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Isolation, Identification and Antioxidant Properties of Anthocyanins Rich Fractions of *Dacryodes edulis* (African pear) Fruit peels

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Original Research Article

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Abstract: Anthocyanins content and antioxidant were determined in the three main pigments toward ripening in *Dacryodes edulis* (African pear) fruit peels. The pigments were extracted with acidified (0.5% HCl) methanol and the extracts were hydrolysed and the applied on 3mm thin-layer chromatograms. The chromatograms were developed subsequently in one direction, using n-Butanol: Acetic acid: Water (BAW) (4:1:5 v/v). The anthocyanidin present in the three pigments, pelargonidin-3-glycoside, was identified according to their Rf values, UV-vis spectrum and reaction with NaOH. The results revealed that the concentration of anthocyanidin in the pink (premature, week4-12) stage was 0.20 mg/g, white (maturing fruit, week 14-16) stage 0.32mg/g and blue-black (matured fruit, week 18-20) stage 0.56mg/g, this results correspond to the capacity of the anthocyanidin to inhibits lipidperoxidation (TBAR) with 76.9% (pink stage), 81.2% (white stage) and 90.0% (blue-black stage). The result demonstrated a positive relationship between Anthocyanins (flavonoids) and antioxidant activity. Hence, *Dacryodes edulis* is better consumed when fully matured.
Keywords: *Dacryodes edulis*, antioxidant activity, Anthocyanins, thin-layer chromatograms.

INTRODUCTION

In Africa, fruits are in high demand, and this is because they are complemented with food to ensure a balanced diet. Fruits serve as sources of fat, carbohydrate, vitamins and minerals hence, they also become important when the functions of these nutrients are being considered in the body [1].

Dacryodes edulis (also called African plum, African pear or Safou) is an indigenous fruit tree in the humid lowlands and plateau regions of West, Central African and Gulf of Guinea countries [2]. *Dacryodes edulis* belongs to the *Burseraceae* family [3, 4]. It is an evergreen tree indigenous to the central Africa and Gulf of Guinea regions. The genus name is derived from the Greek word '*Dakruon*'(a tear) in reference to the resin droplets that appears on the bark surface of its species. The species-specific name *edulis* means edible [2, 4]. The genus *Dacryodes* comprises about 40 species, occurring in the American, Asian and African tropics. In Africa, about 20 species have been described [5]. In Southeast Nigeria, the trees are grown around homesteads and flowering takes place from January to April. The major fruiting season is between May and October [2, 5]. The role of fruits to a healthy and nutritious diet, the world over is a well-established fact. *D. edulis* is a tree cultivated widely for its edible and nutritious fruits. Generally, the fruit may be cooked in hot water, or roasted/baked in an oven at about 50ºC. The cooked fruit can be eaten with maize, plantain, cassava, cocoyam, bread, etc.

The entire plant of *D. edulis* has pharmaceutical properties that are variously exploited by many African communities [6]. Oral treatment against leprosy and it is also gargled as mouthwash for the treatment of tonsillitis. In the western parts of Cameroon, the bark is crushed and used in concoctions against dysenteries while in central Cameroon the bark is used to treat a toothache. The leaves are boiled in combination with Lantana camara, Cymbopogon citratus and *Persea americana* yielding a steam bath taken to treat fever/headaches and malaria in the Republic of Congo. The leaves made into a plaster have been recently reported to treat snake bites in South West Cameroon [7]. The leaves are also crushed and the resultant juice used to treat skin diseases such as scabies, ringworm, rashes, while twigs from branches are sometimes used as chewing sticks [8-10]. The leaves and seed are used in Nigeria for animal feed [9, 11].

Dacryodes edulis possess three stages of pigmentation toward ripening of its fruits which are made up of anthocyanins [12]. Anthocyanins are the

largest group of water-soluble pigments in the plant kingdom [13]. They are responsible for most of the red, blue and purple colours of fruits, vegetables, flowers and other plant tissues or products [13, 14]. Anthocyanins are becoming increasingly important not only as food colorants, but also as antioxidants. Anthocyanins are reported to have therapeutic benefits including vasoprotective and anti-inflammatory [15], anti-cancer and chemoprotective as well as anti-neoplastic properties [15]. Anthocyanins are considered to contribute significantly to the beneficial effects of consuming fruits and vegetables [16]. There is a rising demand for natural sources of food colorants with nutraceutical benefits [17] and alternative sources of natural anthocyanins are becoming increasingly important.

Phenolic compounds are the principal antioxidant constituents of natural products and are composed of phenolic acids and flavonoids, which potent radical terminators are acting by donating hydrogen radicals [18]. Omoregie and Osagie [12], reported that flavonoids are well known anti-oxidants and free radical scanvengers. High potential of polyphenols to scavenge free radicals are because of their many phenolic hydroxyl groups [19]. Phenolic compound plays a major role in antioxidant activity. Kinkela *et al*., [18] reported that natural antioxidant phenolic compound in S*echium edule* with better performance than BHT, known as a very efficient synthetic antioxidant agent and widely used in Food Technology. Obasi and Okolie, [20] reported that the qualitative and quantitative analysis of phenolic compounds in active, extracts showed the presence of flavonoids, flavonol and the chemical composition responsible for the antioxidant effects.

The specific objectives of this study were to isolate and identify the anthocyanin molecules responsible for colouring in the African pear (*Dacryodes edulis*) fruit toward ripening and their antioxidant capacity.

MATERIALS AND METHODS

Plant materials

Matured fruits from *Dacryodes edulis* were collected from private farm land in Okada Town of Ovia North-East LGA of Edo State, Nigeria. These fruits were authenticated by the Department of Botany, University of Medical Sciences, Ondo City. A voucher specimen of each plant was there after deposited in the herbarium of the same Department.

Preparatory of plant extracts

The anthocyanins content were extracted from the fruit peel. The peel sample (2g) was sornicated for 10mins and hydrolysed with 2M HCl in boiling water for 30 minutes followed by extraction with 100% methanol containing 0.5% HCl. The hydrolysates were centrifuged at 300 rpm for 10mins. The supernatant was filtered through filter and concentrated in vacuum and then transfer into sample bottles for analysis.

Chemicals

Methanol, HCl, butanol, acetic acid, TBA (Sigma Chemicals Co, London). All other reagents and chemicals were of analytical grade and obtained locally from BDH and Aldrich in Nigeria.

Estimation of Anthocyanin Pigments in *Dacryodes edulis* Fruit Skin Peels

Anthocyanins are the largest group of water-soluble pigments in the plant kingdom, and they are responsible for most of the colours of fruits, vegetables, flowers and other plant tissues or products [13]. Test for Anthocyanins was carried out as described by [21].

Pear peel extracts (100% methanol with 0.5% HCl) were tested for the presence of anthocyanins by reacting with NaOH. A blue-green colour indicates the presence of anthocyanins in the extracts.

Determination of UV-Visible Spectrum of pigments

Twenty micro litres of samples were diluted with 3ml (0.5% HCl in 100% methanol) and placed in quartz cuvette. Readings were then taking after blanking the machine with 100% methanol containing 0.5% HCl.

Separation of Anthocyanins by Thin-layer Chromatography (TLC)

The extracts were concentrated in vacuum and spotted on 3mm pre-coated plates 2cm from the bottom and 2cm apart. The chromatograms were then developed in BAW (4:5:1, n-butanol: Acetic acid: H_2O) in a saturated chromatography tanks. The chromatograms were then allowed to dry in the dark and the R_f values were calculated. These values were compared with the standards.

Estimation of Anthocyanins Pigment

The skin peels of *Dacryodes edulis* fruits were chopped into pieces with scissors and the fragments ground up in a mortar in a suitable volume of acidified methanol. Filter or centrifuge (3000g for 15 minutes) to remove debris and spectrophotometer was used to measure the absorbance of the extract (which has pH 1.2) at 520nm.

Another volume of the sample and add NaOH (drop wise) were mixed after each drop and checked the pH. Once pH 5 has been reached, the total volume of NaOH used was noted. The solution was centrifuge to remove any debris and before measuring absorbance at 520nm.

Calculations

Anthocyanin concentration (mg cyanidin/g) =
(Abs pH1.2 – Abs pH5) x DF

Where,
Abs pH1.2 = Absorbance at 520nm for pH1.2 sample
Abs pH5 = Absorbance at 520nm for pH 5 sample
DF = Dilution factor (final vol. of extract/ initial vol. of extract)
98.2 = Cyanidin 3-galactoside coefficient of extinction.

Lipid Peroxidation Assay

Lipid peroxidation was determined using a modified thiobarbituric acid reactive species (TBARS) assay [22] as described by [23].

To 0.5ml of 10% v/v egg homogenate was added 0.1ml of various extracts, followed by 1ml of distilled water. The mixture was vortexed and allowed to stand for 30 minutes at room temperature after which 1.5ml of 20% acetic acid and 1.5ml of 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate were added. The resulting mixture was heated in a water bath at 90 C for 60 minutes. After cooling, 4.0ml of butan-1-ol were added to each tube and centrifuged at 3000 rpm for 10 minutes. The absorbance of the organic upper layer was measured at 532nm.

Calculations

Inhibition of lipid peroxidation (%) by the extract was calculated using the formula:

% Inhibition = (1 – E / C) x 100

Where,

C = absorbance value of the fully oxidized control
E = absorbance in the presence of extract.

Statistical Analysis

All statistical analyses were performed using the Graph Pad Instant 3 software (GraphPad Software Inc. San Diego, USA). The results were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used. Significant differences between groups were detected in ANOVA using Bonferroni-Holm posthoc-test at $p<0.05$.

RESULTS

Dacryodes edulis fruits are rich in polyphenols, mainly in the skin. Among the polyphenols, the anthocyanins are pigment of the flavonoids class responsible for the pigment in *Dacryodes edulis* fruits and these flavonoids inhibited the oxidation of lenoleic acid up to 90% when the fruits are fully mature (Table-1).

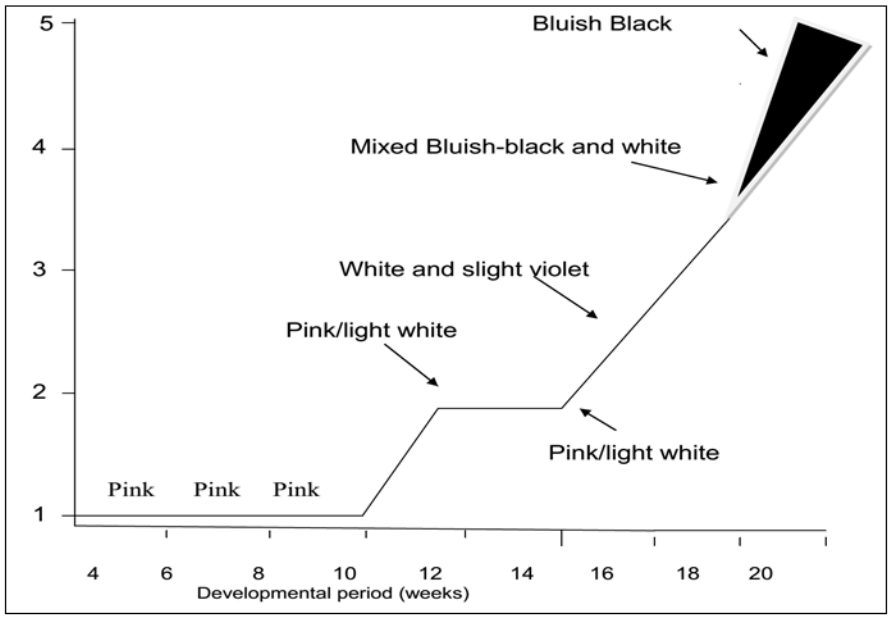

Fig-1: Colour changes observed during the fruit development of *Dacryodes edulis*.

Table-1: Extracts of *Dacryodes edulis* fruit skin peels at different stages of pigmentation tested for the Presence of Anthocyanins. Values are mean ± SEM.

Pigment	Reaction with NaOH	TLC Analysis	RF (cm)	Absorbance 460 – 550 nm
Pink	Blue-Green	Blueish-green Yellow	0.8 ± 0.01 0.76± 0.02	0.716 ±0.02
White	Blue-green	Light -green	0.82± 0.01	1.826 ±0.03
Blue-black	Blue-green	Light-Green yellow	0.85± 0.04 0.75± 0.08	1.738 ±0.02

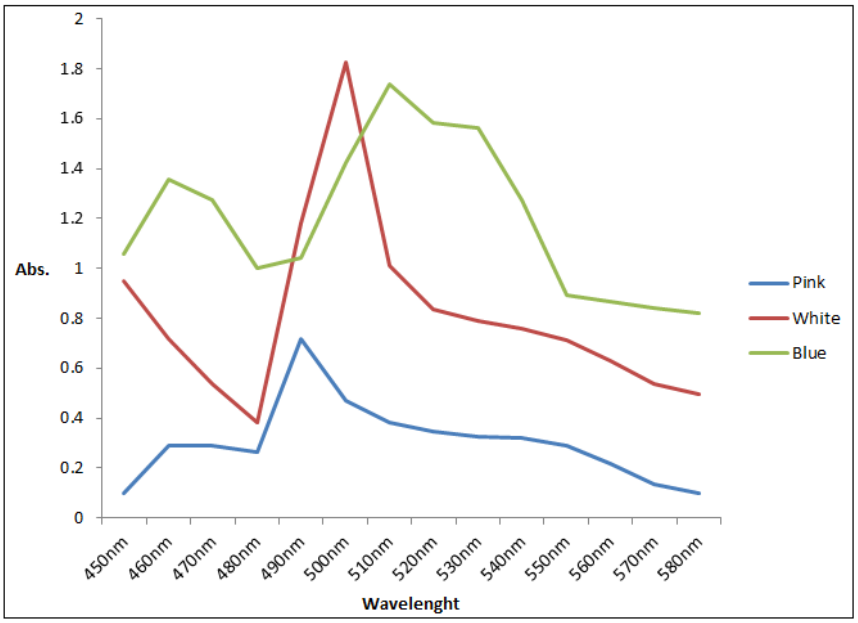

Fig-2: Spectrophotomentary spectra of pink, white and blue pigments from the African pear fruit peel (methanol extract)

Table-2: Estimation of Anthocyanin pigments content and % inhinbition of TBARS during *Dacryodes edulis* fruit development. Values are mean ± SEM (* = P< 0.05).

Pigment	Total anthocyanin Content(mg/g)	% inhibition Lipidperoxidation (TBARS)
Pink	0.20± 0.04	76.9 ± 0.94
White	0.32 ±0.08	81.2 ±0.81
Blue	0.56 ± 0.01	90.0 ± 0.22

DISCUSSION

The main objective of this study was to isolate and identify the anthocyanins molecules responsible for colouring in the peels of Dacryodes edulis. Anthocyanins pigments are usually found as glycosides in plants, however, when they are ingested as food, the sugars are easily hydrolysed from the aglycones.

In the isolation and identification of the anthocyanins responsible for colouring in the *Dacryodes edulis* fruit peel, the isolated anthocyanins molecules in Dacryodes edulis fruit peels from the three stages of pigmentation (pink, white and blue-black) gave blue-green colouration on addition of NaOH which gave a positive test for the presence of anthocyanins [24]. Additional qualitative information was obtained with the aim of spectral characteristics of the chromatographic bands. The spectral data presented in (Table 3.1) show a maximum absorption for the chromatographic peaks for each of the three stages of pigmentation were in the range 480–520nm. These data are corresponding to spectral data of pelargonidin-3-glycoside [13, 24]. Evidence for the identity of anthocyanins can be obtained from the spectral measurements [24]. The wavelength of maximum absorption for most anthocyanins falls in the 460-550nm range, with peak absorbance of about 520nm [24]. Thin layer chromatography revealed two bands in the pink pigment (blue-green and yellow) colours with R_f 0.8 and 0.78 respectively. The white pigment stage had one band with (light blue-green) colour with R_f 0.82 and the blue-black stage had two bands also (blue-black and yellow) colours with R_f 0.85 and 0.75 respectively (Table 3. 1). It has been documented that R_f values for flavonoids is between 0.75 – 0.85 (Ref). The different colours of anthocyanins pigments reflect the nature of their hydroxylation and methoxylation pattern. An increase in hydroxylation is accompanied by an increase in blue colour while methoxylation enhances the red colours [20, 24].

The concentration of anthocyanins in the pink (premature, week4-12) stage was 0.20 mg/g, white (maturing fruit, week 14-16) stage 0.32mg/g and blue-black (matured fruit, week 18-20) stage 0.56mg/g, this results correspond to the capacity of the anthocyanins to inhibits lipidperoxidation (TBAR) with 76.9% (pink stage), 81.2% (white stage) and 90.0% (blue-black stage). The result demonstrated a positive relationship between anthocyanins (flavonoids) and antioxidant activity. The implication of colour differences in Dacryodes edulis fruit peels constitute a biochemical marker which could be used to differentiate fruit maturity, rather than the environmental factors in which the fruits are grown. It is invaluable to link this biochemical differences in the fruit's antioxidant

capacity to the genetic differences in the anthocyanin biosynthetic pathway. Hence, *Dacryodes edulis* is better consumed when fully matured.

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