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**Research Paper**

# Extraction, Partial Characterization and Cytolytic activities of African black pea, *Dacroides aedulis*.

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***ABSTRACT***
*The oil was extracted from the pulp of African pea Dacryoides aedulis using n-hexane as extracting solvent in soxhlet extractor at 60$^{o}$C . The physicochemical properties of the oil was determined. The percentage oil yield was 40.00 ± 0.23. The oil was light yellow in colour and has the aroma of roast pea D. aedulis , and a specific gravity of 0.85 ± 0.03 with a refractive index of 1.42 at 20$^{o}$C . The odour was agreeable, mouthfeel was rated 6.80, and viscosity was 780 (Centipoise). The taste was rated 7.4 and the overall acceptability by the panellists scored 7.5. The acid value of the oil was 6.4 ± 0.05 and the percentage free fatty acid was 3.22 ± 0.03. The peroxide value was 20.00 ± 0.25, iodine value of 39.80 ± 3.20, and the saponification value gave 193.00 ± 3.01. These results suggest that D. aedulis yields appreciable quantity of essential oil. The oil was later used for the cytolytic activity on Bovine red blood corpuscles. This assay showed that D. aedulis oil has a profound cytolytic activity on the red blood corpuscles. It may also be responsible for the bouts of fever that accompany the over-consumption of African pea during its season.*

***KEY WORDS:*** *Pea pulp oil, Cytolytic activity, Spectrophotometer, fatty acids, Spectrin, Bovine erythrocytes.*

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## I. INTRODUCTION

The African pea, *Dacroides aedulis* also called Ube, (Nigeria) and Safou (Cameroon) is an indigenous fruit tree of the humid lowlands and plateau regions of the West, Central Africa and Gulf of Guinea countries. In South-eastern Nigeria, the shrubs and/or trees are grown around homesteads and flowering takes place from January to April. The major fruiting season is between May and October {9; 15}. It is one of the African orphan/under-utilized oil- bearing fruits. Both the pulp and the seed contain appreciable quantity of oil. Most of these oil-bearing fruits have been identified to be of nutritional importance {14}. The African pea is of the family Burseraceae. It is a tropical tree that flowers at the beginning of the rains and bears fruits during 2 through 3 months after flowering. It contains high pectin which makes them useful in lowering blood cholesterol, good in *strengthening* the immune system, and helps relieve pains in various inflammatory conditions. *Dacroides aedulis* has many medical and nutritional uses {6}. The fruit pulp is eaten, and the seeds usually thrown away {2}. There are several studies on African pea that centre mostly on the composition of the edible Fruits pulp that constitute the mainstay of economic value of the fruit {8 ;20}. It was nutritionally suggested that *Dacroides aedulis* pulp oil may contain a cytolytic agent which may be connected with bouts of fever that accompany heavy consumption of the fruit during its season. The present study therefore is undertaken to explore the potentials of *Dacroides aedulis* pulp oil containing a cytolytic agent as well as a facilitator of membrane permeability.

## II. MATERIALS AND METHODS

Mature fruits of African pea *Dacroides aedulis* were purchased in Ogige Market Nsukka, Enugu State Nigeria beween the month of June and September 2017. They were taken to the Department of Botany of the University of Nigeria where a Taxonomist-Mr Alfred Ozioko identified and authenticated the fruits. These fruits were washed, dried and the pulp blended wet with an electric blender (Nakai--462) China. Bovine erythrocytes were collected in an anti-coagulant bottle at Ogige market slaughter house (Abattoir), Nsukka, Enugu State Nigeria.

**Extraction of Dacroides aedulis Pulp Oil**

A quantity, 1000 grammes of the *Dacroides aedulis* fruit pulp was coarse-ground wet using electric blender (Nakai--462) China. An amount 100 grammes of the coarse milled pulp was put into a soxhlet exractor (pyrex) at $70^0$C, and n-hexane as extracting solvent. The extracted oil was concentrated by evaporating the solvent, and the oil was gravimetrically quantified.

**Experimental**

The specific gravity was determined using specific gravity bottle according to the method described by {22}.
The colour, odour and physical of were determined sensorily by 15 Panelists invited from the Department of Food Science and Technology, University of Nigeria, Nsukka .
Acid value was determined for *Dacroides aedulis* oil by dissolving 0.80 gramme of each oil in 10 millilitres of 1;1 volume/volume ethanol ;diethylether solvent and titrating with 0.1N Sodium hydroxide while stirring and by using phenolphthalein as indicator.
Calculation;
Acid value = (56.1 x N X V )/W
Where N = Normality of NaOH
V = Volume (ml) of Na OH used
W = Weight of sample used

The iodine value of the oil was determined by dissolving 0.5 gramme of the oil in 15millilitres carbon tetrachloride in 100millilitres glass stoppered flask. An amount 25millilitres of Wij's solution was added, the flask stoppered and allowed to stand for two hours in the dark at $25^0$C. Also, an amount 20 millilitres of potassium iodide (KI) solution was added and mixture titrated against 0.2N Sodium thiosulphate $Na_2S_2O_3$ using starch indicator. A blank determination was carried out and the iodine value calculated using the formular;
Iodine value =12.69N ($V_2$—$V_1$)W
Where N = Normality of thiosulphate
$V_1$ = Volume (ml) of thiosulphate solution used in blank
W = Weight of the sample (0.5 gramme)

The saponification value of the oil sample was determined as described by AOAC, 2005,and in it ,1.0 gramme of the oil was dissolved in 12.5 millilitres of 0.5% ethanolic KOH and the mixture refluxed for 30 minutes. An amount 1.0 millilitre phenolphthalein indicator was added and the hot soapy solution titrated against 0.5N HCl. A blank determination was also carried out under the same condition and saponification value determined using the equation;
Saponification value = 56.1N ($V_I$—$V_2$) W
Where N = Normality of Hydrochloric acid used
$V_1$ = Volume of hydrochloric acid used in test
$V_2$ = Volume of hydrochloric acid used in blank
W = Weight of oil used (1.0 gramme)

The peroxide value (PV) was determined by weighing 1.0 gramme of the oil sample into a 200 millilitres conical flask, then 25 millilitres 2;1 volume/volume glacial acetic acid ;chloroform solvent was added. Also, 1.0 millilitre of saturated potassium iodide was then added and the mixture left in the dark for one (1.0) . Next, 30millilitres of water was added and the mixture titrated against thiosulphate solution using 5millilitres starch as indicator. A blank was similarly carried out and the peroxide value was calculated using the equation;
Peroxide value; (PV) = (100 ($V_1$-V2) Meg/Kg/) W
W = Weight of sample
$V_1$ = Volume (ml) of thiosulphate used in test
$V_2$ = Volume (ml) of thiosulphate used in blank
N = Normality of thiosulphate ($Na_2S_2O_3$) .

Percentage Free Fatty Acid (%FFA) as (Oleic) was determined by multiplying the acid value with the factor 0.503. Thus, %FFA = 0.503 X acid value.

**Animal Protocol**

Preparation of De-proteinized Extract
A quantity 40 grammes of the African pea pulp was weighed and blended wet using an electric blender (Nakai-462) China .After blending, the extract was poured into a 250 $cm^3$ conical flask. Equal volumes of Trichloroacetic acid (TCA). Exract were mixed for de-proteinization. An amount 20 milliltres of the filtrate

were taken and 10 millilitres were subjected to heating at 60$^{o}$C for 15 minutes and the other 5 millilitres was left neither boiled nor incubated.

**Standardization of Bovine Erythrocytes**

The Bovine red blood was collected from the slaughter house of Ogige Market, Nsukka, Enugu State Nigeria with EDTA anti-coagulant. After this, 5 millilitres of the blood was poured in to three centrifuge tubes and 5 millilitres of normal saline (0.9% NaCl solution) was added also and centrifuged at 10,000 rpm for 10 minutes after which the serum is discarded. The above saline addition to erythrocytes, centrifugation and discarding of serum was done two more times, and this was aimed at standardization of erythrocytes to get them ready for the cytolytic assays.

**Estimation of Cytolytic Activity**

A total of four test tubes were set in a test tube rack and to each of the four test tubes, an amount 8.5millilitres of Tris- saline buffer and 2 millilitres of Bovine erythrocytes are added later, 1.5 millilitres of the boiled, incubated extract, cold extract and distilled water were added to each of the four test tubes respectively. Immediately after 10, 15, 20, and 25 minutes from the addition of the extract and the distilled water, it was observed that the initial red colour of the mixture got changed from red to deep red-black colour in assay I, fairly deep red colour in assayII, red-black colour in assay III, and clear red colour was also obtained in assay IV which served as colour. At this point, a volume 1.5 millilitres of the mixture was poured in to the 2.5 millilitres cuvette of the UV Spectrophotometer (Jenway, 7305) and the optical absorbance (OD) read at 740nm wavelength. The table showing the cytolytic activity of the crude extract of *Dacroides aedulis* is shown in the results section.

## III. RESULTS

**Physical properties of *Dacroides aedulis* pulp oil**

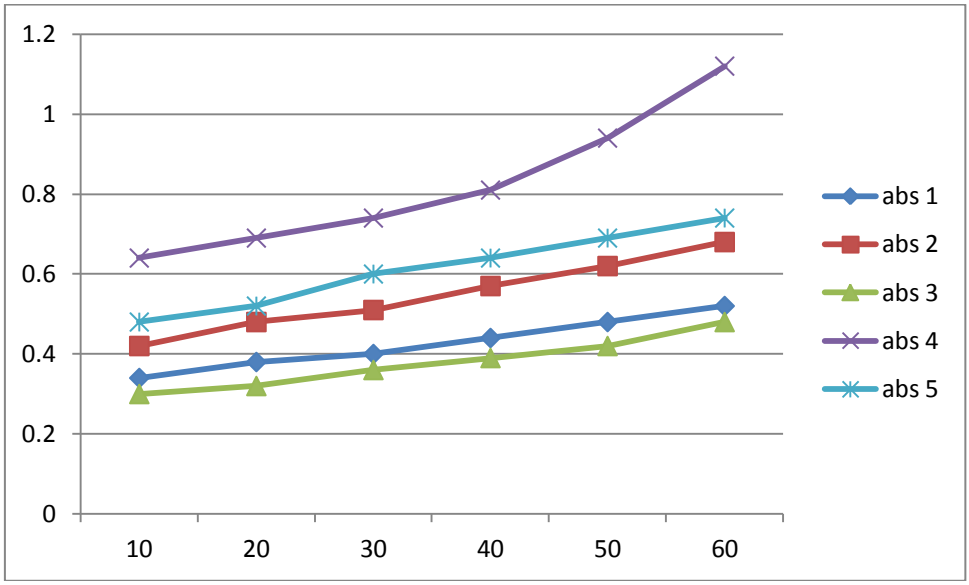
| | |
|---|---|
| Colour | Pale yellow |
| Odour | Agreeable(sweet) |
| Taste | 7.4 |
| Mouthfeel | 6.80 |
| Viscosity | 780 Centipoise |
| Refractive index at 20$^{o}$C | 1.42 |
| Specific gravity | 0.85 ± 0.03 |
| Overall acceptability | 7.5 |

**Chemical properties of *Dacroydes aedulis* pulp oil**

| | |
|---|---|
| Acid value | 6.40 ± 0.05 |
| Iodine value | 39.80 ± 3.20 |
| Peroxide value | 20.00 ± 0.25 |
| Saponification value | 193.00 ± 3.01 |
| Percentage free fatty acids (%age FFAs) | 3.22 ± 0.03 |

**The results showing the cytolytic activity of *Dacroydes aedulis* pulp oil on Bovine erythrocytes**

| Time in secs. | 10 | 20 | 30 | 40 | 50 | 60 |
|---|---|---|---|---|---|---|
| abs 1 | 0.34 | 0.38 | 0.4 | 0.44 | 0.48 | 0.52 |
| abs 2 | 0.42 | 0.48 | 0.51 | 0.57 | 0.62 | 0.68 |
| abs 3 | 0.3 | 0.32 | 0.36 | 0.39 | 0.42 | 0.48 |
| abs 4 | 0.64 | 0.69 | 0.74 | 0.81 | 0.94 | 1.12 |
| abs 5 | 0.48 | 0.52 | 0.6 | 0.64 | 0.69 | 0.74 |




**Key:** abs = absorbance , extract = *D .aedulis* pulp
abs 1 = boiled in hot water
abs 2 = incubated at 60$^{o}$C in an oven
abs 3 = tepid extract
abs 4 = roasted in red hot bonefire
abs 5 = roasted in hot wood ash

## IV. DISCUSSION

The pale yellowish oil extracted from the pulp of *Dacroides aedulis* had a percentage oil yield of 40.00 ± 0..23, which is in agreement with the range described by {21} for most plant oils .The oil has a specific gravity of 0.85 ± 0.03 with a refractive index of 1.42 at 20$^{0}$C which is in agreement with the value of 1.462 for *B. Sapida* oil {3}. This of course does not belong to non-drying oil {7}. The oil odour was agreeable, the taste was rated 7.4, mouthfeel, 6.80, the viscosity was 780 (Centipoise), and the overall acceptability by the panellists scored 7.5. The saponification value of *Dacroides aedulis* pulp oil was found to be 193.00 ± 3.01. This value was in agreement with values obtained for some vegetable oils ranging from 188-196 mg/KOH/g {1} . It was also reported by {22} that oils with higher values contain high proportion of lower fatty acids. The acid value of this oil is 6.40 ± 0.05 mg/KOH/g.

When compared with *Plutenia conophora* (11.5mg KOH/g) as reported by {3}. The peroxide value is 20.00 ± 0.25 and the iodine value is 39.80 ± 3.20 . This therefore contains oxygen and of the class polyunsaturated vegetable oil {18}. The percentage free fatty was 3.22 ± 0.03. However, the results of the partial characterization agrees with the work of Obasi and Okolie 1993 and that of {12} who stated the Dietary reference intake for energy and other nutrients contained in *Dacroides aedulis* .The pulp oil is good in lowering blood cholesterol for it is of plant origin,{13}. Also, it may be polyunsaturated nature of the pulp oil that made it a good enough ingredient in the manufacture of biscuits and other snacks {16}. Beside the above-mentioned properties of Dacryodes aedulis,it is worth noting that the physicochemical properties of Dacryodes aedulis obtained in Nigeria did not have significant difference with that obtained in Cameroon{10; 4}. However, *Dacryodes aedulis* have variations in its keeping properties as well as preserve the germinating properties {9 ; 15}. Dacryodes aedulis has gone beyond use in eating ncereall snacks but has also become a major product tha are marketed localy and locally and international {5}. The cytolytic activity of the crude extract of African pea showed that it contains cytolysin in its pulp. This is in agreement with the work of {20} who reported the pulp of African pea as the repository of the the lipids synthesized by the seeds. However, the Bovine erythrocyte is made up of erythrocyte membrane containing proteins carbohydrates and lipids {17; 21}. Lysis is complete breakdown of a membrane or cell. The colour change that was observed on addition of the crude extract in to the Bovine erythrocyte confirmed the lysing of the erythrocyte membrane component by the cytolysin contained in the crude extract. This agrees with the work of {23} .who disclosed the changes that accompany erythrocyte membrane, hence, the lysis was found to be maximum in the test tube containing the hot bonefire-roasted extract. This may be due to the maximal temperature to which the oil may have been exposed to probably converting the polyunsaturated pulp oil to peharps saturated state—an index to lipid peroxidation and hence erythrocyte membrane lysis.

## V. CONCLUSION:

This study suggests that African pea, *Dacroydes aedulis* pulp oil contained cytolytic agent which break down the membrane of erythrocytes by lipid peroxidation.

**Recommendation:** Consumers of African pea, *D.aedulis* especially in its season should be preparing it with low temperature heat sources so as to lower the degree of inter-conversions of its oil.

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**Authors contribution**

The authors confirm contribution to the paper as follows: the corresponding author conceptualized and, designed and wrote the paper, author number 2 performed the analysis and contributed data or analysis tool.

**Conflict of Interest** The authors of this work has no conflict of interest whatsoever before, during and after this work

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