8H_{16}O$	130	16.63	1.60	27(30%), 41(90%), 43(100%), 57(90%), 70(50%), 84(40%)
13	Benzoic acid, 3-methyl [2-[2-benzotriazoly 1-4-methylphenyl ester 2-(2H-1,2,3-Benzotriazo 1-2-yl)-4-methyl phenyl-3-benzoate	$C_{21}H_{17}N_3O_2$	343	11.38	0.96	65(10%), 91(60%), 343(10%) 119(100%)

Table 2: *Inhibitory effects of the essential oils from the stem bark of Dacryodes edulis*

Pathogens	Concentration of essential oil 1 mg/ml				Mic mg/ml
	50	25	12.5	6.5	
Staphylococcus aureus	10	8	3	-	12.5
Escherichia coli	12	8	6	-	12.5
Streptococcus pneumoniae	8	6	-	-	25
Proteus mirabilis	8	3	-	-	25

Data are means of triplicate determinations
- No inhibition

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[10]. This also supported the use of *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[11,12]. Ascorbic acid may protect against the oxidative damage of light in the eye[13] and may also play an important role in sperm maturation[14]. It helps in stabilizing various plasma components and has been shown to be an effective scavenger of superoxide radical anion (H_2O_2), the hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($O^{\cdot}$) and reactive

*Corresponding Author: e-mail: : okwudonatus@yahoo.com
Received on: 30-10-09 ; Accepted on: 28-12-09

nitrogen oxide (NO)[15]. Fatty acids always occur in plants. The presence of fatty acids, aromatics and alkaloids in *D. edulis* shows the pharmacological properties of the plant. Fatty acids and alcohols in the plant undergoes 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[3,4]. Insecticidal and germicidal activities of the stem barks of *D. edulis* may be due to the synergetic 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 L-(+)-Ascorbic acid 2,6-dihexadecanoate has been reported to have antioxidant, anti-inflammatory and anti-nociceptive properties[10].

The oil exhibited antibacterial activity *in vitro* against some pathogenic microorganisms (Table 2). The oil successfully inhibited *S. aureus, E. coli, S. pneumoniae* and *P. mirabilis*. The observed inhibiting role on these pathogens explains the reason behind the utilization of *D. edulis* in traditional medicine as cough suppressant and wound healing activity[5]. This however, supported the findings of Igoli *et al*[16] who reported that *D. edulis* stem barks extract is used in treating cough in Igede traditional medicine in Nigeria. The stem barks of *D. edulis* possess phyto-constituents capable of inhibiting the growth of microbial wound contaminants; accelerate wound healing and consequently resulting to cell proliferation. *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 *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

1. *OM Agbogidi, EC Enujeke and OF Eshegbeyi (2007) :Germination and seedling Growth of African Pear (Dacryodes edulis Don. G. Lam H.J. As affected by different planting media. American Journal of Plant Physiology 2(4): 282 – 286.*
2. *O Ekpa (1993). The use of Raphia horkeri and Pachylobus odulis in cosmetic formulation. Discovery and innovation 5:312 – 313.*
3. *DE Okwu and FU Nnamdi (2008) Evaluation of the Chemical composition of Dacryodes edulis and Raphia hookeri mann and Wendl exudates used in herbal medicine in South Eastern Nigeria. Afr. J. Trad. CAM 5(1): 194 – 200.*
4. *RRB Leakay (1999) Potential for Novel Food Products from Agro Forestry Trees. A review Food Chemistry 66: 1 – 4.*
5. *E Hutchinson, JMJ Dalziel and FN Hepper (1963) Flora of Africa (Vol. 11) Macmillan Publishers Ltd Lagos Pp 252 – 260.*
6. *RP Adams (2001) Identification of Essential Oil Components Chromatography/Mass Spectroscopy. Allured Publishing Co Carol Stream IL.*
7. *NW Davies (1990) Gas Chromatographic retention indices of monoterpnes and sesuiterpnes on methyl silicone and carbonwax 20 m phase. J. Chromatog 503: 1 – 24.*
8. *WG Jenninas and T Shibamoto (1980) Qualitative analysis of flavour and fragrance volatiles by glass capillary gas chromatography. Academic New York Pp. 201 – 205.*
9. *AJD Britto and PAS Selvakymari (2006). GC-MS study of the oil fraction of methanol extract of the Corm Typhonium roxburghii. Journal of Medicinal and Aromatic Plant Sciences 28: 578 – 579.*
10. *J. Brnneton (1999) Pharmacognosy, Second Edition, Lavoisier Publishers, Inc USA Pp. 310 – 367.*
11. *AC Akinmoladun, EO Ibukun, E Afor, BL Akinsinlola, TR Onibon, AO Akinboboye, EM Obuofor and EO Farombi (2007) Chemical Constituents and antioxidant activity of Alstonia boonei. African Journal of Biotechnology. 6(10): 1197 – 1201.*
12. *S Moncada and EA Higgs (1993) Mechanisms of diseases: The L-arginine nitri oxide Pathway N. Engl J. Med. 329: 2002 – 2012.*
13. *TK Koskela, GR Resis, RF Brubaker, RD Ellefson (1989) Is the high concentration of ascorbic acid in the eye an adaptation to intense solar irradiation. Invest ophthalmol Vis. Sci. 30: 2265 – 2267.*
14. *DH Horning (1975) Distribution of ascorbic acid, metabolites and analogues is man and animals. Annals of the New York Academy of Sciences 258: 103 – 118.*
15. *SR Tannenbanm, JS Wishnok, CD Leaf (1991) Inhibition of nitrosamine formation by ascorbic acid. AM. J. Clin. Nut 53 (Suppl:) 5247 – 250.*
16. *JO Igoli, OG Ogali, TA Tor-Anyim and PN Igoli (2005) Traditional Medicine Practice amongst the Igede people of Nigeria Part II. Afri. J. Trad CAM 2(2): 134 – 152.*
17. *DE Okwu and IN Emenike (2006) Evaluation of the*

***Corresponding Author:** *e-mail: : okwudonatus@yahoo.com*
Received on: 30-10-09 ; Accepted on: 28-12-09

Phytonutrients and Vitamins content of citrus fruits. Int. J. Mol. Med. Advance Sci. 2(1): 1 – 6.

****

Conflict of Interest: NONE

Source of Support: NIL

INTERNATIONAL JOURNAL OF DRUG DEVELOPMENT AND RESEARCH

***Corresponding Author:** *e-mail: : okwudonatus@yahoo.com*

Received on: 30-10-09 ; Accepted on: 28-12-09