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Gas Chromatography-Mass Spectrometry Analysis of Oil Extracted from Seeds of *Dacryodes edulis* (G. Don) H. J. Lam.

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Abstract

There is paucity of information on the bioactive chemical compounds present in oil extracted from seeds of *Dacroydes edulis*. Against the backdrop of the foregoing, this present study was aimed at identifying and quantifying bioactive chemical compounds present in the oil of *D. edulis* seeds. The research was carried out in Akwa Ibom State, Nigeria. Oil was extracted from seeds of *D. edulis*, with η-hexane being used as extracting solvent. The process of oil extraction was carried out at 60 °C. Using Gas Chromatography-Mass Spectrometry (GC-MS), bioactive compounds present in the oil of *D. edulis* seeds were identified and quantified. The yield of oil extracted from *D. edulis* seeds was 40.1%. A total of thirty-two (32) bioactive chemical compounds were detected during the GC-MS analysis. Some of the identified compounds were: Hexadecane, 2,6,10,14-tetramethyl; Octadecane, 3-methyl; Heptadecane, 9-octyl; Tricosane, 2-methyl; Octadecanoic acid; L-Ascorbic acid, Riboflavin, 1,2-Benzenedicarboxylic acid; 2-(5-Thiophen-2-yl-1H-pyrazol-3-yl)-phenol. Of these compounds, L-Ascorbic acid ($C_{24}H_{42}O_7$) was found to have the highest concentration (10.36%) in the oil of *D. edulis* seeds. Oil from seeds of *D. edulis* contains bioactive compounds which are of therapeutic and industrial potential.

Keywords: Seeds, Extraction, Oil**,** Compounds, Sample

1.0 Introduction

Dacryodes edulis, commonly known as African Pear, is a well-known plant in West Africa. In Igbo, it is called *ube*, while the Ibibios call it *eben.* The plant belongs to the family of Burseraceae (Zofou *et al.*, 2013). *D. edulis* can be cultivated widely, because of its ability to

adapt to changes in agronomical variables, photoperiodism, soil, rainfall, temperature and altitudes (Olivier *et al.,* 2016).

Its ripe fruit is blue-black, with unpleasant turpentine smell when unripe. It is oval and matures within the months of May and June. Its fruit consists of large seeds, surrounded by thin mesocarp (Olivier *et al.,* 2016). The pulp of the fruit is very rich in protein, fat, fibre, minerals, and essential amino acids. Consumption of the fruits usually takes place between the first and third quarters of the year (April – September) (Onuegbu *et al.,* 2016). In some cultures, heat is applied using hot water or ash, to soften the fruits; and they are consumed as a side dish to bread, roasted or boiled corn and yam. The seed is, however, not eaten and are often discarded as waste. They eventually get consumed by birds and domestic animals, just before decomposition takes place (Onuegbu *et al.,* 2016).

In African traditional medicine, *D. edulis* is multipurpose. This is because various parts of the plants are employed in the treatment of a number of ailments, according to researches. For instance, the bark of *D. edulis*, when pulverized, can be applied on a wound to cicatrize it (Okunomo and Egho, 2010). The bark of *D. edulis* can be employed in the treatment of anaemia, dysentery, stiffness, debility, leprosy, tonsillitis, etc. (Igoli *et al.,* 2005). Ajibesin *et al.* (2008) reported that widespread skin diseases such as ringworm and scabies are treated with the juice obtained from crushed leaves of *D. edulis*. Moreso, stem or stem twigs of *D. edulis* can be used as chewing stick (Ajibesin *et al.,* 2008).

Koudou *et al.* (2008) evaluated volatile components, antioxidant, and antimicrobial properties of the essential oil of *D. edulis* from Gabon. In 2015, Ogboru *et al.* analysed the quality and quantity of phytoconstituents inherent in the bark of *D. edulis*, sourced from Benin City and South-South Nigeria. The study revealed that alkaloids (18.13 mg/kg), Phenolic compounds (22.01 mg/kg), Flavonoids (60.91 mg/kg), Tannins (18.16 mg/kg), Saponins (3.16 mg/kg), Anthraquinones (12.16 mg/kg), Cardiac glycosides (0.81 mg/kg) and steroids (0.91 mg/kg) were significantly present in the sample. The presence of these phytochemicals gives credence to the medicinal benefits that the bark of this plant has been used for in the past years (Ogboru *et al.,* 2015). Okwu and Nnamdi (2008) reported the phytochemical contents and importance of *D. edulis* and *Raphia hookeri* exudates in traditional medicine. From results of previous studies, it can be inferred that indigenous people derive food from various parts of *D. edulis*; and potential feedstock from its exudates can be of immense benefit to the pharmaceutical industry (Olivier *et al.,* 2016).

Although the consumption of *D. edulis* fruit has been widespread in the southern part of Nigeria, there is paucity of information on the characterization of oil in the seed. The study, therefore, was aimed at identifying and quantifying bioactive chemical compounds present in the oil of *D. edulis* seeds.

The scientific classification for *D. edulis* is as follows: Kingdom-Plantae, Phylum-Tracheophyta, Class –Magnoliopsida, Order-Spindales, Family-Burseraceae, Genus-*Dacryodes*, Species-*D. edulis* (G. Don.) H. J. Lam (Ajibesin *et al.*, 2008).

2.0 Materials and Methods

2.1 Sample Collection and Preparation

Eight (8) fresh and healthy seeds of *D. edulis,* were sourced from Akpan Andem Market in Uyo Local Government Area of Akwa Ibom State.

Fruits of *D. edulis* were harvested; cleaned, extraneous materials washed off and exfoliated. Foreign materials and dirt found in the seeds were removed by handpicking. These were done according to the methods of Adebayo *et al.* (2012). The dressed seeds were broken down into smaller pieces using mortar and pestle. The chopped seeds were then dried under room temperature in the Department of Botany Laboratory, Akwa Ibom State University, Mkpat Enin, Nigeria. Upon drying, the seeds were pulverized with a laboratory blender (LEXUS MG-2053 OPTIMA).

2.2 Extraction of the Oil

Soxhlet extraction as described by Adebayo *et al.* (2012) was employed in extracting oil from the experimental seeds. A round bottom flask was filled with 300 ml of the solvent (η-hexane); 10 g of the milled sample was then loaded into a thimble. This was placed in the middle of the extractor. A temperature range of 40 – 60 °C was used to heat the Soxhlet.

Upon vapourization of the solvent, the extract was removed, dried, cooled and weighed, to determine the amount of oil extracted. On completion of extraction, the resultant mixture was heated to recover solvent from the oil. The oil was then concentrated by heating in the oven at 60 °C to ensure complete evaporation of the solvent and latter cooled in a desiccator. The percentage oil yield for the seed sample was estimated using the expression:

$$\text{Oil yield (\%)} = \frac{weight\ of\ oil\ extracted}{weight\ of\ sample}\ x\ 100 \quad \text{.....Equation 2.1}$$

All chemicals used for the experiments were of analytical grade and products of British Drug House, Poole, England.

2.3 Gas Chromatography-Mass Spectrometry (GC-MS) Screening

2.3.1 Column Program

The Agilent 8860 gas chromatography connected to a 5977B MSD was used for the analysis. One microlitre of the sample was injected in the pulsed spitless mode onto a 30 x 0.25mm ID DB 5MS coated fused silica column with a film thickness of 0.15 micrometre. Helium gas was used as a carrier gas. The column head pressure was maintained at 20psi to give a constant of 1ml/minute. Other operating conditions were preset. The column temperature was initially held at 55 °C for 0.4 minute, increased to 200 °C at a rate of 25 °C/minute, then to 280 °C at a rate of 8 °C/minute and to a final temperature of 300 °C at a rate of 25 °C/minute, held for 2 minutes for a total run time of 12.09 minutes. Identification of bioactive components was based on retention time. Components with lower retention time were the first to elute. These were followed by components with higher retention time.

2.3.2 Sample Preparation

Fifty (50) mg of the sample was weighed into a beaker. 10 ml of the solvent mix (1:1 – Hexane: Dichloromethane) was measured into the beaker to homogenize the sample. The homogenized sample was cleaned up with 100 – 200 mm mesh silica gel and 3 g of anhydrous sodium sulfate in a well-packed column, conditioned with hexane to form a slurry. 1 microlitre of the sample was injected through the injection port into the GC-MSD.

3.0 Results

Pulverization of the seed sample produced a mass of 60.0 g. When without oil, mass of the flask was 30.0 g. Upon addition of oil, a mass of 54.06 g was obtained. In essence, this means that mass of the oil alone was 24.06 g. The percentage yield of oil, calculated using equation 2.1, therefore, was 40.10%.

Table 3.1: Result of Oil Extraction from Seeds of *D. edulis*, using η-Hexane

Experimental seeds	Mass of pulverized sample (g)	Mass of flask without oil (X) (g)	Mass of flask with oil (Y) (g)	Mass of oil alone (Y – X) (g)	Yield of oil (%)
Dacryodes edulis	60.0	30.0	54.06	24.06	40.10

Table 3.2: Bioactive Chemical Compounds Identified in *D. edulis* Seed Oil extracted, using η-Hexane

S/N	Compound Name	Molecular Formula	Molecular Weight ($kg.mol^{-1}$)	Concentration (%)
1	Octadecane	$C_{18}H_{38}$	254	3.799
2	Dodecane, 2,6,11-trimethyl	$C_{15}H_{32}$	212	9.779
3	Hexadecane, 2,6,10,14-tetramethyl	$C_{20}H_{42}$	282	7.451
4	Octadecane, 3-methyl	$C_{19}H_{40}$	268	4.236
5	Heptadecane, 9-octyl	$C_{25}H_{52}$	352	7.451
6	Docosane, 2,21-dimethyl	$C_{24}H_{50}$	338	8.937
7	Tricosane, 2-methyl	$C_{24}H_{50}$	338	9.393
8	Octadecanoic acid	$C_{18}H_{36}O_2$	284	10.470
9	L-Ascorbic acid, 6-octadecanoate	$C_{24}H_{42}O_7$	442	10.363
10	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{39}H_{76}O_5$	624	9.651
11	Octadecanoic acid, propyl ester	$C_{21}H_{42}O_2$	326	0.675
12	Octadecanoic acid, 10,12-bis[(trimethylsilyl)oxy]-, methyl ester	$C_{25}H_{54}O_4Si_2$	474	7.408
13	Octadecanoic acid, 10,13-bis[(trimethylsilyl)oxy]-, methyl ester	$C_{25}H_{54}O_4Si_2$	474	5.186
14	Octadecanoic acid, 9,10-dihydroxy-, methyl ester, bis(trifluoroacetate)	$C_{23}H_{36}F_6O_6$	522	3.646
15	Octadecanoic acid, 9,10-dichloro-, methyl ester	$C_{19}H_{36}Cl_2O_2$	366	0.077
16	Riboflavin, 2',3',4',5'-tetraacetate	$C_{25}H_{28}N_4O_{10}$	544	0.104
17	Riboflavin	$C_{17}H_{20}N_4O_6$	376	0.074
18	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	166	1.301
19	Phthalaldehydic acid, oxime	$C_8H_7NO_3$	165	0.0001
20	2-Pyridinecarbonitrile	$C_6H_4N_2$	104	0.0001
21	3H-Bisthiazolo[4,5-b:5,4-E]thiopyran-2,6-dione	$C_7H_2N_2O_2S_3$	242	0.0001
22	2-(5-Thiophen-2-yl-1H-pyrazol-3-yl)-phenol	$C_{13}H_{10}N_2OS$	242	0.0001
23	Silanediol, dimethyl-, diacetate	$C_6H_{12}O_4Si$	176	0.0001
24	Silane, trimethyl[(1-methyl-1-phenylethyl)dioxy]	$C_{12}H_{20}O_2Si$	224	0.0001
25	Cyanic acid, phenyl ester	C_7H_5NO	119	0.0001
26	Pyrazolidin-3-one, 2-(4-methylbenzoyl)-1-phenyl	$C_{17}H_{16}N_2O_2$	280	0.0001
27	3-Pentanone, 1-hydroxy-2-methyl-1-phenyl	$C_{12}H_{16}O_2$	192	0.0001
28	2-Butanone, 4-hydroxy-3,3-dimethyl-4-phenyl	$C_{12}H_{16}O_2$	192	0.0001
29	N-Phenylglycine methyl ester	$C_9H_{11}NO_2$	165	0.0001
30	Benzeneacetic acid, α-amino-, methyl ester	$C_9H_{11}NO_2$	165	0.0001
31	3β,17β-Bis(trimethylsilyloxy)-5α-androstane	$C_{25}H_{48}O_2Si_2$	436	0.0001
32	5α-Androstane-3α,17β-diol, bis(trimethylsilyl) ethe	$C_{25}H_{48}O_2Si_2$	436	0.0001

A total of thirty-two (32) bioactive chemical compounds were detected during the GC-MS analysis. The concentration of the bioactive compounds ranged from 0.0001% to 10.47%. Octadecanoic acid and L-Abscorbic acid were among the bioactive compounds with the highest concentration, while cyanic acid, N-Phenylglycine methyl ester were among the compounds with the lowest concentration (Table 3.2).

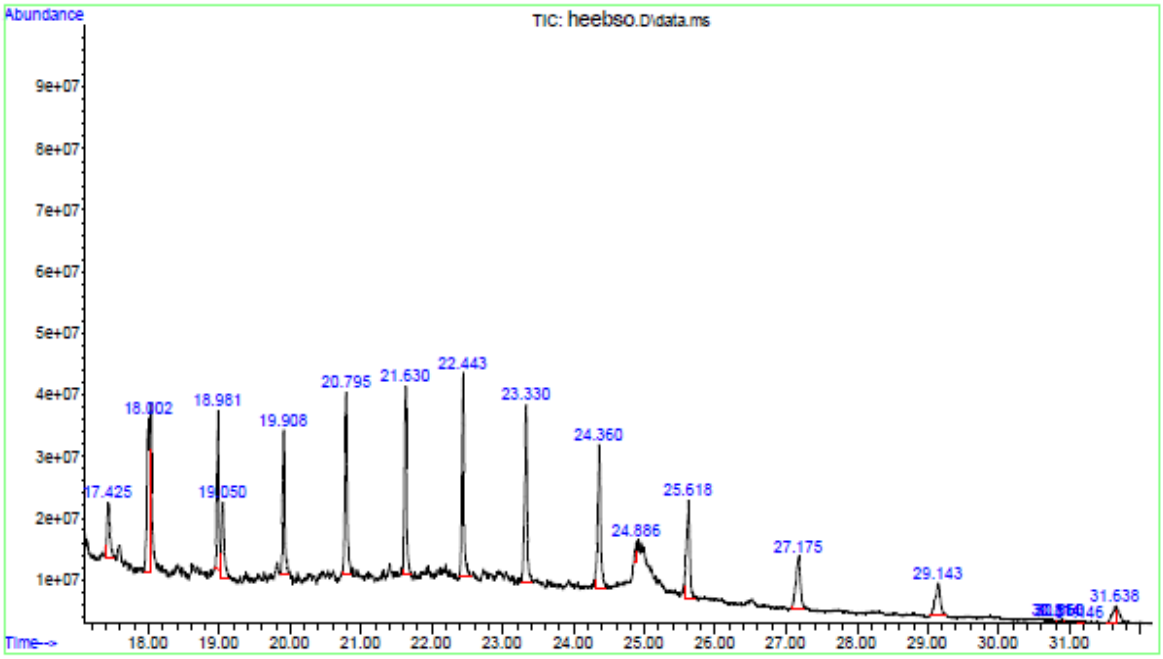

Figure 3.1**:** GC-MS Chromatogram of *D. edulis* Seed Oil, extracted with η-Hexane

4.0 Discussion

The percentage oil yield from the seeds of *D. edulis* was 40.1%. This result was higher than that recorded by Ekissi *et al.* (2016) for *D. edulis* seed oil extracted using η-Hexane (34.85%). The result was, however, similar to those reported by Kyari (2008) for *Schorocarya birrea* (42%). These variations between oil yields in seeds could be attributed to their climate of cultivation, ripening stage, harvesting time and the extraction method employed (Egbekun and Ehieze, 1997).

GC-MS is a powerful technique used for many applications, which has very high sensitivity and specificity (Hussein and Hameed, 2016). GC-MS, being able to separate and confirm identity of components by elucidation of spectral structure, has made this research possible (Iko *et al.*, 2015).

GC-MS analysis of the seed oil of *D. edulis* showed several peaks in form of total ion current (TIC) chromatogram. These peaks represent an important tool for the identification of individual components (Montalbino, 2012). Ascorbic acid ($C_{24}H_{42}O_7$) was identified and quantified in *D. edulis* seed oil. It was found to have the highest peak (10.36%). This result corroborates with findings from Khalid *et al.* (2024), who analysed ascorbic acid of several samples involving fruits, vegetables and Lee *et al*., (2014) who observed high levels of ascorbic acids in D.*edulis*.

Commonly named vitamin C, ascorbic acid is popular for certain qualities like acting as a potent antioxidant and a free-radical scavenger (Santos *et al*., 2022). As noted by Ali *et al.* (2024), ascorbic acid (vitamin C) is the body's most crucial antioxidant, containing ample health-related benefits. It is needed for the production of collagen in the human body- so many connective functions are facilitated by the protein called "collagen". The collagen-containing materials and structures of the body include: the bone, binding materials in the skin muscle or scar tissue, and the gums (Walingo, 2005). Other importance of this vitamin include: the detoxification of toxic substances in the system, fighting of infections in the blood and proper functioning of the immune system (Khalid *et al.*, 2024). As an antioxidant, it reduces symptoms of inflammation by reacting with compounds like histamines and peroxides; due to its anti-inflammatory, anti-cancer, anti-viral, anti-oxidant, immune-supportive properties, it prevents humans from diseases (Ali *et al*., 2024).

Other bioactive compounds identified and quantified in the seed oil were: octadecanoic acid (10.47%), dodecane, 2,6,11-trimethyl (9.779%), octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (9.651%), tricosane, 2-methyl (9.39%), docosane, 2,21-dimethyl (8.937%) and hexadecane, 2,6,10,14-tetramethyl (7.451%).

4.0 Conclusion

This present study was aimed at identifying and quantifying bioactive compounds present in the oil of *D. edulis* seeds, extracted using η-hexane. A total of thirty-two (32) bioactive chemical compounds were detected during the Gas Chromatographic-Mass Spectrometry (GC-MS) analysis of the seed oil of *D. edulis*. All the components identified could contribute to the human biological system singly or in combination. They could be anti-glycemic, anti-cancer or anti-inflammatory mostly in synergy of action.

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References

Adebayo, S. E., Orhevba, B. A., Adeoye, P. A., Musa, J. J. and Fase, O. J. (2012). Solvent Extraction and Characterization of Oil from African Star Apple (*Chrysophyllum albidum*) Seeds. *Academic Research International,* 3 (2): 178 – 183.

Ajibesin, K. K., Rene, N, Bala, D. N. and Essiett, U. A. (2008). Anti-microbial Activities of the Extracts and Fractions of *Allanblackia floribunda*. *Biotechnology,* 7: 129 – 133.

Ali, A., Riaz, S., Khalid, W., Fatima, M., Babar, Q., Manzoor, M. F., Khalid, M. Z. and Madilo, F. K. (2024). Potential of Ascorbic Acid in Human Health against different Diseases: An Updated Narrative Review. *International Journal of Food Properties*, 27 (1): 493 – 515.

Egbekun, M. K. and Ehieze, M. U. (1997). Proximate Composition and Functional Properties of Fullfat and Defatted Beniseed (*Sesamum indicum* L.) Flour. *Plant Foods for Human Nutrition*, 51: 35 – 41.

Hussein, H. J. and Hameed, I. H. (2016). Gas Chromatography-Mass Spectrum Analysis of volatile Components of Methanolic Leaves Extracts of *Corida myxa. International Journal of Current Pharmaceutical Review and Research*, 7 (6): 400 – 406.

Igoli, J. O., Ogaji, O. G., Tor-Anyiin, N. P. (2005). Traditional Medicinal Practices among the Igede People of Nigeria II. *African Journal of Traditional Complementary and Alternative Medicine*, 2: 134 – 152.

Iko, W., Eze, S. and Oscar, O. (2015). Gas Chromatography-Mass Spectrometry of *Quassia undulata* Seed Oil. *Nigerian Journal of Biotechnology*, 30: 53 – 58.

Khalid, W., Ali, A., Riaz, S., K., Fatima, M., Mubeen, U. And Babar, Q. (2024). Potential of Ascorbic Acid in Human Health against different Diseases: An Updated Narrative Review. *International Journal of Food Properties*, 27 (1): 493 – 515.

Koudou, J., Edou, P., Obame, L. C., Bassole, I. H. and Figueredo, G. (2008). Volatile Components, Antioxidant and Antimicrobial Properties of the Essential Oil of *Dacryodes edulis* from Gabon. *Journal of Applied Sciences*, 8: 3532 – 3535.

Kyari, M. Z. (2008). Extraction and Characterization of Seed Oils. *International Agrophysics*, 22: 139 – 142.

Tee, L. H., Yang, B., Nagendra, K. P., Ramanan, R. N., Sun, J., Chan, E. S., ... & Jiang, Y. (2014). Nutritional compositions and bioactivities of Dacryodes species: A review. *Food Chemistry*, *165*, 247-255.

Montalbino, S. (2012). Pyrolysis-Chromatography Mass Spectrometry and Chemometric Analysis for the Characterization of Complex Matrices. Ph.D. Thesis in Chemical Sciences, University of Bologna. p. 19.

Ogboru, R. O., Okolie, P. L. and Agboje, I. (2015). Phytochemical Screening and Medicinal Potentials of Bark of *Dacryodes edulis* (G. Don) H. J. Lam. *Journal of Environmental and Analytical Chemistry,* 2: 158 – 166.

Okunomo, K. and Egho, E. O. (2010). Economic Importance of some underexploited Tree Species in Nigeria: Urgent Need for separate Research Centers. *Continental Journal of Biological Sciences,* 3: 16 – 32.

Okwu, D. E. and Nnamdi, F. U. (2008). Evaluation of the Chemical Composition of *Dacryodes edulis* and *Raphia hookeri* Exudates used in Herbal Medicine in Southeastern Nigeria. *African Journal of Traditional Complementary and Alternative Medicine*, 5: 194 – 200.

Olivier, T. T., Moise, F., Jackson, S. A. and Francis, W. T. (2016). Review of Traditional Uses, Phytochemical and Pharmacological Profiles, Spiritual and Economic Values, and Toxicity of *Dacryodes edulis*. *Journal of Drug Discovery and Therapeutics,* 6 (5): 84 – 90.

Onuegbu, N. C., Nwuka, M. U., Ojukwu, M. and Kabu, N. O. (2016). Nutritional Properties of African Pear Seed and Performance of Defatted Cake in Poultry Feed Formulations. *Journal of Animal Research and Nutrition,* 1 (2): 1 – 3.

Santos, K. L., Bragança, V. A., Pacheco, L. V., Ota, S. S., Aguiar, C. P. and Borges, R. S. (2022). Essential Features for Antioxidant Capacity of Ascorbic Acid (vitamin C). *Journal of Molecular Model,* 28 (1): 1 – 8.

Walingo, K. M. (2005). Role of Vitamin C (Ascorbic Acid) on Human Health: A Review. *African Journal of Food, Agriculture, Nutrition and Development*, 5 (1): 1 – 13.

Zofou, D., Tene, M., Ngemenya, M. N., Tane, P. and Titanji, V. P. (2013). In-vitro Antiplasmodial Activity and Cytotoxicity of Extracts of selected Medicinal Plants used by Traditional Healers of Western Cameroon. *Malaria Research and Treatment,* 12 (3): 1 – 6.