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Antioxidant properties of *Canarium schweinfurthii,* (including its quality indices), and *Elaeis guineensis* oils on rats exposed to paraquat-induced lipid peroxidation

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Abstract: *The antioxidant property of Canarium schweinfurthii (C.s) oil in comparison with palm oil was studied in albino Wistar rats pretreated orally with the oils for 16 consecutive days. Thereafter, 20 and 80mg/kg body weight dose of paraquat was administered to the animals intraperitonealy (i.p) and were maintained for 3 hour period before sacrifice. At 20mg/kg body weight dose, the mean malondialdehyde (MDA) concentration (nM/dm^3) for groups fed palm oil and C.s oil were: 0.0007.22±2.6 and 0.0035.11±2.1 respectively (P=0.05) compared to control group. Catalase activity (µMole/min) were 326.8±5.4, and 258±7.2 in that order (P=0.05) relative control group. At 80mg/kg body weight paraquat dose, the mean concentration of MDA for groups fed palm oil, C.s oil were 0.000001.3±5.37and 0.001.±9.2 in that order (P=0.05) compared to control group. Activity of catalase was 137±6.2, and 189.2±1.0 respectively (P=0.05) relative control group values. The quality indices for C.s oil showed saponification, free fatty acid, and iodine values of 200.22mg KOH/1g of oil, 3.42%, and 70.32 respectively. Refractive index was 1.45 whereas moisture content was 0.56%. C.s oil appeared to be a more efficient antioxidant than palm oil at 20mg/kg body weight paraquat dose but at 80mg/kg body weight paraquat dose, the reverse was the case. The result showed that both oils reversed peroxidation to some degree. Their consumption can be beneficial against peroxidation and thence oxidative stress.*
KEY WORDS: *Antioxidant, Malondialdehyde, Catalase, Radicals, Paraquat, Peroxidation*

I. Introduction

Plants are good sources of antioxidants such as glutathione, ascorbate, fibre as well as all the biomolecules required by the cell. They serve as indispensable constituents of human diet supplying the body with vitamins, minerals and certain hormone precursors, in addition to protein and energy [1]. Plant rich diets are associated with lowering the risk of cancer, diabetes, atherosclerosis, and dementia [2].

Canarium schweinfurthii (*C.s*) is a plant popular in Plateau State of Nigeria. It is eaten cooked and the fruit is oily [3]. The plant grows to 36.6m high with very slight blunt buttress. A ripe fruit is brown/purplish plum-like containing a hard seed. The fruit pulp contains 30-50% oil use as essential oil and believed to have analgesic effects [4]; [5].

II. Materials and Methods

2.1 Materials

Fresh *Canarium schewainfurthii (C.s)* fruit were purchased from Pankshin Market in Plateau State, Nigeria. Eighteen male albino Wistar rats were purchased from the animal farm, Department of Pharmacology, University of Jos. The rats were fed with pelletised vital feed "grower mash", and tap water and allowed to grow and aclimatise for ten weeks. The chemicals were of analytical grade and were purchased from chemical stores in Jos, Plateau State. Reagents were obtained from the Department of Biochemistry, University of Jos, Department of Chemistry, organic chemistry division, University of Jos, and the laboratory division, Grand cereals and oil mill Nigeria Limited, Jos Plateau State, Nigeria.

2.2 Chemicals and Reagents

All chemicals and reagents used for determination of iodine value, saponification value, free fatty acids, catalase activity, and membrane lipid peroxidation were of analytical grade and included: tetrachloromethane, 10% Potassium iodide solution and 0.1N Thiosulphate solution (iodine value). For the determination of saponification value, reagents were: 0.25 alcoholic KOH, 0.5N HCI and Phenolphthalein indicator. In the case of free fatty acids, reagents used included 0.10N NaOH, diethyl ether and Phenolphthalein indicator. The assay of catalase activity was done using 0.059M H_2O_2, 0.05M potassium phosphate buffer (pH 7.0). For the determination of membrane lipid peroxidation, 15% (W/V) Trichloroacetic acid, 0.375 %(W/V) Thiobarbituric acid, and 0.25N Hydrochloric acid were the reagents used.

2.2 Extraction of Oil

The warm pressing extraction method was used for the extraction of the oil as described by [6] with slight modification. The fruit were sorted to remove any dirt or foreign material present in them. They were then washed in cold water to remove any dirt adhering to the surface and were softened by applying hot water at 60^0C on to them in a clean bowl and allowed to stand for 8minutes. The seeds were then removed by gentle pounding using ceramic mortar and pestle. The pounded pulp was then transferred into a water bath and maintained between temperature range of 60-75^0C for 10minutes. As heating progressed, the oil floated on top of the water and was carefully collected into a dish using a spoon. The oil was then transferred to a hot air oven at 80^0C for 1hour to remove any free moisture.

Saponification value, iodine value, moisture content and free fatty acid value of the oil were determined thus:

2.3 Determination of Saponification Value

2g of sample was weighed, and added on to 25ml of alcoholic potassium hydroxide solution (40g of KOH in 100ml of ethanol). The flask was connected to air condenser and boiled for 60min with intermittent shaking. While solution was still hot, 1ml of phenolphthalein was added and titrated against excess alkali with 0.5M HCl. Saponification value was calculated using the formula:

$$SV = \frac{(b - a) \times N \times 56.10}{W}$$

where, b = blank titre, a = sample titre, N = Normality of HCl,
w= weight of sample.

2.4 Determination of Iodine Value

0.2g of sample as dissolved is 15ml CCl_4 in a conical flask followed by the addition 25ml of Wijs solution.

The conical flask was closed with a round glass stopper, mixed and kept in the dark at room temperature for 60 minutes. Thereafter, 20ml of 10% aqueous potassium iodide solution followed by addition of 150ml distilled water. Two sets of blank determinations were conducted in the same manner but with distilled water instead of oil.

The Iodine value (IV) was calculated using the formula

$$IV = \frac{(b - a) \times N \times 12.692}{W}$$

Where, b = blank titre, a = sample titre, N = Normality of KI, w = weight of the sample.

Preparation of Wijs Solution

8g of iodine trichloride was dissolved in 200ml glacial acetic acid. 9g of iodine was dissolved in 300ml carbon tetrachloride. The two solutions were then mixed and diluted to 1000ml with glacial acetic acid.

2.5 Determination of Free Fatty Acids

25ml diethyl ether was mixed with 25ml alcohol and 1ml phenolphthalein solution (1%). 0.1M NaOH was added carefully. 7g of oil was dissolved in the mixed neutral solvent and titrated with aqueous 0.1ml NaOH shaking constantly until a pink colour which persisted for is seconds was obtained.

2.6 Determination of Acid Value

This was calculated using the formula:

$$\text{Acid value} = \frac{\text{Titre (ml)} \times 5.61}{\text{Weight of sample used}}$$

Moisture Content

The moisture content was determined by difference in weights.

2.7 Determination of Refractive Index

The refractive index correlates with iodine value and saponification value according to the equation of [7].

Administration of the Oils

20 white male albino Wistar rats with mean weight of 168.3g were randomly grouped into four thus: A, B, C, D of 6, 6, 4, 4 rats respectively. Groups A and B rats were given a daily oral dose of 5ml/kg *C.s* oil and palm oil respectively alongside their normal meal of pelletised vital feed and tap water for a period of sixteen

consecutive days. The rats in group C were given only the meal of pelletised vital feed and water for sixteen consecutive days. Group D was baseline control fed with neither paraquat nor the oils.
Groups A, B and C were further divided randomly into sub-groups of 3, 3 and 2 rats each as follows: A_1 A_2; B_1 B_2 and C_1 C_2. Groups A_1 B_1 and C_1 were administered an oral dose of 20mg/kg body weight paraquat; groups A_2 B_2 and C_2 were administered 80mg/kg body weight oral dose of paraquat. Treatments at both levels of paraquat dose lasted three hours after which the rats were sacrificed. Activity of catalase and plasma malondialdehyde concentrations were determined using spectrophotometric technique as described by [8] and [9] respectively.

Collection of Blood Sample

Chloroform was applied to cotton wool in a dessicator and made air tight. Rats were anaesthesised by putting them into chloroform-saturated dessicator for 10 minutes individually. Thereafter, the rats were dissected from the diaphragm through the chest using forceps and scissors. Using 10ml syringe and needle, blood samples were obtained by direct cardiac puncture. Samples were then transferred into labeled plastic EDTA containers.

2.8 Assay of catalase activity

The method of [8] was applied to determine the activity of this enzyme with temperature maintained at 25^0C at pH of 7.0.

2.9 Measurement of membrane lipid peroxidation in plasma

The method of [9] was used to determine the extent of the peroxidation. Beer-Lambert law was applied to determine the concentration of malondialdehyde (MDA). Extinction coefficient of MDA is $155nM^{-1}C^{-1}M^{-1}$.

Dilution of Whole Blood

Whole blood was diluted ×500fold with 0.05M potassium phosphate buffer (pH 7.0). Thereafter, the diluted whole blood was diluted further by measuring 0.01ml and mixed with 4.99ml of the buffer to give a total volume of 5.0ml.

Statistical Analysis

The InStat3 statistical software was used; ANOVA was chosen to analyse (compare means of test groups with the control) data obtained. P value of 0.05 was considered significant.

Table 1: Chemical property of *Canarium schweinfurthii* oil

Parameter	Value for C. *schweinfurthii* crude oil
Free fatty acid (%)	3.42 ± 0.1
Iodine value (mg/100g)	70.32 ± 0.2
Saponification value (mgKOH/1g)	200.22 ± 0.3
Moisture content (% water)	0.59 ± 0.1
Refractive index	1.45 ± 0.0

Values are means of three determinations (± SEM)

Table 2: Malondialdehyde concentration (nM/dm^3) in rats plasma for both control and test groups

	Paraquat (20mg/kg)	Paraquat (80mg/kg)
MDA Conc. in plasma of palm oil fed rats	6.92×10^{-4}*±$0.02.58 \times 10^{-5}$	$1.34X10^{-3}$*±$5.37X10^{-5}$
MDA Conc. in plasma *C.s* oil fed rats	3.89×10^{-4}* ±2.26×10^{-5}	$1.88X10^{-3}$* ±9.19×10^{-5}
MDA Conc. of plasma of rat given PQ alone	$104 X10^{-3}$*±1.20×10^{-3}	389×10^{-3} ±1.20×10^{-3}
MDA Conc. in plasma of normal control	3.1×10^{-4}* ± 6.50×10^{-6}	3.10×10^{-4}± 6.50×10^{-6}

Values are means of three determinations (± SEM) ;
*Values in the same column differ significantly (P = 0.05).

Table 3: Mean plasma catalase activity (µMole/min) in rats exposed to paraquat

	Rats fed with *C.s* oil	Rats fed with palm oil	Rats fed with no oil
PQ 20mg/kg	326.8 ±5.940*	258.0 ± 7.01*	103.3 ± 2.56*
PQ 80mg/kg	137.6 ± 6.15*	189.2 ± 0.98*	51.6 ± 0.02*

Values are mean ± SEM, (n = 6); * values differ significantly (P=0.05).
Note* actual catalase activity value is multiplied by a factor of: 10^{-2}units

III. Discussion

Evaluating the antioxidant attributes of *C.s* oil alongside that of palm oil was the thrust of this work. The chemical properties of *C. s* oil are as presented in table 3. The saponification value obtained for the oil was 200.22mg KOH/100g of oil. This value is within the range of values for palm oil, 190-205, [10], soybean oil, 190-210, [11]. Oils from groundnut and corn have saponification values of 188-196 and 187-196 respectively [12]. The high saponification value of *C.s* oil is an indication that the oil could be suitable for soap making. The free fatty acid value of *C.s* oil was 3.42% and the corresponding acid value was 6.84%. Generally, free fatty acid values are a function of oxidative rancidity of oils. The value obtained for *C.s* oil is comparatively higher than the value for palm oil, 2.73-2.89 [13] and soybean oil, 1-2.8, [11]. However, the value is lower than the maximum free fatty acid content of edible oils, 3.5%, [10].

Iodine value is a measure of the extent of unsaturation of given oil. The iodine value obtained for *C.s* oil was 70.32; this value is lower than that of groundnut oil, 84-99, and castor oil, 81-91, [12], but higher than that of palm oil, 48-53, [13]. Unsaturated lipids are assimilated and broken down easily than saturated lipids [14] and the higher the iodine value, the more unsaturated the oil. However, higher iodine value reduces the stability of oils but enhances oxidation. Lipid peroxidation has been reported as the major contributor of the loss of cell function under oxidative stress condition and it is usually indicated by TBARS in animals [15]. Considering that typical reactions during ROS-induced damages involve the peroxidation of unsaturated fatty acid, the result obtained clearly showed that exposing rats to paraquat for 3 hours led to oxidative stress which was directly proportional to increase in the concentration of paraquat.

Refractive index is the degree of deflection of a beam of light when passing from one medium into another. It is directly proportional to the number of carbon atoms [16]. Refractive index value of 1.45 obtained for *C.s* would mean that *C.s* oil is composed of long chain unsaturated hydrocarbons. The moisture content of the oil was 0.56%, a value comparatively lower than the value for palm kernel oil, 5.7±0.2 [17]. The low moisture content of the *C.s* oil sample is critical with respect to its stability and storage.

The role of free radicals and reactive oxygen is becoming increasingly recognized in the pathogenesis of the many human diseases including cancer, aging, and atherosclerosis [18]. Free radicals can also cause lipid peroxidation of membranes especially the Red Blood Cell membrane because it lacks cell organelles such as mitochondria [19]. Although there are some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), these compounds are associated with side effects. Determining natural sources of antioxidants and the antioxidant potential of plants, in this case *C.s* oil, products is imperative. The results showed a significant difference in MDA concentration for the test groups, at 20 and 80mg/kg body weight, relative the control group ($P = 0.05$) implying both oils possess antioxidant attributes. At 20mg paraquat/kg body weight dose administered, *C.s* oil appeared to be a better antioxidant than palm oil since the concentration of MDA in the group was lower than that of group fed palm oil. At 80 mg paraquat/kg body dose administered, palm oil fed groups had lower concentration of MDA relative group fed *C. s* oil implying palm oil is a better antioxidant compared to *C.s* oil at this dose. Lipid peroxidation may not have been completely inhibited in this work, but the oils exhibited significant capacity to mitigate this phenomenon. Perhaps higher quantity of the oils could confer more efficient antioxidant effect.

Catalase is an enzyme involved in the breakdown of toxic hydrogen peroxide to water and molecular oxygen. This enzyme's activity is useful as a biomarker of certain disease conditions including ovarian cancer [20]. The result for the activity of catalase indicated treatment with both oils led to high activity of the enzyme relative control group values ($P = 0.05$). Specifically, *C.s* oil appeared to be a more efficient antioxidant at 20mg/kg body weight paraquat dose where the mean catalase activity value was 3.26±1.21 µMole/min as against 2.580±1.31 µMole/min for palm oil ($P = 0.05$). At 80mg/kg body weight paraquat dose, mean activity of catalase in group fed palm oil was 1.89±2.21 µMole/min whereas group fed *C.s* oil had catalase activity value of 1.37±1.56 µMole/min ($P = 0.05$).

IV. Conclusion

The results obtained showed that the quality of *C.s* oil was within the range of values for many edible oils. The result also showed C.*s* oil protected cells of rats from peroxidation *in vivo* at paraquat concentration of 20mg/kg. Thus, degenerative diseases which arise owing to the effect of reactive oxygen species on tissue/cells over time may be obliterated by ample consumption of *C. schweinfurthii* oil. By what mechanism(s) these oils exert their efficient antioxidant functions as reported at low (*C.s*) and high (palm oil) dose of paraquat constitute further research on these oils.

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