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# Physicochemical studies on the hydrolysis and saponification products of *Dacryodes edulis* exudate

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**ABSTRACT**

*The physicochemical properties of the exudate of Dacryodes edulis and those of the saponification and acid hydrolysis products have been determined. The value of the purified exudate, acid hydrolysis product and saponification product where respectively: melting point ($^oC$) (73.33, 62.20, and 60.33): flash point ($^oC$) (110, 100, 100): Charring point ($^oC$) (200, 190. 190) and density ($g/cm^3$) (0.75, 0.88, 0.95). The exudate has a high intrinsic viscosity. The chemical constituents of the exudates gum were determined using standard methods. It was found that n-hexane was the most suitable solvent for purifying the exudate and for dissolving the modified products which were insoluble in water. The TLC $R_f$ value of the purified exudate extracts shows that exudate is a mixture of many chemical substances while IR spectra indicate the presence of –COOH (carboxylic), -OH (alcohol), N-H and C-N (protein) functional groups in the purified exudate, acids hydrolysis and saponification products, suggesting the presents of resin acid, fatty acid, fatty alcohol and protein.*

**Keywords:** *Dacryodes edulis*, exudates, hydrolysis, saponification, physicochemical properties, and IR spectroscopy

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## INTRODUCTION

Environment is endowed with many resources necessary for the good of man, and for the benefit of the society. One of the resources is *Dacryodes edulis* (African pear) also known as Eben (Ibibio), Ube (Igbo), which its exudates will be considered in this work. Exudates can be defined as sap liberated from special pores present in plants [1]. It is the viscous fluid formed spontaneously at sites of injury in plants, and which become hydrated to give hard clear modular, consisting largely of polysaccharides [1]. The yield and the quantity of the exudate increase as the weather becomes warmer [2]. *Dacryodes edulis* exudate with gummy or mucilaginous characteristics has wide and varied applications locally.

Local African pear (*Dacryodes edulis)* is a common fruit tree and grows readily on the tropical rain forest, but there are other sources of exudates, some are rubber, pines, raffia palm and cashew tree [3]. The most important product from *Dacryodes edulis* is the fruit called African pear or African plum, which is a popular source of food (bush butter) in the Southern part of Nigeria, in addition, this plant produces exudate (whitish resin) continuously, which has its peculiar smell and pale gray colour ones the bark of the plant is cut. The exudate is a renewable natural resource and can easily be obtained manually. *Dacryodes edulis* exudate is usually sticky (gummy) when touched with the hand, it also has a sickening odour. The exudates should be treated with acid or alkali to modify its gummy nature so that its sticky properties are withdrawn and mucilaginous properties are introduced to enhance its dispersing ability in water and other solvent.

Some works have been reported on some physicochemical properties of exudate [4], [5], however not much is known about the physicochemical properties of the saponification and the acid hydrolysis products. It is seen from the traditional view that the raw African pear exudate can be used as fuel in providing flame, when burnt as a primitive lamp oil or a bush candle [6]. It has been used as fuel as well as adhesives in many parts of South Eastern States of Nigeria where the plant is mostly found. It has also be shown that the exudate is used as pitch on calabashes and for mending earthen wares, roofs and ships to stop water getting through indeed, *Dacryodes edulis* exudate also has a vital application in the paper industry as the sizing and binding agent to increase the resistance to penetration of water and related fluid in paper [7]. The use of *Dacryodes edulis* exudates in the preparation of candle has been reported [8]

The exudate of *Dacryodes edulis* has been found useful as paper sizing agent [2], but during its preparation for paper sizing either in the alkaline or acidic condition, an oily substance is usually encountered when the sizing preparation is hot. This substance solidifies into hard brittle solid on cooling. In this work, the physicochemical characteristics of the solid product obtained either by sodium hydroxide (saponification) or mineral acid (HCl or $H_2SO_4$; and hydrolysis) treatment on the exudate of the *Dacryodes edulis* that are studied with the intension of establishing proper uses. However, in order to correlate the properties of these products with those of the crude exudate, it is necessary to determine the physicochemical properties of the crude exudate of *Dacryodes edulis*.

## MATERIALS AND METHODS

***Raw Materials Collection and Purification***

The sample crude exudate from African pear tree (*Dacryodes edulis)* was collected randomly from three pear trees at Afua in Nsit Ibom Local Government Area of Akwa Ibom State. Many incisions were made on the bark of the trees and after some few minutes the exudate oozed out of the tree. The fresh exudate was a milky, highly viscous liquid and this was collected with help of a knife blade and dropped into a container. It was observed that the exudates ooze out faster in rainy season than dry season. The sample was air dried for two hours for it to lose some of its moisture before the purification treatment was carried out. The purification of the exudates was essential due to the bark of the tree and other impurities.

***Preliminary Test for Solubility***

1g of each crude exudate was put into six test tubes. 5ml of the different solvents were added to the exudate in the test tube and shaken vigorously for 10mins to observe their solubility in each of the solvents. The solvents used where water, ethanol, petroleum ether, n-hexane, ethanol-benzene (1:2) and benzene. The mixtures where warmed in hot water bath and there solubility pattern observed. This was repeated with the saponification product and acid hydrolysis products.

***Extraction of Exudate from Dacryodes edulis***

The methods reported by Chukwu and Nwankwo [9] were adopted for the extraction of exudate from *Dacryodes edulis.*

***Elemental Screening with Atomic* Absorption *Spectrophotometer***

The sample was digested in order to prepare the crude exudates extract for Atomic Absorption Spectrophotometer (AAS). 1g of the dry ground and sieved sample was weighed into a 100ml beaker; 10ml of conc. $HNO_3$ and 5ml of conc. $HClO_4$ were added in the ratio of 2:1. It was covered with a watch glass. The mixture was placed in a hot plate and heated to dryness with the colour changing to white. After allowing the sample to cool, the residue was leached with 5ml of 20% $HNO_3$. It was littered and the volume made up to 20cm$^3$ with distilled water. A blank was prepared using the similar method above but with the omission of the sample [1]. The metal elements in the sample were then determined by reading their absorbance in the spectrophotometer and comparing with their standard. This was repeated with direct extract, column eluate, acid hydrolysis product and saponification product.

***Determination of Physicochemical properties***

***Browning and Charring Temperature***

These were determined in a melting point apparatus (Gallenkamp). One gram sample of the exudate was placed on the hot stage of the apparatus. The temperature was gradually and incrementally raised by 10$^o$C. The temperature at which colour changes in the exudate gum was observed and recorded. Five replicate determinations were made and the average taken.

***Determination of Density, Moisture Content and Ash Content.***
Two tenth gram of extract exudate obtained was weighed in the weighing balance. The volume was found by immersing the mass in some de-ionise water contained in graduated cylinder 100ml. The volume of water displaced was noted. Division of the weight of solid by the volume of water displaced by the exudate gives the density.

The moisture content was determined in an Ultra X Moisture Balance (Model US 2010, Germany). 5g quality of the exudate was used. The equipment was operated at 60$^{o}$C until constant weight of the sample was obtained. Samples were then weighed to determine the percentage of moisture absorbed. The tabulated values in Table 2 are the average of three determinations. Ash content was determined using the method reported by [9].

***Determination of Viscosity***
A U-tube Ostwald Viscometer (BS 188) having internal tube diameter of 0.88nm and capillary length of 12.5cm and flow time of 120 seconds for the solvent bank (0.1M Sodium Chloride solution) was used to determine the flow time of 10ml dilute solutions (0.05 to 1.0%) of the ethanol extract in 0.1M Sodium Chloride solution. A five point flow time determination was made for each extract and the average used to calculate the intrinsic viscosity according to Huggins equations [10] given below.

$$\eta_{red} = [\eta] + K' [\eta]^2 \quad \text{..... (1)}$$

$\eta_{red}$ = reduced viscosity
$[\eta]$= intrinsic viscosity
K' = Huggins Constant
C = Concentration of exudate solution

***Rosin and Starch Test***
The Libermann Torch was used for rosin test. 5ml of acetic anhydride was added to 1g of each of the samples in a test tube and boiled till the volume decreases to 1ml. It was poured into a crucible and allowed to cool at room temperature. Concentrated sulphuric acid (1 drop) was allowed to run down the side of the crucible. When the acid met the anhydride, a red violet colour shows the presence of rosin.

In determining starch, one quarter gram of sample was boiled with 5ml distilled water, filtered and allowed to cool at room temperature. One drop of dilute iodine solution was added, a blue-black colouration was observed, which indicated a positive test, hence the presence of starch in each Sample.

***Qualitative Analysis of Lignin***
Lignin was detected using phloroglucinol solution as describe by [11]. The solution was prepared using 0.2g phloroglucinol. And 1.0g of n-hexane extract was placed in a 10cm$^3$ beaker and a solution of phloroglucinol was added drop wise to the 10cm$^3$ mark the mixture was allowed to stand for 10mins for the exudates to settle at the bottom of the beaker. The characteristics violet-red colour was gradually observed which increased in intensity with time and finally reached the maximum intensity after a very long time. The presence of lignin is confirmed by a characteristic violet-red colouration. The intensity of the colour is an indication of the amount of lignin present in samples.

***Electrical Conductivity***
The conductivity meter HACH was switched on and the electrode was rinsed with distilled water. 50ml of the crude sample solvent in hexane was measured into a beaker and the electrode was immersed in the crude, the reading was taken directly from HACH conductivity/ TDS meter with corresponding temperature. This was repeated with both acid hydrolysis and saponification products, direct purified extract and chromatography column purified extract.

***IR Spectroscopic Analysis***
Infrared spectra of *Dacryodes edulis* was recorded on Shimadzu FTIR – IR Prestige-21 (200VCE) using potassium bromide (KBr) pellets. 1mg of the sample was intimately mixed with 100mg of dry powdered KBr. Mixing was effected by grinding thoroughly in a smooth agate mortar. The mixture was pressed with special dice under a pressure of 13000psi into transparent disks (pellets). The KBr pellets were inserted into the sample compartment of the instrument and scanned between 4000-400cm$^{-1}$. The instrument was calibrated with polystyrene film before the spectral scan was made for the samples.

## RESULTS AND DISCUSSION

***Chemical composition of the exudate***.

*Dacryodes edulis* exudate was obtained as an off-white fluffy, odourless and tasteless powder. The yield was 30% in the direct extract and between 34.68 – 61.12% yields in column extract. Table 1 present the elemental contents of crude exudate, including those of direct extract, column eluate, saponification products as well as acid hydrolysis products. All the five samples contain some metallic elements in varying degrees. The prominent inorganic elements found in the exudates are potassium, manganese, sodium, calcium and magnesium. These metallic elements may occur in the form of cation in the resin soap present in the exudates. Calcium is found to be of high proportion. It is known that the amount of element is not fixed for exudate gum but very depending upon the soil type, husbandry of the bush, age of the parent tree, amount of rainfall received, time of exudation, also the heavier the pruning condition, the higher the elemental composition [12]. The different pruning style is said to affect the yield of the shoots of the leaf, therefore affecting the quality of the exudate gum [13].

**Physicochemical Properties**

Some physiochemical properties of the crude exudate, direct extract, chromatography column, saponification and acid hydrolysis products are presented in Table 2. The browning and Charing temperatures are between 190 $\pm$ 7.9 to 200 $\pm$ 7.9$^{o}$C and 150 $\pm$ 5.7 to 170 $\pm$ 5.7$^{o}$C respectively. This temperature which indicates the thermal stability of excipients is above the temperature encountered during processing, storing and proper handling of materials. The densities of the direct and column chromatography of crude exudate are 0.75 $\pm$ 0.02 and 0.84 $\pm$ 0.02gkm$^{3}$ respectively, while those obtained from acid hydrolysis product and saponification products are 0.88 $\pm$ 0.02 and 0.95 $\pm$ 0.02 gkm$^{3}$ respectively. Density is a measure of packing characteristics of powdered material. An increase in density is good in tableting because of reduction in the fill volume of the die cavity of the tablet machine. The saponification product is more crystalline due to added sodium ion ($Na^{+}$) with no impurities. In hydrolysis products, the density increase more than that of the crude exudate.

The moisture content of crude exudates is 1.31%, same value with both direct extract and chromatography column. It decreases from crude exudates to acid hydrolysis product (1.03%). The moisture content is low in all cases because of the resinous nature of the exudates but reflects the affinity of a material for moisture. High moisture sorption is a disadvantage when moisture sensitive active ingredients are to be formulated [14].

Lignin contents was 7.82% in both crude exudate and direct purified extract, 6.92% in column extract, 5.22% in saponification product in the form of soda-lignin and 6.25% in acid hydrolysis product as lignosulphuric acid. More lignin is present in the crude exudate than in the modified products because soda lignin and lignosulphuric acid are slightly soluble in the liquors.

The amount of ash in the crude exudate lies between 1- 3%. Studies however, revealed that the major components in wood ash are calcium, magnesium, potassium and silicate [15], [16]. The ash content in the crude exudates and direct solvent extract sample is 1.8% higher than the ash in the column chromatography and modified products. The difference in the value is due to the fact that the crude sample and the direct extracted samples may contain some impurities which may contribute to the increase in the amount of the ash.

The electrical conductivity µS/cm in the purified exudate are similar, while there is an increase in the value of the saponification and acid hydrolysis products (29.76 and 28.36 µS/cm) respectively. The electrical conductivity could be explained in terms of the presence of some metal ions of $K^{+}$, $Mg^{2+}$, and $Na^{+}$ as major charge carriers in the exudates gum while the increase in both saponification and acid hydrolysis products is due to the ions from the reactants ($H_2SO_4$ and NaOH), Conductivity value is important to know because if the exudate is used as a paper sizing agent during paper making, there is need to know how much such paper can conduct electricity in order to determine the application of the paper.

**Table 1: Metal elements present in the exudates and modifies samples (mg/g)**

| Inorganic Element | Crude Exudates | Direct Extract | Column Eluate | Saponification Products | Acid Hydrolysis Products |
|---|---|---|---|---|---|
| Calcium (Ca) | 50,000.00 | 5,100.00 | 5,200.00 | 3,800.00 | 5,080.00 |
| Sodium (Na) | 90.45 | 90.48 | 91.46 | 72.80 | 85.28 |
| Potassium (K) | 15.46 | 15.68 | 16.66 | 10.90 | 14.78 |
| Magnesium(Mg) | 46.20 | 46.38 | 47.36 | 43.65 | 45.97 |
| Manganese(Mn) | 26.20 | 26.22 | 27.20 | 25.97 | 27.00 |

From Table 1, it is seen that the exudates contain the inorganic elements, K, Mn, Na, Ca, and Mg. These metals may occur in the form of cation in the resin soap present in the exudates. Calcium is found to be of high proportion owing to the fact that it is the most abundant element in plant [1].

**Table 2: Some Physicochemical Properties of Crude and Modified Exudates.**

| Property | Crude Exudates | Direct Extract | Chromatog-raphy Column | Saponifica-tion Plant | Acid Hydrolysis Product. |
|---|---|---|---|---|---|
| Charing Temp ($^oC$) | 200 ± 7.9 | 200 ± 5.7 | 200 ± 3.4 | 190 ± 5.7 | 190 ± 7.9 |
| Browning Temp ($^oC$) | 170 ± 7.9 | 170 ± 5.7 | 169 ± 3.4 | 150 ± 5.7 | 150 ± 7.9 |
| Moisture Content (%) | 1.31 | 1.31 | 1.31 | 1.10 | 1.03 |
| Av. Densities ($g/cm^3$) | - | 0.75 ± 0.02 | 0.84 ± 0.02 | 0.88 ± 0.02 | 0.95 ± 0.02 |
| Ash Content (%) | 1.8 | 1.8 | 1.5 | 1.4 | 1.2 |
| Electrical Conductivity (µS/cm) | 27.60 | 28.40 | 28.00 | 32.07 | 32.99 |
| Lignin Content (%) | 7.82 | 7.82 | 6.92 | 5.22 | 6.25 |
| Flash Point ($^oC$) | 110 ± 7.9 | 110 ± 5.7 | 110 ± 3.4 | 100 ± 3.2 | 100 ± 32 |

It is known that the amount of element is not fixed for exudates gum but vary depending upon the soil type, husbandry of the bush, age of the parent tree, amount of rainfall received, time of exudation and type of exudation. Also, the heavier the pruning condition, the higher the elemental composition [20]. The different pruning is said to affect the yield of the shoots of the leaf therefore affecting the quality of the exudates gum [19].

**Viscosity**

Table 3 present the viscosity data obtained from the exudates gums as reduced times of flow. This data is similar to the reduced viscosity of grewia gum, trangacanth, acacia and methyl-cellulose [4].

Huggins equation (Eqn.1) was used to evaluate the intrinsic viscosity by extrapolation of finite dilution. The least square method was used to evaluate the ordinate of the straight line generated from equation 1, this represents the intrinsic viscosity.

An intrinsic viscosity of 0.14dl/g was obtained from ethanol extract. This is higher than the intrinsic viscosity of 0.10, 0.08 and 0.04dl/g obtained for ethanol-benzene (1:2), n-hexane and petroleum ether extracts respectively, but lower than the 0.29dl/g obtained from benzene. The correlation coefficients (r) are quite significant. The intrinsic viscosity is the intrinsic ability of a polymer to increase the viscosity of solvent at a given temperature. It is a measure of the effective hydrodynamic interactions between segments of the same polymer molecules. It usually falls within the range 0.3 (for good polymer solvent pairs) to 0.5 (for poor polymer solvent pairs) [15]. Values of Huggins constants obtained for the exudates gums are within this range.

**Table 3 : Viscosity Data for Exudate Gum Solution in 0.1 NaCl at $25^0C$**

| Concentration g/dl | Ethanol $t\text{-}t_o/t_oC$ dl/g | Benzene $t\text{-}t_o/t_oC$ dl/g | Petroleum Ether $t\text{-}t_o/t_oC$ dl/g | n-hexane $t\text{-}t_o/t_oC$ dl/g | Ethanol Benzene 1:2 $t\text{-}t_o/t_oC$ dl/g |
|---|---|---|---|---|---|
| 1.00 | 0.90 | 0.80 | 0.88 | 1.18 | 0.91 |
| 0.80 | 0.74 | 0.70 | 0.75 | 1.10 | 0.72 |
| 0.60 | 0.60 | 00.60 | 0.64 | 0.86 | 0.43 |
| 0.40 | 0.46 | 0.50 | 0.43 | 0.62 | 0.29 |
| 0.20 | 0.27 | 0.20 | 0.24 | 0.50 | 0.28 |
| 0.10 | 0.20 | 0.17 | 0.19 | 0.41 | 0.20 |
| 0.05 | 00.18 | 0.05 | 0.16 | 0.32 | 0.17 |

**Table 4: IR spectral bands of exudate and the modified products of *dacryodes edulis***

| Exudate | Acid group (COOH) | | | Alcohol (-OH) | Nitro Group ($NO_2$) | | | Amino Group ($NH_2$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | O-H | C=O | C-O | O-H | N=O (sym) | N =O (asy) | C=N | N-H | N-H (bend.) | C-N |
| A | 294 | 1710 | 1210 | 3500-3400 | 1380 | 1460 | 850 | 1650 | 890 | 1245 |
| B | 2940 | 1710 | 1210 | 3480-3380 | 1380 | 1460 | 880 | 1640 | 670 | 1240 |
| C | 2940 | 1710 | 1210 | 3480-3380 | 1380 | 1458 | 880 | 1655 | 660 | 1245 |
| D | 2950 | 1700 | 1210 | 3500-3360 | 1380 | 1458 | 850 | 1655 | 750 | 1245 |
| E | 2960 | 1710 | 1210 | 3500-3360 | 1380 | 1460 | 880 | 1650 | 665 | 1245 |
| F | 2945 | 1750 | 1210 | 3480-3400 | 1380 | 1455 | 880 | 1650 | 675 | 1245 |

*A = crude exudates, B = n-hexane (Acid hydrolysis product), C = Benzene (chromatography column exudates), D = Benzene (saponification product), E= Ethanol (Direct purified exudates), F = Ethanol (saponification product).*

The ability of plant exudates gums to increase the viscosity of solutions is direct dependent on their molecular weight. This relationship was used to obtain molecular weight of 316,000 for grewia gum [4]. High intrinsic viscosity and hence molecular weight for polymers are applied in suspension [17] and emulsion technology [18].

The binding properties of polymers used in tableting are dependent on molecular weight [14]. High molecular weight could prolong tablet disintegration, drug dissolution and biovailality [19], in this case, the rate of hydration and the solubility of the drug are decreased. This concept is applied in the sustained release formulation.

The IR spectra bands (absorption frequency) of the exudate samples, the acid hydrolysis and saponification products are presented in Table 4. The listed absorption frequencies are shown to be related to their respective functional groups.

For the alcohols, the broad O–H stretching vibration of alcohol group of all the exudates were in the region 3500-3360cm$^{-1}$, this indicates a secondary alcohol with probably intermolecular hydrogen bonding. The C-O stretch of alcohol was in the region 1070-1060cm$^{-1}$. The spectra of all the exudates also indicate the presence of a carboxylic acid.

The broad and intense O-H band stretch absorption of the acid was observed in the region 2940-2920cm$^{-1}$ in all the exudates. The C=O stretch, 1710cm$^{-1}$ and C–O stretch, 1210cm$^{-1}$ of the acid also confirm the presence of the acid.

Two absorption bands, symmetric and asymmetric stretching vibrations of nitro group, (N=O) were observed in the region 1380-1375cm$^{-1}$ and 1470-1455cm$^{-1}$ respectively. The C-N stretch of the nitrogen group was found in the region 880-850cm$^{-1}$ indicating the presence of nitro group in exudates. The presence of amino group was detected for the exudates. The only detectable absorption bands of the amino group were N-H bending 1650-1640cm$^{-1}$, C-N stretch 1245-1240cm$^{-1}$ and N-H wagging vibrations, 890-660cm$^{-1}$.

The weak absorption band in the region 3500-3400cm$^{-1}$ representing asymmetric and symmetric stretching vibrations of O-H alcohol. The N-H of the amino group were absent probably due to the strong and broad O-H stretch absorption from alcohol and carboxylic aid.

These absorption bands of all the exudates samples indicate mainly the presence of carboxylic fatty acid and O-H of the fatty alcohol, while the absorption band of C-N and N-H indicate the presence of some protein materials; some nitro group may also be present in the exudates.

From the above information, *Dacryodes edulis* exudates consists of mixed compounds mainly of resin acids, resin soap of the metals, fatty alcohols and some protein materials while the sponification product is the sodium salts of the acids, the acid hydrolysis products and the resin acids. Since the band does not indicate any absorption of the C-H aromatic, it may not contain aromatic compounds even though it is soluble in benzene.

## CONCLUSION

The physicochemical properties of the exudate gum showed that the exudate consists of glucose, resin acid and fatty alcohol with traces of metals. It has a high intrinsic viscosity and appreciable lower density. The n-hexane is the most suitable solvent for the crude *Dacryodes edulis* exuate and should be used in purification by column chromatography. The exudate is easily saponified or acid hydrolyzed into solid substances which can easily seal up crack when applied in molten form. The plant from which the exudate is derived grows widely in Nigeria and can also be cultivated. The isolation technique is simple, economical and effective. The yield of the exudate gum is encouraging. These qualities should warrant consideration of this plant polymer as sizing agent in paper making and it should be partially saponified or hydrolyzed for ease of spreading on the paper surface during drying.

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