Assessment of *In vivo* Antioxidant properties of *Dacryodes edulis* and *Ficus exasperata* as Anti-Malaria Plants

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Abstract: The potential anti-oxidant properties of two plants; *Dacryodes edulis* and *Ficus exasperata* hitherto linked with treatment of other ailments but increasing implicated in anti-malaria "Agbo" preparation, were evaluated. The anti-oxidant effects of ethanol leaf extracts of *D. edulis* and *F. exasperata* were examined using albino rats (*Rattus norvegicus*) pre-treated with Tween 80 (placebo), CCl_4 (-ve control), Vit. E (+ve control) and 50mg/kg, 100mg/kg, 200mg/kg plants extracts for 7days; intoxicated previously with CCl_4 for 2 days. Animal blood and liver tissues were examined for Thiobarbituric Acid Reactive Substances (TBARS), Reduced Glutathione (GSH) and Catalase (CAT) activities. Phytochemical screening showed reducing sugars, flavonoids, saponins, tannins in the plant species; except cardiac glycosides in *D. edulis* and alkaloids and terpenoids in *F. exasperata*. The plant species recorded significant anti-oxidant activites comparable to Vit E. particularly at 200mg/kg treatment ($P<0.05$). Ethanol extracts of *D. edulis* and *F. exasperata* showed significant anti-oxidant action comparable to Vit E and is dose dependent with the 200mg/kg pretreatment showing the most oxidative stress suppressive action and this justifies their inclusion amongst the plants employed for the traditional preparation of the anti-malarial remedies.
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1. Introduction

Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free-radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long term complication of several diseases (Sabu and Kuttan, 2002, Tsao *et al,* 2004, Atawodi, 2005).

The blood is the chief vehicle for transport in the body and the liver a vital organ in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges like xenobiotics, drugs, viral infections, and related assaults. The natural protective mechanisms of the liver provides a major defense line against such attacks, however, when compromised by the high degree of such assaults, tissue or organs injuries result, making oxidative stress either instigator or associate to the complications of several illnesses (Marcel, *et al*, 2010; Ramakrishna. *et al*, 2011).

In Nigeria and along the West African region, herbal medicine practitioners have made several claims regarding the diverse pharmacological properties of several plant species; which is often the rationale behind their usage in the management of disease conditions and the lure to access them in pharmaceutical researches for the development of new drugs. (Ekanem and Udoh, 2009). In these areas, the anti-malarial concoction "Agbo" is a popular remedy that is prepared with a variety of plant species, some of which were previously employed for management of other diseases. Coupled with the destructive attributes of the parasites to the red blood system; is the effect of oxidative stress, which is linked with the development of anaemia in malaria. Indeed, increase total antioxidant status is shown to be important in recovery from malaria. (Ayoola *et al,* 2008). Hence, there is the need to continuously search for new drugs and thus new plants, since majority of anti-malaria medications have been from plant sources and where these anti-malaria plant species have good reactive oxidative species (ROS) scavenging status, they will be good candidates for further anti-malaria drug screening.

The present study seeks to determine the phtyochemical properties and ascertain the antioxidant potentials of *Dacryodes edulis* and *Ficus exasperata* and thus efficacy on the management of malaria and inclusion in the preparation of the anti-malaria concoction "Agbo".

2. Material and Methods

All plants were collected from forests and farmland; Southern Nigeria, during an Ethnobotanical field survey extending from August – September 2010.

Animal Collection

Albino rats (*Rattus norvegicus*) average weight 100 – 170g, were acclimatized with normal rat feed (Laymore Concentrate) and tap water *ad libitum*. The animals were treated daily for 7 days.

Extraction of Plant Samples

Plant materials were air dried at room temperature (28-32°C) for two weeks and blended into uniform powder. The ethanol extracts were prepared by soaking 100g of each of the dry powdered plant materials in 1L of ethanol at room temperature for 48 hours. The extracts were concentrated with a rotary evaporator with water bath at 40°C.

Preparation of Plant Extracts Solution

The extracts solutions were prepared by dissolving 0.2g of the evaporated extract in 10 ml of 5% Tween 80, to give an effective concentration of 20 mg/ml.

Preparation of Liver Homogenates

About 0.5g of liver tissue was homogenized separately using 4.5ml 0.4M Phosphate buffer solution and centrifuged at 3500rpm for 4 minutes.

Preparation of Reagents for Malondialdehyde Assay (MDA)

Tissue Homogenate (Tris –HCl buffer Ph 7.5). TBA-TCA-HCl Reagent: TBA (Thiobarbituric acid 0.37%), 0.25N HCl, 10% TCA (10g of TCA in 100ml of distilled water)

Preparation of Reagents for Reduced Glutathione Assay (GSH)

10% TCA: 10g of TCA in 100ml of distilled water) Ellman's Reagent: 19.8mg of Dithiobisnitrobenzoic acid (DTNB) in 100ml of 0.1% Sodium Nitrate, 0.2M Phosphate buffer ph 8.0: 6.8g of KH_2PO_4 and 17.9% of $Na_2HPO_4.12H_20$ dissolved in 500ml distilled water.

Preparation of Reagents for Catalase Assay (CAT)

0.01M Phosphate Buffer Ph 7.0: 1.36g of KH_2PO_4 and 3.58g of Na_2HPO_4 dissolved in 1litre of distilled water. 2M Hydrogen Peroxide (H_20_2), 5% Potassium dichromate and Glacial acetic acid (ratio 1:3): 5% potassium dichromate dissolved in 100ml of distilled water.

Determination of Antioxidant Activity *in vivo*

Animal Treatment: The rats were randomly selected and sorted according to their weights. A set of three animals were assigned to each of the six study groups: three test and three control groups.

The three test groups were administered 50mg/kg, 100mg/kg and 200mg/kg dose concentrations of aqueous extracts of *D. edulis,* and *F. exasperata*. The three control groups consist of positive control group - treated with Vitamin E; negative control group – treated with Carbontetrachloromethane (CCl_4) and placebo – treated with 5% Tween 80.

Test and control group animals were intoxicated with Carbontetracholoromethane (CCl_4) for 2 days followed by 7 days test and control pre-treatments. After the treatments the animals were starved overnight and sacrificed under mild chloroform anesthesia. Blood and liver tissues were harvested stored in a biofreezer for analysis.

Estimation of Thiobarbituric Acid Reactive Substances (TBARS)- Malondialdehyde Assay (MDA)

Lipid peroxidation as evidenced by the formation of TBARS was measured using the methods of Jiang *et al*, (1992). 0.1ml of liver tissue homogenate and blood samples were treated separately with 2ml of (1:1:1 ratio) TBA-TCA-HCl reagent (Thiobarbituric acid 0.37%), 0.25N HCl, 10% TCA) and placed in a water bath for 15minutes, cooled and centrifuged at room temperature for 10 minutes at 1000 rpm. The absorbance of clear supernatant was measured against reference blank at 535nm. MDA activity calculated.

Determination of Non-enzymatic Antioxidant Status Estimation of Reduced Glutathione

Reduced glutathione (GSH) was determined by Moron *et al,* (1979). 10% TCA was added to the liver homogenate and blood samples separately and centrifuged. 1.0ml of the supernatant was treated with 0.5ml Ellman's reagent and 3.0ml of phosphate buffer. The absorbance was read at 412nm.

Estimation of Catalase

Catalase (CAT) was assayed colorimetrically at 620nm and expressed as μmoles of H_20_2 consumed per min/mg protein as described by Sinha (1972). 1.5ml of the reaction mixture, comprising of 1.0ml, 0.01M pH 7.0 phosphate buffer; 0.1ml tissue homogenate and 0.4ml, 2M H_2O_2. The reaction was stopped by the addition of 2.0ml (1:3) dichromate and glacial acetic.

Statistical Analysis

Graphs plotted and Computation for Significance were carried out using Microsoft Excel (2007) and SPSS (15.1) for Windows.

3. Results

Effect of Plant Extracts on Lipid Peroxidation

TBARS (Thiobarbituric Acid Reactive Substances) concentrations expressed as MDA in blood and liver samples of all experimental rats are shown in figures 1 and 2. Elevated MDA levels were observed for the CCl_4 treated group (-ve control) than for other control samples, with the Vit E pre-treatment recording the least MDA levels.

Test samples (50mg/kg – 200mg/kg) for the blood and the 200mk/kg pre-treatment recorded lower MDA levels comparable to Vit E for *D. edulis* (Figure 1). MDA levels recorded for *F. exasperata* pre-treatments were not comparable to Vit E. Significantly

higher values were recorded for the Blood and liver samples, except the results for the 200mg/kg liver homogenates pretreatments, which recorded significantly lower than for Vit E (Figure 2).

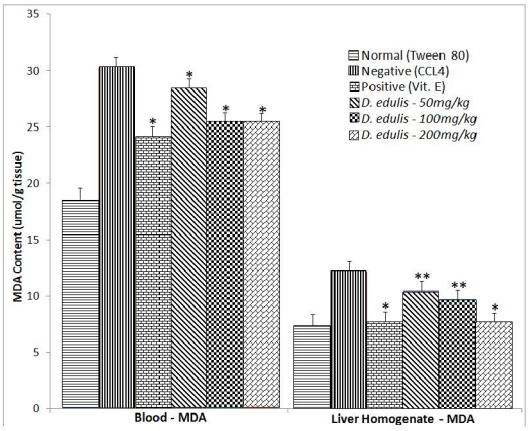

Figure 1: Effect of 50mg/kg, 100mg/kg and 200mg/kg *Dacryodes edulis* extracts and controls: 5% Tween 80; CCl_4 and Vit E on the MDA levels prior to 2 days of CCl_4 intoxication. ($\alpha = 0.05$)

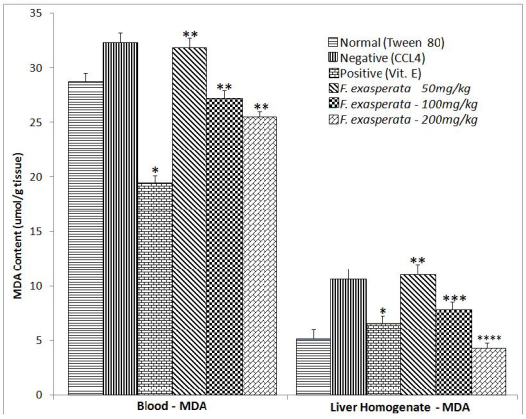

Figure 2: Effect of 50mg/kg, 100mg/kg and 200mg/kg *Ficus exasperata* extracts and controls: 5% Tween 80; CCl_4 and Vit E on the MDA levels prior to 2 days of CCl_4 intoxication. ($\alpha = 0.05$)

Effect of Plant Extract on Catalase (CAT) Activity

CAT activities in the plasma samples of rats for all experimental groups are shown in the figures 3, and 4. The CAT activity in the plasma samples of CCl_4 treated rats was considerably lower than the of normal control. The CAT levels in blood and liver tissues of *D. edulis* extracts pretreated rats were comparable to Vit E except for the 200mg/kg pretreatment, significantly higher than Vit E (Figure 3). *F. exasperata* extracts instigated lower catalase levels than normal (placebo) and significantly higher catalase activities than Vit E in blood and liver homogenates of rats except for the 50mg/kg pre-treatments (Figure 4).

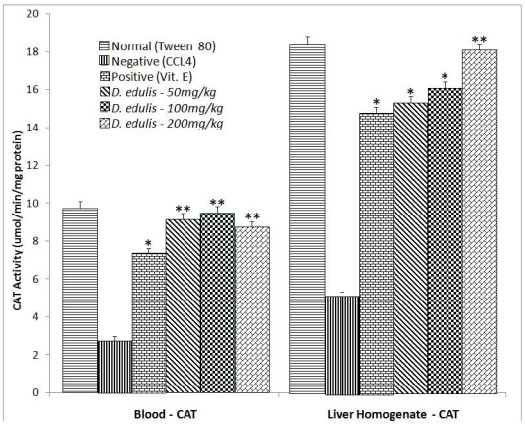

Figure 3: Effect of 50mg/kg, 100mg/kg and 200mg/kg *Dacryodes edulis* extracts and controls: 5% Tween 80; CCl_4 and Vit E on the CAT levels prior to 2 days of CCl_4 intoxication. ($\alpha = 0.05$)

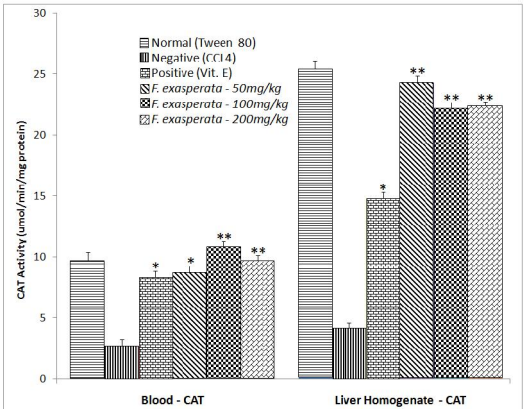

Figure 4: Effect of 50mg/kg, 100mg/kg and 200mg/kg *Ficus exasperata* extracts and controls: 5% Tween 80; CCl_4 and Vit E on the CAT levels prior to 2 days of CCl_4 intoxication. ($\alpha = 0.05$)

Effect of Plant Extracts on Glutathione Level (GSH)

Effects of the plant extracts on GSH level for all experimental groups are shown in figures 5, and 6. CCl_4 treatment caused a decrease of GSH level in blood plasma compared to the normal group.

Pretreatment of 50mg/kg, 100mg/kg, and 200mg/kg of *D. edulis* extracts for 7 days, significantly enhanced the level of GSH when compared to the control groups for blood tissues; and recorded levels comparable to Vit E for liver tissues. The *F. exasperata* extracts pre-treatments recorded significantly higher levels in the 100mg/kg, 200mg/kg pre-treatments for blood and liver homogenates.

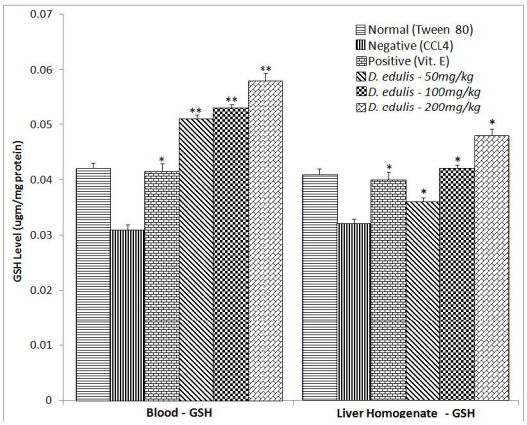

Figure 5: Effect of 50mg/kg, 100mg/kg and 200mg/kg *Dacryodes edulis* extracts and controls: 5% Tween 80; CCl_4 and Vit E on the GSH levels prior to 2 days of CCl_4 intoxication. ($\alpha = 0.05$)

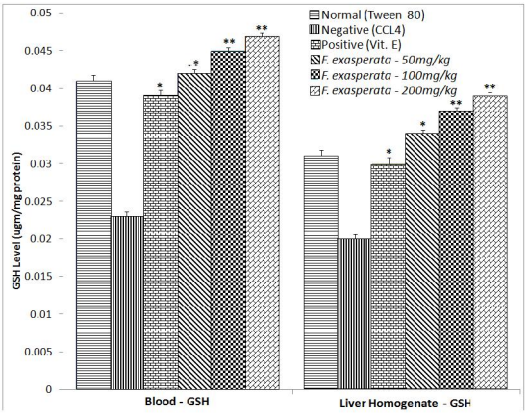

Figure 6: Effect of 50mg/kg, 100mg/kg and 200mg/kg *Ficus exasperata* extracts and controls: 5% Tween 80; CCl_4 and Vit E on the GSH levels prior to 2 days of CCl_4 intoxication. ($\alpha = 0.05$)

Cumulative Dose-Dependent activity of the Plants extracts

The cumulative plant extracts dosage, which is the mean dose administered for the blood and liver tissues and the Vit E pre-treatments are shown in Figure 7. The plot comparatively evaluates the plants extracts and Vit E, effects on the MDA levels in the rats' tissues and organs, after 7 days plants. The figure gives an overview of the *D. edulis* and *F. exasperata* extracts effects on oxidative stress conditions. No significant difference in MDA levels were recorded between *D. edulis* and *F. exasperata* 50mg/kg, 100mg/kg and 200mg/kg pre-treatments. Similarly, the various pretreatments recorded levels comparable to Vit. E with the 200mg/kg pretreatments recording lower MDA levels for *D. edulis* and *F. exasperata.*

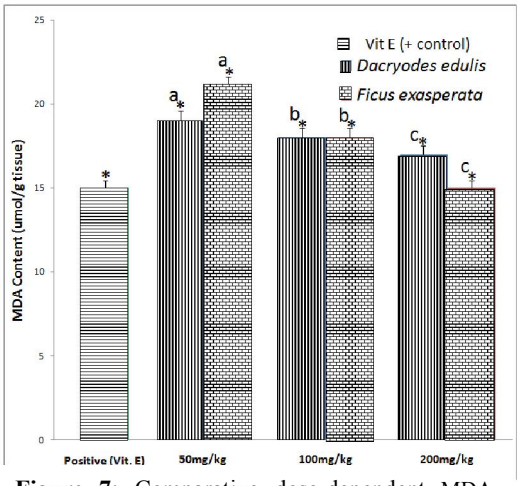

Figure 7: Comparative dose-dependent MDA levels of combined *D. edulis* and *F. exasperata* extracts for blood and liver homogenates against Vit E. ($\alpha = 0.05$)

4. Discussions

In the present study, phytochemical screening of the plants species yielded alkaloids, tanins, cardiac glycosides, reducing sugars, saponins, flavanoids and terpenoids. *D. edulis* did not record cardiac glycosides, and *F. exasperata* did not record alkaloids and terpenoids. Similar results were reported by Ayoola *et al,* (2008) for *Carica papaya*, *Psidium guajava*, *Vernonia amygdalina* and stem bark of *Mangifera indica.*

The primary defence mechanism of the body is monitored by the indicative expression of glutathione (GSH), catalase (CAT) and malondialdehyde (MDA) levels amongst several other constitutes a mutually supportive team of defense against ROS (Bandhopadhy *et al,* 1999; Rajesh and Latha, 2004). MDA is the major oxidation product of peroxidized poly-unsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation and ultimately tissue damage by series of chain reactions. The increased MDA levels recorded in the present study for the negative control (CCl_4 treated rats) is evident of the high level of lipid peroxidation, the analogous level of the failure of the antioxidant defense mechanism and thus the degree of tissue damage (Souza *et al*, 1997). However, decreased levels observed for the plants extracts pre-treatments significantly reduced with increasing *D. edulis* and *F. exasperata* extracts administration; with the 200mg/kg dose recording the most promising recovery effect

from blood and liver tissues damages. Similarly, Ramakrishna. *et al,* 2011; reported reduced MDA levels for CCl_4 intoxicated rats liver tissues, treated with *Mallotus Philippensis* leaves extracts.

Widely distributed in all animal tissues including RBC and liver is the enzymatic antioxidant; catalase (CAT), which decomposes hydrogen peroxide and helps protect the tissues from highly reactive hydroxyl radicals. The levels of calatalase measured in the tissues like MDA, is indicative of the degree of damages the tissues is undergoing or the degree of protection offered by the protective enzymatic agents against ROS. He, *et al,* (2102) recorded significantly reversed CAT, GSH and MDA levels using ethanolic extract of *Meconopsis quintuplinervia* on CCl_4-induced oxidative stress in mice. In the present study, the leaf extracts of *D. edulis* and *F. exasperata* showed significantly higher CAT levels in blood and liver tissues of experimental rats. The 200mg/kg pre-treatment recorded higher CAT levels, showing improve protective potential with increased concentration of the plants extracts.

Cellular Glutathione (GSH) levels is maintained by glutathione reductase (GR) and NADH activities, and excessive peroxidation causes increased glutathione consumption, leading to reduced levels. Where these levels are successfully reversed, protective action against oxidative stress is achieved, upholding the antioxidant machineries of the liver and in the blood (Fahmy *et al*, 2011). In the present study, the plants extracts recorded significantly higher GSH values that the normal and Vit E, an indication that the extracts are effective in restoring CCl_4-intoxicated kidney and blood tissues enzymes towards regular levels and thus good at combating oxidative stress *in vivo*. Manna *et al*, (2006), reported similar levels of GSH in serum, kidney and liver tissues for *Terminalia arjuna* bark extract treated mice prior to CCl_4 intoxication.

Cumulative Dose-Dependent activity of the Plants extracts

In the cumulative plant extracts dose analysis, the protective potentials of the plants extracts were clearly dose-dependent and the effect amplified with increased dosage. Ethanolic extracts of *Hybanthus enneaspermus* exhibited significantly reduced oxidative stress in liver tissues by recording higher restorative activity at 500 mg/kg body weight dosage (Anad and Gokulakrishnan, 2012). Similarly, in the present study higher restorative activities were recorded for *D. edulis* and *F. exasperata* at concentrations of 200mg/kg body weight. The oxidative stress suppressive effects of the plants species are comparable to Vit. E even at the lower concentration of 50mg/kg body weight; showing the plants species possess effective restoring prowess against CCl_4-induced intoxication in blood and liver tissue. Previous studies have shown *D. edulis* to exhibit considerably high antiplasmodial and *F. exasperata* anti- antileishmanial activities, and as multipurpose plants in African folk medicine, They have been implicated in the treatment of several diseases conditions, particularly, *D. edulis* (Mpiana *et al*, 2007, Zofou *et al*, 2011).

The inclusion of these plants amongst others used for malaria treatment and other illnesses is therefore not unconnected with the anti-oxidative stress exhibited by the plants, which is directly linked with antioxidant potential of several of the phytochemicals of the plants species (Farombi *et al,* 2001; Okwu and Nnamdi, 2008, Ajibesin, 2010; Song et 2010, Anago *et al*, 2011; Marcel, *et al*, 2011; De Martino *et al*, 2012).

Conclusively, ethanol extracts of *D. edulis* and *F. exasperata* possess antioxidant phytochemicals, that recorded significant effects on the MDA levels, GSH and CAT activities in blood and liver tissues of rats. The study suggests that *D. edulis* and *F. exasperata* are effective in bringing about restorative activity against CCl_4-induced oxidative stress and tissues damages blood and liver tissues. The plants ethanol extracts may act through antioxidants enzymes such as GSH and CAT as well as reduced lipid peroxidation. Clearer mode of action will require further studies. The oxidative stress suppressive effect may justify the plants inclusion in traditional formulation of antimalarial preparations "Agbo" in the West African region particularly, Nigeria and Cameroun.

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