

Comparative Study of Antioxidant Activity and Hepato-Protective Effect of Extracts of *Tetracarpidium conophorum* and *Dacryodes edulis* on Sodium Arsenate Exposed Rats

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Abstract: This study comparatively evaluated the *in vitro* antioxidant activity and the hepato-protective effect of extracts of two locally consumed fruits, *Dacryodes edulis* (African pear) and *Tetracarpidium conophorum* (African walnut) in sodium arsenate exposed rats. Antioxidant activity was determined by spectrophotometric methods using 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical, hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) assays. Total phenol, flavonoids and anthocyanins content of extracts were also determined. Results from the study indicated that DPPH and H₂O₂ scavenging capacity as well as percentage inhibition of MDA were significantly higher (P<0.05) in extract of *T. conophorum* (48.34%, 46.9% and 53.5%, respectively) when compared with *D. edulis* extract (45.47%, 18.1% and 45.3%, respectively). The anthocyanins content was significantly higher (P<0.05) in *D. edulis* extract than in *T. conophorum*. Whereas, *T. conophorum* extract recorded higher phenol content (52.3GAE/100g) compared with *D. edulis* extract (22.5GAE/100g). The flavonoids content of *T. conophorum* extract was comparable to that of *D. edulis*. The hepato-protective effects of the extracts were examined *in vivo* in male Wister rats challenged with sodium arsenate. Results showed that the rats fed with the two extracts had significant reduction (p<0.05) in lipid peroxidation, increase in SOD and catalase activities relative to control values. These results suggest that rat fed with *Dacryodes edulis* and *Tetracarpidium conophorum* extracts possess varied degree of potent antioxidant activity and may serve as important sources of antioxidants in food, cosmetics and pharmaceutical industries.

Keywords: *Dacryodes edulis*, *Tetracarpidium conophorum*, antioxidants, sodium arsenate, hepato-protective.

INTRODUCTION

For decades, the screening of medicinal plant materials for their therapeutic values has continued to represent potential sources of new effective medicine. Besides, evidence from epidemiological studies have suggested that high consumption of fruits and vegetables may be linked to reduced risk of developing most oxidative stress induced diseases such as cancer, diabetes mellitus, protein energy malnutrition (PEM), cataract, infections and other degenerative diseases of aging [1-3]. Naturally, there is a dynamic balance between the amounts of free radicals produced in the body and antioxidants to scavenge or quench them to protect the body against deleterious effects. The amount of antioxidant principles present under normal physiological conditions and may be insufficient to neutralise free radical generated under pathological conditions. Therefore, it is important to enrich our diets with antioxidants to protect against harmful with diseases. Hence, there has been an increased interest in

the food industries and preventive medicine in the development of "natural antioxidants" from plant materials.

Tetracarpidium conophorum (Mull. Arg.) Hutch and Dalziel syn. *Plukenetia conophorum* commonly called African walnut and *Dacryodes edulis*, named "African pear" or "Safou" are tropical trees producing consumable fruits which softens when heated, and then would be eaten with corn or as dessert [4, 5].

Dacryodes edulis is an attractive tree, usually 8-12 meter in height, and some time reaching 20-25 meter in dense forest stands. "Safou" belongs to the family *Burseraceae* [6, 7]. The wounded bark exudes a limpid resin that becomes opaque while solidifying [5]. *Dacryodes edulis* has a long history of use in folk medicine. Traditional healers in Nigeria use the plant to treat various infections; it is used in traditional medicine

as a remedy for parasitic skin diseases, jigger, mouthwash, tonsillitis and drepanocytosis [4, 8].

Tetracarpidium conophorum is a climber and belong to *Euphorbiaceae*, found in West Africa in general. Its habitat is usually large trees; the fruits are greenish with four round seeds in each fruit. The seed testa is hard, and the cotyledons are white in colour [4, 9].

The fruits are edible, the plant is medicinal and used for various purposes [4]. The leaves, bark, and fruits of *T. conophorum* are used medicinally, and their uses include masticatory, giddiness, thrush, antihelminthic, syphilis, dysentery, and antidote of snake bite [9].

In Southern Nigeria ethno-medicine, African walnut is used as a male fertility agent and in the treatment of dysentery [10]. The methanol and ethyl acetate extract of *T. conophorum* leaves have been shown to possess good antibacterial activities especially Gram +ve organism [10].

The objective of this work is to determine and compare the antioxidant activities of the fresh fruits of *T. conophorum* and *D. edulis* by investigating DPPH, hydrogen peroxide and MDA level and some antioxidant enzymes- SOD and CAT.

MATERIALS AND METHODS

Plant materials

Matured fruits of *Tetracarpidium conophorum* and *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.

Preparation of plant extracts

Methanol: chloroform extracts of each plant fruits was prepared according to previous method of Bligh and Dyer [11] as reported by Bafor and Osagie [12]. The sterilised mesocarp was macerated three times in 500ml of $\text{CHCl}_3/\text{MeOH}$ (1:2 v/v) using MSE homogeniser. The homogenate was filtered each time and the filtrate residue re-blended with the $\text{CHCl}_3/\text{MeOH}$ solution. Appropriate volume of chloroform and 0.72% NaCl solution were added to the combine filtrate to form a biphasic system chloroform/methanol/water in volume 1:1:09. The chloroform layer was withdrawn, and concentrated to dryness on a rotary evaporator at 30°C under reduced pressure and then stored at 40°C for subsequent analysis.

Animals and treatment

30 male rats of Wister strain weighing between (100-120) g were obtained from the animal house unit of the Department of Biochemistry, University of Medical Sciences, Ondo City, Nigeria.

The animals were divided into three sub-groups having five animals each. Animals in sub-group 1 were fed with 50mg/kg of the extract for seven days, while sub-group 2 and 3 was given corn oil. At the 8th day, sub-group 1 and 2 rat were injected intraperitoneally with 1mg/kg of Sodium Arsenate, while sub-group 3 was given distilled water. 24 hours later, the animals were sacrificed by cervical dislocation. The livers were removed at once, blotted dry, weighed and stored at 4°C .

Preparation of homogenates

1g of the liver tissue was homogenised in 10ml of ice-cold physiological saline to obtain 10% (w/v) homogenates. The resulting homogenates were centrifuged at 5000g for ten minutes and the supernatants obtained were used for determination of superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation.

Chemicals

DPPH (2, 2-diphenyl-2-picrylhydrazyl), Gallic acid, folin-ciocalteau's reagent were obtained from Sigma Chemical company, USA. All other reagents and chemicals were of analytical grade and obtained locally from BDH and Aldrich in Nigeria.

Phytochemicals study

The chemical constituents in the fruits extracts of *T. conophorum* and *D. edulis* were classified qualitatively and quantitatively using phytochemicals reagents according to procedures described by Trease and Evans [13]. The fruits were screen for the presence of phenolics, flavonoids and anthocyanins.

Determination of *in vitro* antioxidant activities TBARS

Thiobarbituric acid reactive substances (TBARS) modified assay was used to measure the lipid peroxide formed, using egg yolk homogenates as lipid rich media. Malondialdehyde (MDA), a secondary end product of the oxidation of polyunsaturated fatty acids, reacts with two molecules of thiobarbituric acid (TBA) yielding a pinkish red chromogen with an absorbance maximum at 532nm [14].

Inhibition of lipid peroxidation (%) by concentrates was calculated with formula:

$$(C-E) / C \times 100$$

where C is the absorbance value of the fully oxidized control and E is ($\text{Abs}_{532\text{ TBA}} - \text{Abs}_{532\text{-TBA}}$)

DPPH Radical Scavenging Activity

The radical scavenging activity of the fruit extracts against 2, 2-diphenyl-2-picryl-hydrazyl radical was determined by measuring UV absorbance at 532nm. Radical scavenging activity was measured by a slightly modified method of Brand-Williams [15]. The following 0.02, 0.04, 0.06, 0.08 and 0.1mg/ml concentration of samples were placed into test tubes and 0.5ml of 1mM DPPH solution in methanol was added after. The experiment was carried out in triplicate. The test was incubated for 15 minutes at room temperature and the absorbance was read at 517nm. A blank solution was prepared and measured containing the same amount of methanol and DPPH. Lower absorbance of reaction of the reaction mixture indicates higher radical scavenging activity. The radical scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity \%} = \frac{[AB - AA]}{AB} \times 100$$

Where, AB = Absorption of blank sample and AA is the absorption of tested extract solution.

Hydrogen peroxide (H₂O₂) Determination:

The hydrogen peroxide scavenging assay was carried out following the procedure [16] as described in [17]. Crude extract at 20μ/ml concentration in 3.4ml phosphate buffer (0.1M, pH 7.4) was added to 0.6ml of H₂O₂ solution (43Mm). The absorbance was taken at 230nm. Blank solution contain phosphor buffer without H₂O₂. The concentration of hydrogen peroxide (mM) in the assay medium was determined using a standard curve.

CALCULATIONS

$$\% \text{ scavenging of hydrogen peroxide} = \frac{(A_0 - A_1)}{A_0} \times 100$$

A₀ - Absorbance of control

A₁ - Absorbance in the presence of plant extract

Determination of in vivo antioxidant activity:

Superoxide dismutase (SOD) assay:

SOD was assayed in the liver according to the method of Misra and Fridovich [18], based on the rapid auto-oxidation of adrenalin due to the presence of superoxide anions. This is measured spectrophotometrically at 420nm and SOD concentration is expressed as units/g tissue.

Catalase activity

Catalase activity in the liver was determined as residual H₂O₂ after incubation with the enzyme according to Kaplan and Groves [19].

Estimation of Lipid Peroxidation:

Lipid peroxidation in the tissue involves the determination of thiobarbituric acid reactive species (TBARS) which is the indicator of membrane lipid peroxidation. Values for TBARS were reported as Malondialdehyde (MDA) and quantified using a Molar extinction coefficient of 1.5 X 10⁵ Mcm³ and expressed as mmole MDAg⁻¹ of tissue [20].

Statistical Analysis

Data were expressed as mean ± standard error of mean (SEM). One way analysis of variance (ANOVA) was performed to test for differences between the groups mean. Significance differences between the mean were determined by Duncan's multiple range test and p-value <0.05 were regarded as significance [21].

RESULTS

Estimation of total phenolic, total flavonoids content, and anthocyanins content of *Tetracarpidium conophorum* nuts and *Dacryodes edulis* fruit pulp are represented in (Table 1and 2). The results shows high level of total phenolic content (TPC) expressed in garlic acid equivalent (GAE) in the *Tetracarpidium conophorum* and *Dacryodes edulis*. The total flavonoids content expressed as quercetin equivalent (QE) was found to be very high in both *Tetracarpidium conophorum* and *Dacryodes edulis*.

Table-3 represent the antioxidant activity of the fruit plants were evaluated by determining the percentage inhibition of thiobarbituric acid reactive species (TBARS), 1,1-diphenil-2-picrylhydrazyl (DPPH) and hydrogen peroxide (H₂O₂) scavenging activity by the plants whole fruit samples and the extracted oils from the plants. The oil extract were found to possess the highest inhibitive activity of TBARS, DPPH and H₂O₂.

The effects of various extracts on hepatic catalase (CAT) and superoxide dismutase (SOD) activities are represented in Table 4-5. These enzymes were considered increased (P<0.05) in the rats treated with both fruits extracts when compare with the controls. *T. conophorum* fruits extracts had effects than the *D. edulis*.

The hepatic Malondialdehyde (MDA) level of the rats is shown in Table 4-5. The (MDA) was considered reduced (P<0.05) or inhibited in the both extract treated rats as against the control.

Table-1: Phytochemical composition (Qualitative) of *Tetracarpidium conophorum* and *Dacryodes edulis* fruits extracts

Phytochemical substances	<i>T. conophorum</i>	<i>D. edulis</i>
Flavonoids content	++	++
Phenolics content	++	++
Anthocyanins content	+	+++

+ = Detectable; ++ = moderately present; +++ = highly detectable

Table-2: Phytochemical composition (Quantitative) of *Tetracarpidium conophorum* and *Dacryodes edulis* fruits extracts

Phytochemical substances	<i>T. conophorum</i>	<i>D. edulis</i>
Flavonoids content ^a	60.5* ± 2.34	80.0 ± 0.36
Phenolics content ^b	52.3* ± 2.01	22.5 ± 0.24
Anthocyanins content ^c	66.0* ± 3.11	161 ± 3.41

a = expressed as mg/100g; b= expressed as mg GAE/100g; c= expressed as mg/100g.
Values are expressed as mean ± SEM (*P<0.05).

Table-3: *In vitro* Antioxidant Activity of *Tetracarpidium conophorum* and *Dacryodes edulis* fruits extracts

Fruit extract	H ₂ O ₂ % scavenging ability	DPPH % scavenging ability	MDA % inhibition
<i>Tetracarpidium conophorum</i>	46.91* ± 0.77	48.34* ± 0.38	53.3* ± 1.02
<i>Dacryodes edulis</i>	18.14 ± 2.02	40.70 ± 1.34	45.3 ± 0.21

Values are expressed as mean ± SEM (*P<0.05)

Table-4: *In vivo* Antioxidant Activity of *Tetracarpidium conophorum* fruits extracts

Fruit extract	SOD Activity	CAT Activity	MDA U/L
<i>Tetracarpidium conophorum</i>	0.048* ± 0.01	0.775* ± 0.04	0.666* ± 0.09
+Ve Control	0.059 ± 0.02	0.777 ± 0.03	0.618 ± 0.01
-Ve Control	0.039 ± 0.01	0.290 ± 0.07	1.121 ± 0.09

Values are expressed as mean ± SEM

Table-5: *In vivo* Antioxidant Activity of *Dacryodes edulis* fruits extracts

Fruit extract	SOD Activity	CAT Activity	MDA U/L
<i>Dacryodes edulis</i>	0.048 ± 0.01	0.775* ± 0.02	0.635* ± 0.05
+Ve Control	0.059 ± 0.03	0.777 ± 0.08	0.618 ± 0.05
-Ve Control	0.040 ± 0.02	0.290 ± 0.09	1.105 ± 0.09

Values are expressed as mean ± SEM (*P<0.05)

DISCUSSION

In this study, two commonly consumed fruits were evaluated for their *in vitro* antioxidant activities. The antioxidant activities of the various extracts were also assessed *in vivo* in the rats exposed to Sodium Arsenate.

The DPPH test provided information on the reactivity of test compounds with a stable free radical. Due to its odd electron, 2,2-diphenyl-2-picryl-hydrazyl (DPPH) radical gives strong absorption band at 517nm in visible spectroscopy. As the electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes; the resulting decolouration is stoichiometric with respect to a number taken up. Thus, the efficacies of antioxidant are often associated with the ability to scavenge stable, highly reactive, free radical. This may be useful in the treatment of radical

related pathological damages. These highly reactive free radicals have been implicated in the pathology of different numbers of diseases in humans such as diabetic mellitus, atherosclerosis, cancer, Parkinson's disease and other neurodegenerative disorder. It is also reported that antioxidant compounds such as phenols and other phytochemicals play a vital role in removing free radicals and inhibition of lipid peroxidation [22, 23].

In the present study, the both fruit extracts exhibited DPPH free radical scavenging ability. *Tetracarpidium conophorum* had significant increase (P<0.05) free radical scavenging ability higher than that of *Dacryodes edulis*. This may be a reflection of the total activities of various components present in the extracts [24, 25]. Indeed, several studies have reported that the antioxidant activities of most plants with therapeutic properties may be due to the presence of

natural substances mainly phenolic compounds [26, 24]. Plant phenolic which may occur widely in the plant kingdom, especially in fruits and vegetables constitutes the major group of compounds acting as primary antioxidant or free radical terminators [27]. There is a strong relationship between total phenolic content and total antioxidant activity in selected fruits, vegetables and grains products. The antioxidant capacity of phenolics compounds is mainly attributed to their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators [28]. In this study, the phenolic content of *T. conophorum* fruit extracts was found to have higher level of phenols (52.3 GAE/100g) compared to *D. edulis* (22.5 GAE/100g). Flavonoids in both fruits were comparatively high (60.5 mg/100g for *T. conophorum* and 80.0mg/100g for *D. edulis*) ($P < 0.05$) and may account for parts of the benefits associated with the consumption of fruits and vegetables. They have been reported to interfere with the activities of the enzymes involved in ROS generation, quenching of free radicals, chelating metals, and rendering them redox inactive in the Fenton reaction [23]. 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 [29].

Anthocyanins are polymeric flavonium ions. They are effective antioxidant which provides several health benefits, including the prevention of cancer, urinary tract infection and cardiovascular diseases as well as inhibition of LDL oxidation [29]. The results from this study showed that both *Tetracarpidium conophorum* and *Dacryodes edulis* have high anthocyanins with the highest values observed in *D. edulis* extracts.

The animal study shows appreciably increased activities of the antioxidant enzymes (SOD and CAT) and as well as reduced the level of Malondialdehyde in the liver [2]. Malondialdehyde is the major oxidation product, peroxidised PUFAs and increased MDA level is an important indicator of lipid peroxidation. Catalase on the other hand, is an enzymatic antioxidant widely distributed in all animal tissues including the red blood cell (RBC) and liver. Catalase decomposes H_2O_2 and helps protect the tissues from highly reactive hydroxyl radicals. SOD, another antioxidant enzyme, removes superoxide radical by converting it to H_2O_2 [22].

The present study involved a comparative evaluation of the antioxidative activity of *Dacryodes edulis* and *Tetracarpidium conophorum* fruits extracts. The overall antioxidative capacity of the extracts suggest a positive correlation as well as synergistic effects with respect to DPPH scavenging activity, total phenol, flavonoids and anthocyanins components of the extracts. This indicates that these components are more likely to contribute to the antioxidant potential of the

extracts [30]. It is possible that the in vivo antioxidant capacity observed in these extracts was not solely from the phytochemicals alone, but could be due to the presence of some other compounds like essential fatty acids and ascorbic acid.

These results suggest that *Dacryodes edulis* and *Tetracarpidium conophorum* extracts possess varied degree of potent antioxidant activity and may serve as important sources of antioxidants in food, cosmetics and pharmaceutical industries.

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