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Proximate and Phytochemical Studies of Exudate of *Dacryodes edulis*

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Abstract

Proximate and Phytochemical analysis of exudates of Dacryodes edulis has been studied and proximate analytical result of exudate of Dacryodes edulis showed that the major chemical components present in exudate and the modified products were lipids and carbohydrate; while some of the mineral elements identified in purified, hydrolysed and saponified products were potassium, sodium, manganese, calcium and magnesium. Phytochemical screening showed the presence of alkaloids, saponins and resins as toxic substances. The IR spectra indicated the presence of –COOH (carboxylic), -OH (alcohol), N-H and C-N (protein) functional groups in the purified exudate, acid hydrolysis and saponification products, suggesting the presence of resin acid, fatty acids, fatty alcohols and protein in the exudate. From the results, Dacryodes edulis exudate could be useful in production of drugs for treatment of some ailments.

Keywords: Proximate, Phytochemical, Hydrolysis, Saponification, Purified exudates, Mineral elements.

Introduction

Exudate consists of small amount of sugars and other organic and inorganic substances besides water. Conditions necessary for exudate formation include abundant supply of water, favourable temperature, the presence of living cells in the roots and transpiration control. If the roots are dead, no exudation can take place[1,2]. It can be source of light when used as an ancient lantern or shrub candle[2]; it can be used on calabashes and on stitching clay products; protective covering, and vessel for carrying passengers to ensure water-proof state. In the newspaper manufacturing firm, exudate can be used as a fastening material to raise the resistance to wet penetration[3]. The exudate is used in treatment of wounds and other ailments in some villages.

Extracellular plant gums are prepared with various white crystalline carbohydrates that are derived from monosaccharide bonds[4]. Having core fundamental bond usually of D-galactose or D-glucuronic acid units, attached by monosaccharide bonds that are comparatively firm to acidic medium. The sugar units as side chains attached to the central nucleus can easily be isolated by acidic medium. Galactoglocomannans and glucomannans gum so extracted have comparatively straightforward arrangement. These types of bonding units are smaller in number and their simple structural units are to a larger extent less branched[5]. Compounds with stable sharp melting points can signify the purity of the compounds. The elemental analyses of the compounds can work together to give an inside of the composition suggested for the compounds.

Other common sources of exudate include *Dacryodes edulis*, pines, raffia palms, cashew and rubber trees[6], and their components can be found in various parts of the plant. The major constituents of plant exudate which are of industrial and medicinal importance are the phenolic derivatives, cardio active glycosides, alkaloids, tannins, flavanoids and saponins etc[7].

Exudate exists as extractives in plants. The components of the extractives which become exudate as the plant surface is breached constitute secondary metabolites of industrial and medicinal importance.

There are many reports on the usefulness of the exudate of *Dacryodes edulis*, but not much study appears to have been done on the proximate and phytochemical components and the hydrolysis and saponification products. This research therefore aims at investigating the proximate, phytochemical composition, hydrolysis and saponification products of the African pear tree (Eben in Efik, Ube in Igbo) exudate in order to establish their place in medicine and the industry[8].

Produce from African pear tree is softened by harmful organisms lowering the properties. This can happen within few seconds especially when at 60–85°C; at ordinary room temperature it may take 7–10 days but as it is expose to microorganisms it reduces the time to 3 days. As temperature keeps increasing the enzyme hardens the pulp[9]. The fruit properties change in various physical states varies greatly in size, shape, colour and composition. The ratio of seed to pulp is about 2:3 for the tiny ones and changes to over 5 for the big ones. The pulp composition are 59% water per 100 g dry sample: oil 32–44 g, protein 14–26 g, carbohydrates, fibres and other matter 32–38 g, ash 4–10 g and mineral elements like potassium 12-18µg/g, sodium 80-100µg/g, calcium 100-

5000µg/g and magnesium 30-50µg/g. The oil content of the fruit pulp is very high 30–60% on dry sample basis; oleic acid (45–60%), palmitic acid (30–35%), linoleic acid (15–20%), and stearic acid (2%) constitute about 95% of the pulp oil[10].

As the oil from the fruits are extracted out it is notice to form two layers, solid layer is found below while liquid layer is up. The composition of fatty acid in both layers looks the same. Unsaponifiable extracts constitute only 2%, whereas sterols 20%, triterpene alcohols (34%) and small amounts of tocopherols[11]. Comparing with other oily fruits extracts, the seed per 100g dry sample are alike in the pulp oil composition. There is a useful animal feed usually left out after the extraction and called cake. The main component of the African pear fruit is the oil which is about 1.5%. Other constituents are: myrcene (45%), α-pinene (9%), α-terpineol (8%) and germacrene-D (4%); minor compounds include: E-α-cadinol, δ-cadinol and β-eudesmol. Another part of African pear tree of economic importance is the wood which is most often hard to work with because of silica that blunts sawing[12] tools and the inorganic metal phosphates have interesting properties like alkali metal conductivity[1].

Materials and Methods

The exudate used in the research work was obtained from African pear tree in Afua-Mbiabong in Ibiono, Nigeria. Various cuttings into the body tissues were made on the woody stems of the living tree and the exudate freely oozed out. All reagents and chemical used were of analytical grade.

The following analyses were carried out on the sample of purified exudate, hydrolysis and saponification products obtained from *Dacryodes edulis*.

Proximate and Mineral Elements Analysis: Percentage Moisture, ash, crude fibre, fat (lipid), protein and carbohydrate contents, caloric value and mineral elements were determined using standard procedures of the Association of Official Analytical Chemist[13]. For mineral elements, 2 g of fine ground oven dried sample of crude exudate 105^{o}C was used while digested ethanol extract was used for AAS.

Phytochemical Screening: Chemical tests were carried out on 0.5 g sample of n-hexane exudate extracts of *Dacryodes edulis* for the detection of alkaloids, cardiac glycoside, flavonoids, resin, carbohydrate and saponins using the standard test procedures[14].

Electrical Conductivity: The 2g sample was dispersed in 50ml distilled water and Hath Conductivity Meter was used for electrical conductivity measurement by dipping electrode into 50ml of the flesh tapped gummy crude sample in a beaker[15].

IR Scan: Infrared spectral of *Dacryodes edulis* exudate was recorded on Shimadzu FTIR.

Results and Discussion

Exudate from *Dacryodes edulis* was characterized and the results obtained for proximate composition of purified exudate, acid hydrolysis and saponification products are presented in Tables 1 and data showed that, The moisture content was low with the highest recorded for purified exudate (2.70±0.20 % dry weight). The ash content was 0.40 ± 0.05% (saponification product), 0.35±002% (acid hydrolysis) and 0.31±0.05% (purified exudate). Ash content is an indication of the mineral composition of the sample. The lipid content was interestingly very high: crude sample contained 92.30±1.07%, acid hydrolysis product 81.73± 1.07% and saponification 78.65±5.22%. This confirms that the sample is a good source of lipid[16]. Protein was found to be low: 1.46±0.05% for purified sample and 0.73±0.05% for both the hydrolysis and saponification products. The exudate is not a good source of protein. The carbohydrate content was 5.83±1.08% for the purified sample, 17.05±1.08% for hydrolysis product and 20.07±2.68% for saponification product. The caloric value was high due to the high content of lipid. The results revealed that *Dacryodes edulis* exudate is not a good source of food, but the lipid content can be extracted and used in industry in the form of resin soap or sizing agent in paper making[17].

Table–1
Proximate composition of purified exudate, acid hydrolysis and saponification products

Sample	% Moisture	% Ash	% Fibre	% Lipid	% Protein	% CHO	Caloric Value (Kcal/100g)
Acid hydrolysis product	1.30 ± 0.02	0.35± 0.02	0.12± 0.02	81.75 ± 1.07	0.73± 0.05	17.05± 1.08	806.87 ± 1.25
Saponification product	1.30± 0.05	0.40± 0.05	0.15± 0.02	78.65± 5.22	0.73± 0.05	20.07± 2.68	791.05± 1.25
Purified exudates	2.70± 0.20	0.31± 0.05	0.10± 0.02	92.30± 1.07	1.46± 0.05	5.83± 1.08	859.85± 1.25

Each data is a mean of three replicate determination ± SD

Phytochemical analyses give the exposure and consideration of phytocomponents present in the plant exudate, and maintain the biological constituents which can be analyzed, determined or standardized qualitatively. The intricate materials of particular constitution varied and are found in food stuffs in tiny quantity pre 100 grams or mg or µg/Kg of samples. Their contribution to the body calorie is not significant although they are many in form. Some constituents of plants are carbohydrates, proteins, fats and oils; they are put to use as food by man and animals. Other constituents in plants that differ from those mentioned above are phytochemicals. Such compound frequently makes use of uncharacteristic, exceptional, precise dynamic properties responsible for their therapeutic and pharmacological functions. Behaviors of this physically happening compound are normally dependable for an event that occurs, which are useful to assure man's requirement.

These phytochemical are applied mostly for precautionary and remedial purposes. The phytochemcal screening of the n-hexane extract of the crude and purified extracts of *Dacryodes edulis* as presented in Table-2 reveals the presence of alkaloids, saponins, carbohydrates and resins. Flavonoids and glycosides were not detected. The presence of these phytochemical components in *Dacryodes edulis* exudate while making it useful in treating some medical ailments imposes limitation in it use in food preparation.

The metallic elements K, Mn, Na, Ca and Mg content in the exudates. Table-3 shows that the metals may be found as cations in the resin soap present in the exudate. Calcium was in very high proportion since it is the most abundant element in plant[18]. The elements depend on the pruning conditions, the heavier the pruning the higher the elemental composition[19,20]. The different pruning state or method is said to affect the yield of the shoots of the leaf hence affecting the quality of the exudates gum[21,22]. Analysis of variance showed significant difference ($p \leq 0.05$) in each of Mg, Na, K, Ca and Mn.

Table-2
Phytochemical screen of the n-hexane extract of the crude and purified exudates

Experiment	Observation	Inference	
		Crude Exudate	Purified Extract
Alkaloid			
1. Drangendorff's Reagent	Pink precipitate	+++	+++
2. Mayer Reagent	Milky colour solution	+++	+++
3. Picric Reagent	White precipitate	+++	+++
Saponins			
1. Frothing test	Persistent frothing occurred for more than 15 minutes	+++	+++
Cardiac Glycoside			
1. Sofowora test	Reddish brown colour at interphase	--	--
Flavonoid tests			
1. Mg-metal/HCl test	No effervescence formed and no orange coloration	--	--
Carbohydrate			
1. Molisch test	A reddish brown ring observed at the interphase	++	++
Tannins			
1. Bromine water test	No decolorisation	--	--
2. Ferric chloride test	No dark green precipitate	--	--
Resin test	Green colouration	+++	+++

Key: + = Low concentration, ++ = Medium concentration, +++ = High concentration, -- = Negative

Table-3
Metal ion content of the exudate, acid hydrolysis and saponification products

Inorganic element	Crude exudate (µg/g)	Direct extract (µg/g)	Column eluate (µg/g)	Acid hydrolysis product (µg/g)	Saponification product (µg/g)	WHO (2002) µg/g
Potassium (K)	16.54	16.68	17.66	11.90	13.98	12-18
Sodium (Na)	90.25	90.40	91.26	72,60	85.00	80 -100
Calcium (Ca)	500.56	510.00	520.00	380.00	508.00	100 - 5000
Magnesium (Mg)	40.60	46.30	47.37	47.65	44.97	30 -50

Key: Crude Exudate = Sample without Treatment, Direct Extract = Treatment with solvents

Table-4
Electrical conductivity data for crude, direct, column chromatography, acid hydrolysis and saponification products

Solvent system	Crude Exudate	Direct extract (µS/cm)	Column chromatography (µS/cm)	Saponification product (µS/cm)	Acid hydrolysis product (µS/cm)
Ethanol	27.60	28.02	28.00	30.00	32.02
n-hexane	27.62	28.80	28.60	30.02	32.08
Benzene	27.60	28.40	28.40	29.80	32.06
Petroleum ether	27.61	28.40	28.20	29.60	32.04
Ethanol-Benzene (1:2)	27.61	28.20	28.00	29.40	32.00
Average	27.61	28.36	28.24	29.76	32.04

The electrical conductivities of the crude, purified and modified products is shown in Table - 4. The electrical conductivities in the purified exudate are similar. There is increase in the value for the saponification and acid hydrolysis products. The conductivity could be explained in terms of the presence of some ions of K^+, Mg^{2+}, and Na^+ as the major charge carriers in the exudate gum. The conductivities (µS/cm) in the purified exudates are similar while there is increase in the saponification (29.79 µS/cm) and the acid hydrolysis products (28.36 µS/cm) due to the added ions from the reactant (H_2SO_4 and NaOH).

The IR spectral bands scan of the purified exudate sample, acid hydrolysis and saponification products are presented in Figures-1 to 3 shown below.

Their frequencies are related to the functional group similar to that reported by Udo et al.[21]. The broad O-H stretching vibrations of alcohol group of the entire exudate sample were in the region 3500-3360cm^{-1}, and the C-O stretch of alcohol was in the region1070-1060cm^{-1}. Other functional groups observed were: C=O stretch 1210cm^{-1} confirming the presence of the acid group, N=O symmetric stretching 1380- 1375cm^{-1} and asymmetric stretching 1470-1455cm^{-1}. C-N stretch 880-850cm^{-1} indicates the presence of nitro group in exudate, N-H bending 1650-1640cm^{-1}, C-N stretch 1245-1240cm^{-1} and N-H wagging vibration 890-660cm^{-1}. The bands of the crude exudate, acid hydrolysis and saponification products indicate mainly the presence of carboxylic fatty acid and O-H of fatty alcohol, while the absorption band of C-N and N-H shows the presence of some protein materials.

Conclusion

Phytochemical screening of exudate of *Dacryodes edulis* revealed the presence of components like alkaloids, saponins, carbohydrates and resins which could be useful in production of drugs for treatment of some ailments. The metal ions compositions are within the range specified by WHO. *Dacryodes edulis* exudate can be used medicinally and in the industry.

Acknowledgment

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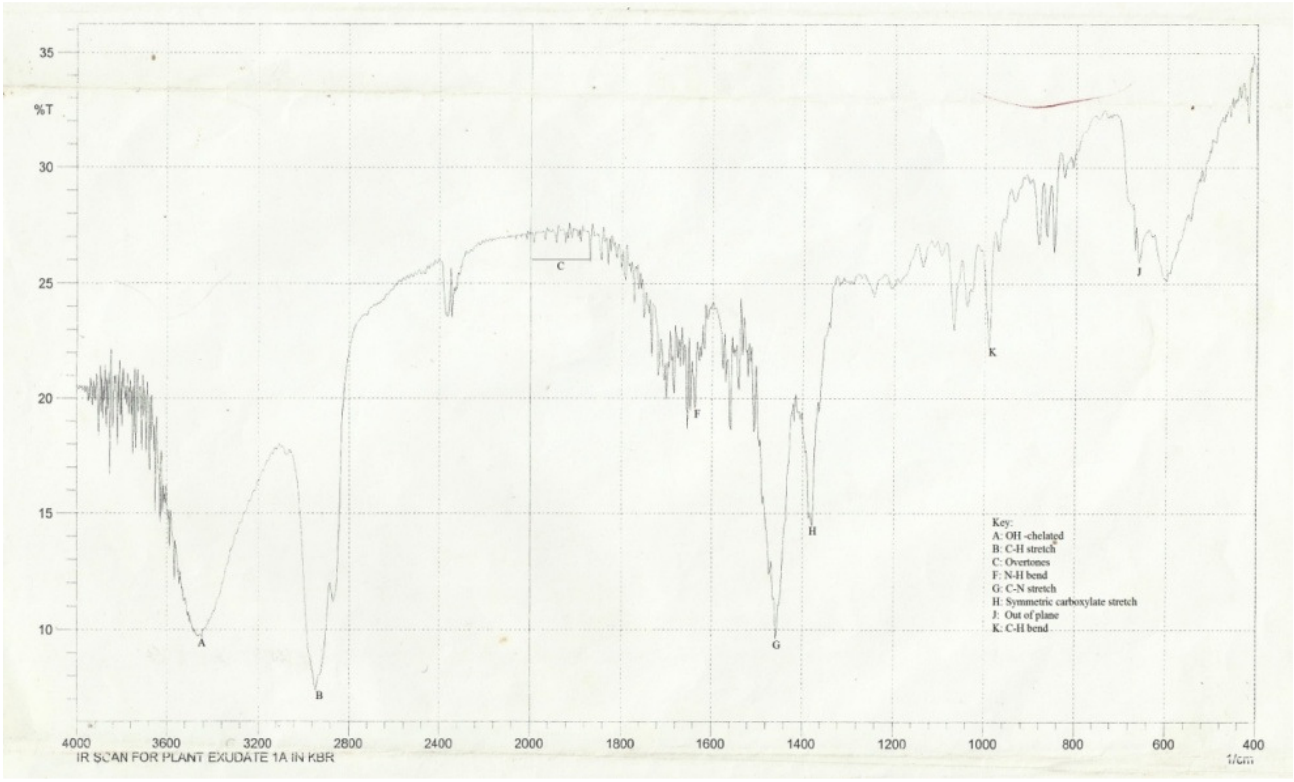

Figure-1
IR Scan of *Dacryodes edulis* Purified Product

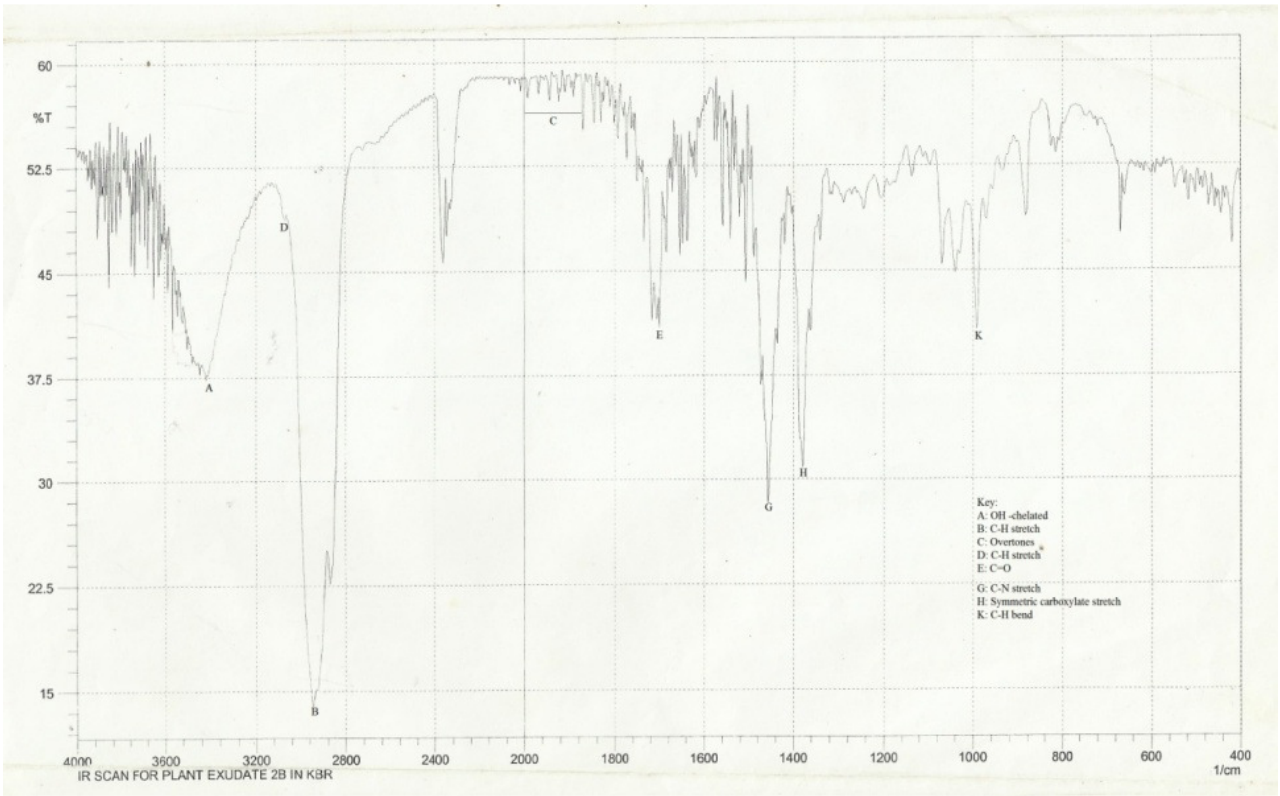

Figure-2
IR Scan of *Dacryodes edulis* Acid Hydrolysis Product

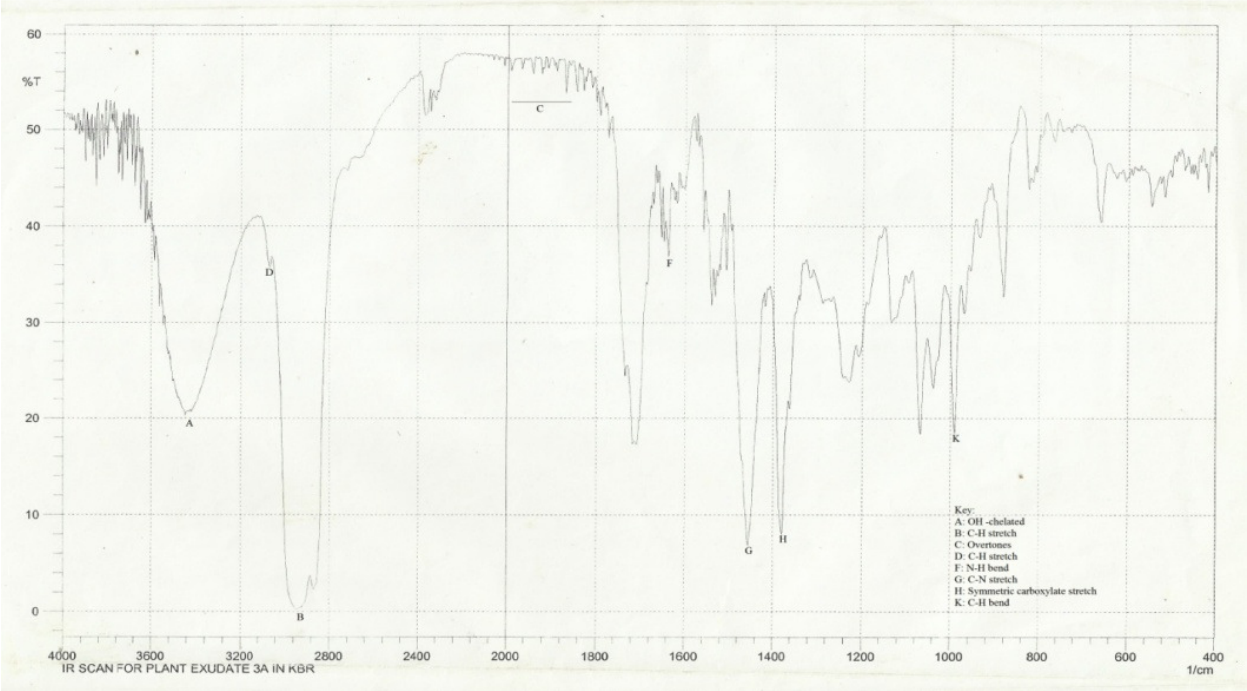

Figure -3
IR Scan of *Dacryodes edulis* Saponification Product

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