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## Assessment of *In vivo* antioxidant properties of *Dacryodes edulis* and *Ficus exasperata* as anti-malaria plants

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## PEER REVIEW

**Peer reviewer**

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**Comments**

This is a good study in which the authors evaluated the phytochemical profile and potential anti-oxidant properties of *D. edulis* and *F. exasperata*. The oxidative stress suppressive effects of the plants extracts are comparable to vitamin E even at the lower concentration of 50 mg/kg body weight, which proved that the plants materials possess effective restoring prowess against CCl<sub>4</sub> induced intoxication in blood and liver tissues.

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## ABSTRACT

**Objective:** To evaluate the phytochemical profile and potential anti-oxidant properties of *Dacryodes edulis* and *Ficus exasperata*.

**Methods:** Six groups of Albino rats were intoxicated with tetrachloromethane (CCl<sub>4</sub>) for 2 d, prior to 7 d administration of 50 mg/kg, 100 mg/kg, 200 mg/kg ethanol extracts of plants and three control remedies which is Tween 80 (placebo), CCl<sub>4</sub> (negative control) and vitamin E (positive control). Tissue homogenates were employed in assessing the thiobarbituric acid reactive substances expressed as malondialdehyde (MDA) levels, reduced glutathione (GSH) and catalase (CAT) activities.

**Results:** Phytochemical profiling of the plants showed the presence of reducing sugars, flavonoids, saponins and tannins, except alkaloids and terpenoids in *F. exasperata* and cardiac glycosides in *D. edulis*. Generally, significantly different values ( $P < 0.05$ ) were recorded for blood than for liver homogenates. Elevated MDA levels were observed for the CCl<sub>4</sub> treated group (negative control). However, lower MDA levels comparable to vitamin E (positive control) were recorded for all *D. edulis* and the 200 mg/kg *F. exasperata* pre-treatments. CAT levels were significantly ( $P < 0.05$ ) raised in the 100 mg/kg and 200 mg/kg pretreatments for blood and the 200 mg/kg pretreatments for liver, than for Vit E. CCl<sub>4</sub> reduced GSH levels were reversed significantly ( $P < 0.05$ ) in blood by *D. edulis* and by the 100 mg/kg and 200 mg/kg *F. exasperata* pretreatments in blood and liver tissues. The mean dose-dependent analysis shows increasing fall in MDA levels with dosage.

**Conclusions:** The plant extracts exhibited dose-dependent oxidative stress suppressive action. This may justify their use for the traditional preparation of anti-malarial remedies.

## KEYWORDS

*In vivo*, Anti-oxidant, Anti-malaria, *Dacryodes edulis*, *Ficus exasperata*

### 1. Introduction

Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders. Oxygen free-radicals 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 complications of several diseases<sup>1–3</sup>.

The blood is the chief vehicle for transport in the body and the liver is a vital organ in the maintenance of metabolic functions and detoxification from exogenous and endogenous challenges like xenobiotics, drugs, viral infections, and related assaults. The natural protective mechanisms of the liver provide a major defense line against such attacks, however, when compromised by high degree of such assaults, tissue or organs become injuries, implicating oxidative stress either because of the instigator or the complications of several

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illnesses<sup>[4,5]</sup>.

In Nigeria and the West African regions, 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<sup>[6]</sup>. 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 used for management of other diseases but now increasingly listed as anti-malarial plants.

Coupled with the destructive attributes of the malaria parasites to the red blood system is the effect of oxidative stress, which is linked with the development of anaemia in malaria. Elevated total antioxidant status is shown to be important in recovery from malaria<sup>[7]</sup>. Hence, there is a need to continuously search for new drugs and thus new plants with appreciable antioxidant properties. Majority of anti-malaria medications have been from plant sources and such plant species have good reactive oxidative species (ROS) scavenging properties. The present study sought to determine the phytochemical profile, ascertain the antioxidant potentials, and thus ascertain efficacy of leaves extracts of *Dacryodes edulis* (*D. edulis*) and *Ficus exasperata* (*F. exasperata*) on the management of malaria and their inclusion in the preparation of the anti-malaria concoction “Agbo”.

## 2. Materials and methods

### 2.1. Plant samples

Leaves of *D. edulis* and *F. exasperata* were collected from forests and farmland in Southern Nigeria in an ethnobotanical field survey during August and September 2010.

### 2.2. Experimental animals

Albino rats with average weight 100–170g, were acclimatized with normal rat feed (Laymore Concentrate) and tap water *ad libitum* for 28 d.

### 2.3. Extraction of plants

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

### 2.4. Preparation of plant extracts solution

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

### 2.5. Preparation of liver homogenates

About 0.5 g of liver tissue was homogenized separately using 4.5 mL 0.4 mol/L phosphate buffer solution and centrifuged at 3500 r/min for 4 min.

### 2.6. Preparation of reagents for malondialdehyde (MDA) assay

Tissue homogenate (Tris-HCl buffer, pH 7.5). TBA-TCA-HCl reagent: thiobarbituric acid (TBA, 0.37%), 0.25 N HCl, 10% TCA (10 g of TCA in 100 mL of distilled water).

### 2.7. Preparation of reagents for reduced glutathione (GSH) assay

10% TCA: 10 g of TCA in 100 mL of distilled water; Ellman's reagent: 19.8 mg of dithiobis nitrobenzoic acid in 100 mL of 0.1% sodium nitrate; 0.2 mol/L phosphate buffer (pH 8.0): 6.8 g of  $\text{KH}_2\text{PO}_4$  and 17.9% of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  dissolved in 500 mL distilled water.

### 2.8. Preparation of reagents for catalase (CAT) assay

0.01 mol/L phosphate buffer pH 7.0: 1.36 g of  $\text{KH}_2\text{PO}_4$  and 3.58 g of  $\text{Na}_2\text{HPO}_4$  dissolved in 1 L of distilled water. 2 mol/L hydrogen peroxide ( $\text{H}_2\text{O}_2$ ); 5% potassium dichromate and glacial acetic acid (ratio 1:3): 5% potassium dichromate dissolved in 100 mL of distilled water.

### 2.9. Determination of antioxidant activity in vivo

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 50 mg/kg, 100 mg/kg and 200 mg/kg concentrations of aqueous extracts of *D. edulis* and *F. exasperata*, respectively. The three control groups consist of positive control group-treated with vitamin E; negative control group-treated with  $\text{CCl}_4$  and placebo-treated with 5% Tween 80.

Test and control group animals were intoxicated with  $\text{CCl}_4$  for 2 d followed by 7 d 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 and stored in a biofreezer for further analysis.

### 2.10. Estimation of thiobarbituric acid reactive substances (TBARS)-MDA

Lipid peroxidation was estimated by the formation of TBARS.

A volume of 0.1 mL of liver tissue homogenate and blood samples were treated separately with 2 mL of TBA–TCA–HCl reagent (1:1:1 ratio, 0.37% TBA, 0.25 N HCl, 10% TCA) and placed in a water bath for 15 min, cooled and centrifuged at room temperature for 10 min at 1000 r/min. The absorbance of clear supernatant was measured against reference blank at 535 nm. MDA activity was calculated<sup>[8]</sup>.

### 2.11. Determination of non-enzymatic antioxidant status

The non-enzymatic antioxidant status was determined by estimating the reduced glutathione (GSH). 10% TCA was added to the liver homogenate and blood samples separately and centrifuged. One millilitre of the supernatant was treated with 0.5 mL Ellman's reagent and 3.0 mL of phosphate buffer. The absorbance was read at 412 nm<sup>[9]</sup>.

### 2.12. Estimation of catalase

CAT was assayed colorimetrically at 620 nm and expressed as  $\mu\text{moles of H}_2\text{O}_2$  consumed per min/mg protein. A volume of 1.5 mL of the reaction mixture, comprising of 1.0 mL, 0.01 mol/L pH 7.0 phosphate buffer; 0.1 mL tissue homogenate and 0.4 mL, 2 mol/L  $\text{H}_2\text{O}_2$ . The reaction was stopped by the addition of 2.0 mL (1:3) dichromate and glacial acetic<sup>[10]</sup>.

### 2.13. Statistical analysis

Graphs were plotted and computation for ANOVA and standard deviation (SD) were carried out using Microsoft Excel (2010) for Windows and Cgi-bin.

## 3. Results

### 3.1. Phytochemical profile

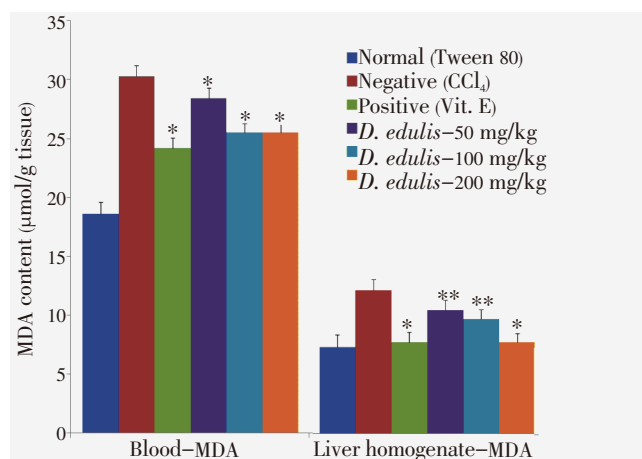
Phytochemical screening of the ethanol extracts of *D. edulis* and *F. exasperata* revealed the presence of alkaloids, tannins, cardiac glycosides, reducing sugars, saponins, flavanoids and terpenoids.

### 3.2. Effect of plant extracts on lipid peroxidation

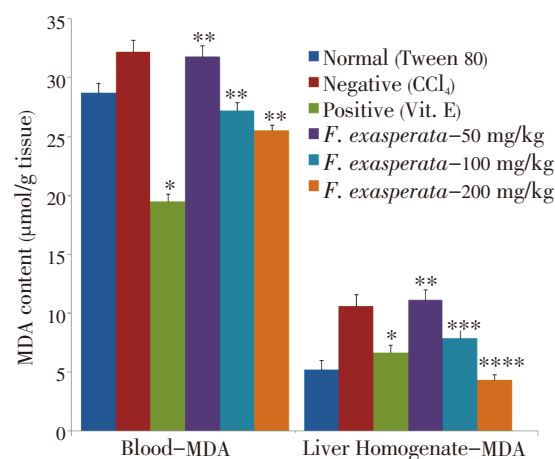
TBARS concentrations expressed as MDA in blood and liver samples of all experimental rats are shown in Figures 1 and 2. Elevated MDA levels were observed for the  $\text{CCl}_4$  treated group (negative control) than for other control samples, with the vitamin E pre-treatment showing the least MDA levels.

Test samples (50 mg/kg–200 mg/kg) for the blood and the 200 mg/kg pre-treatment for liver showed lower MDA levels

comparable to vitamin E for *D. edulis* (Figure 1). MDA levels recorded for *F. exasperata* pre-treatments were not comparable to vitamin E. Significantly higher values were recorded for the blood samples than for liver samples, except the results for the 200 mg/kg liver homogenates pretreatments, which showed significantly lower values than for vitamin E (Figure 2).



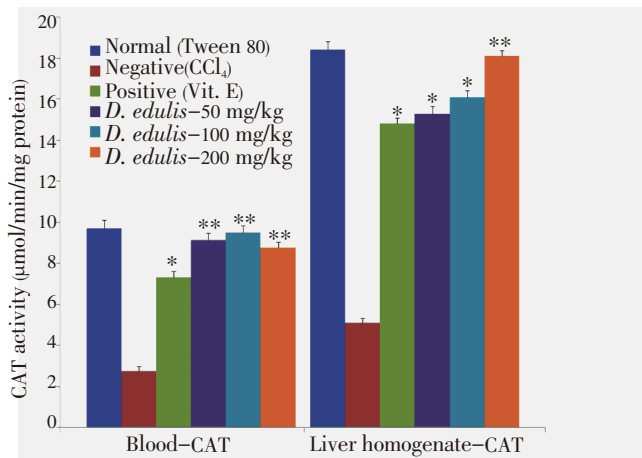
**Figure 1.** Effect of 50 mg/kg, 100 mg/kg and 200 mg/kg *D. edulis* extracts and controls on the MDA levels prior to 2 days of  $\text{CCl}_4$  intoxication ( $\alpha=0.05$ ).



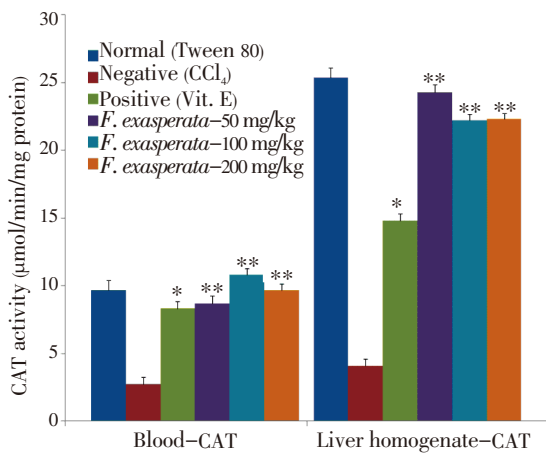
**Figure 2.** Effect of 50 mg/kg, 100 mg/kg and 200 mg/kg *F. exasperata* extracts and controls on the MDA levels prior to 2 days of  $\text{CCl}_4$  intoxication ( $\alpha=0.05$ ).

### 3.3. Effect of plant extract on catalase (CAT) activity

CAT activities in the plasma samples of rats for all experimental groups are shown in Figures 3 and 4. CAT activity in the plasma samples of  $\text{CCl}_4$  treated rats was considerably lower than for the normal control. The CAT levels in blood and liver tissues of *D. edulis* extracts pretreated rats were comparable to vitamin E except for the 200 mg/kg pretreatment with significantly higher value than for vitamin E (Figure 3). *F. exasperata* extracts instigated lower CAT levels than that was recorded for the normal (placebo) except for the 50 mg/kg pre-treatments, significantly higher catalase activities were recorded in blood and liver homogenates than that was recorded for vitamin E (Figure 4).



**Figure 3.** Effect of 50 mg/kg, 100 mg/kg and 200 mg/kg *D. edulis* extracts and controls on the CAT levels prior to 2 days of CCl<sub>4</sub> intoxication ( $\alpha=0.05$ ).



**Figure 4.** Effect of 50 mg/kg, 100 mg/kg and 200 mg/kg *F. exasperata* extracts and controls on the CAT levels prior to 2 days of CCl<sub>4</sub> intoxication ( $\alpha=0.05$ ).

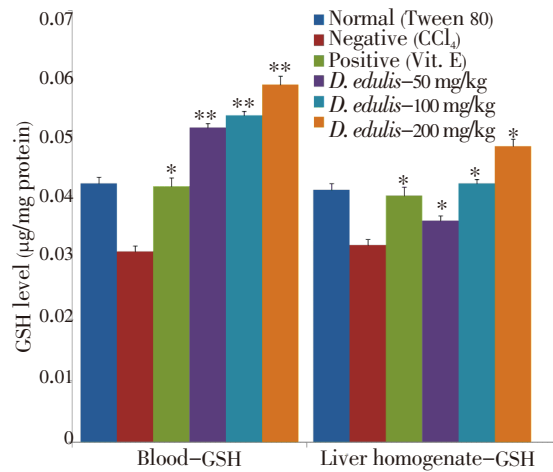
### 3.4. Effect of plant extracts on glutathione level (GSH)

Effects of the plant extracts on GSH level for all experimental groups are shown in Figures 5 and 6. CCl<sub>4</sub> treatment caused a decrease of GSH level in blood plasma when compared to that of the normal group treatment. The 50 mg/kg, 100 mg/kg, and 200 mg/kg *D. edulis* extracts pretreatments for 7 days, significantly enhanced the level of GSH when compared to the control groups for blood tissues, and recorded levels is comparable to vitamin E values for liver tissues. The *F. exasperata* extracts pre-treatments recorded significantly higher levels in the 100 mg/kg and 200 mg/kg pre-treatments than for the 50 mg/kg pretreatment for blood and liver homogenates.

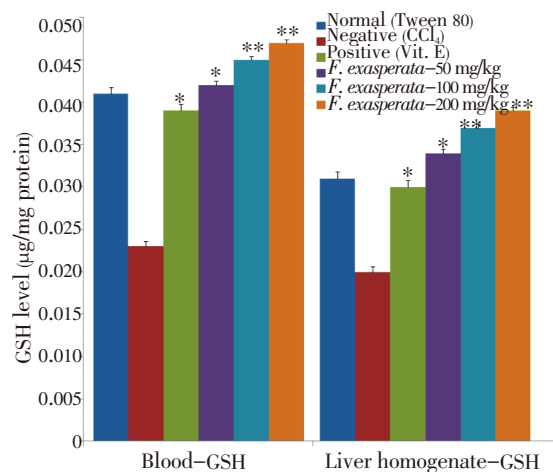
### 3.5. Cumulative dose-dependent activity of the plants extracts

The cumulative plant extracts dosage, which is the mean dose administered for the blood and liver tissues and the vitamin E pre-treatments are shown in Figure 7. The plot comparatively evaluates the plants extracts and vitamin E, effects on the MDA levels in the rats' tissues and organs after 7 d. Figure 7 gives an overview of the *D. edulis* and *F. exasperata* extracts effects on oxidative stress conditions. No significant difference in MDA

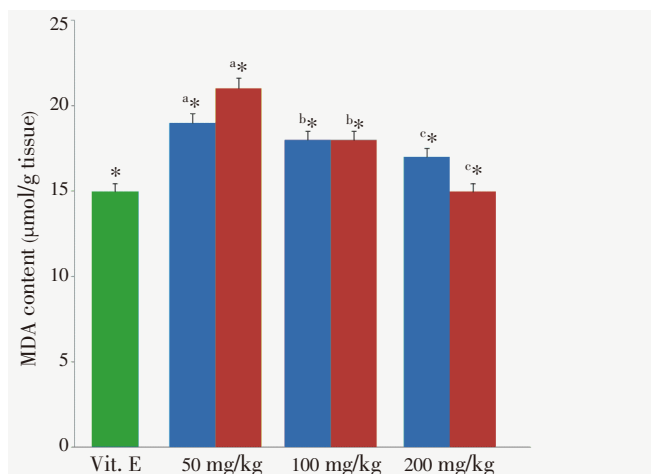
levels were recorded between *D. edulis* and *F. exasperata* 50 mg/kg, 100 mg/kg, and 200 mg/kg pre-treatments. The various pre-treatments however, recorded levels comparable to vitamin E with the 200 mg/kg pretreatments recording the least MDA levels for *D. edulis* and *F. exasperata*.



**Figure 5.** Effect of 50 mg/kg, 100 mg/kg and 200 mg/kg *D. edulis* extracts and controls on the GSH levels prior to 2 days of CCl<sub>4</sub> intoxication ( $\alpha = 0.05$ ).



**Figure 6.** Effect of 50 mg/kg, 100 mg/kg and 200 mg/kg *F. exasperata* extracts and controls on the GSH levels prior to 2 days of CCl<sub>4</sub> intoxication ( $\alpha = 0.05$ ).



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

#### 4. Discussion

In the present study, phytochemical screening of the ethanol extracts of *D. edulis* and *F. exasperata* yielded alkaloids, tanins, cardiac glycosides, reducing sugars, saponins, flavanoids and terpenoids. However, cardiac glycosides were not recorded for *D. edulis*, and alkaloids and terpenoids were not recorded for *F. exasperata*. Earlier studies reported similar results with some variance for methanolic, ethanolic and water extracts of root, stem and leaves of *D. edulis* and *F. exasperata*[11–17].

The primary defence mechanism of the body is monitored by the indicative expression of GSH, CAT and MDA levels amongst several other compounds/enzymes, which constitutes a mutually supportive team of defense against ROS[18,19]. 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<sub>4</sub> treated rats) is evident of the high level of lipid peroxidation caused by CCl<sub>4</sub>. It is analogous of the level of failure of the antioxidant defense mechanism and thus the degree of tissue damage that can result from the action of the intoxicant or those of related compounds[20]. However, significantly reduced MDA levels were recorded with increasing *D. edulis* and *F. exasperata* extracts dosage, with the 200 mg/kg dose showing the most promising recovery effect from blood and liver tissues damages. Reduced MDA levels for CCl<sub>4</sub> intoxicated rats liver tissues, treated with *Mallotus philippensis* leaves extracts shows MDA levels as good indicator of the degree of CCl<sub>4</sub> activity[5].

Widely distributed in all animal tissues including RBC and liver is the enzymatic antioxidant CAT which decomposes hydrogen peroxide and helps protect tissues from highly reactive hydroxyl radicals. Like MDA levels, CAT measured in tissues is indicative of the degree of damages the tissues are undergoing or the degree of protection offered by the protective enzymatic agents against ROS. Significantly reversed CAT, GSH and MDA levels were recorded for CCl<sub>4</sub>-induced oxidative stress in mice treated with ethanolic extract of *Meconopsis quintuplinervia*[21]. In the present study, the leaf extracts of *D. edulis* and *F. exasperata* showed significantly higher CAT levels in blood and liver tissues in experimental rats. The 200 mg/kg pre-treatment recorded higher CAT levels, showing increasing protective potential with increased concentration of the plants extracts.

Cellular GSH levels are maintained by glutathione reductase and NADH activities, and excessive peroxidation causes increased glutathione consumption, leading to reduced GSH levels. Where these levels are successfully

reversed, protective action against oxidative stress is presumably achieved and one may suppose that the antioxidant machineries of the liver and in the blood are upheld[22]. In the present study, the plants extracts recorded significantly higher GSH values than the normal (placebo) and vitamin E, indicating that the extracts are effective in restoring CCl<sub>4</sub>-intoxicated kidney and blood tissues enzymes towards regular levels and thus are good at combating oxidative stress *in vivo*. Similar levels of GSH in serum, kidney, and liver tissues were recorded for mice treated with *T. arjuna* bark extract prior to CCl<sub>4</sub> intoxication[23].

In the cumulative plant extracts dose analysis, the protective potentials of the plants extracts were clearly dose-dependent and the effect was 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[24]. In the present study, higher restorative activities were recorded for *D. edulis* and *F. exasperata* at concentrations of 200 mg/kg body weight. The oxidative stress suppressive effects of the plants extracts are comparable to vitamin E even at the lower concentration of 50 mg/kg body weight, which showed that the plants materials possess effective restoring prowess against CCl<sub>4</sub>-induced intoxication in blood and liver tissues. In earlier study similar increasing dose-dependent effect on blood and liver homogenates were recorded for *Allamanda cathartica*, however, *Bixa orellana* showed a reverse activity (poor antioxidant) trend[10].

The inclusion of these plants amongst others used for malaria treatment is not unconnected with the anti-oxidative stress exhibited by the plants, which is directly linked with antioxidant potential of several phytochemicals of the plants species[4,7,12,25–28]. Previous studies have shown that the plants was multipurpose species in African folk medicine in the treatment of several diseases conditions. *D. edulis* exhibits considerably antiemetic, antiplasmodial, antidrepanocytary, (anti-sickle cell anaemia) anti-inflammatory, antifungal (dermal) effect and *F. exasperata* is known for its anti-leishmanial, antidiabetic, anticonvulsant, antiinflammatory, antimicrobial, hypolipidemic, antioxidant, antiulcer, anxiolytic and hypotensive activities[17,25,29–31].

Ethanol extracts of *D. edulis* and *F. exasperata* possess antioxidant phytochemicals that showed 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<sub>4</sub> induced oxidative stress and tissues (blood and liver) damages in rats. The ethanol extracts of the plants may act through antioxidants enzymes such as GSH and CAT as well as reduced lipid peroxidation. However,

clearer understanding of the mode of action will be required in further studies. The oxidative stress suppressive effect may justify the plants' inclusion in folk preparation of the popular antimalarial potion– “Agbo” in the West African region, particularly in Nigeria and Cameroun.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

The authors are grateful to Dr. S Rotimi for his assistance in the Laboratory. The project was partially funded by Covenant University Centre for Research & Development (CUCERD seed, Grant no. CUCERD–0012–34/09).

### Comments

#### Background

Herbal medicine practitioners have made several claims regarding the diverse pharmacological properties of several plant species.

#### Research frontiers

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<sub>4</sub> induced oxidative stress and tissues (blood and liver) damages in rats. The plants ethanol extracts may act through antioxidants enzymes such as GSH and CAT as well as reduced lipid peroxidation.

#### Related reports

Previous studies have shown the plants as multipurpose species in African folk medicine that have been implicated in the treatment of several diseases conditions. *D. edulis* exhibits considerably antiemetic, antiplasmodial, antidrepanocytary, (anti-sickle cell anaemia) anti-inflammatory, antifungal (dermal) effect and *F. exasperata* is known for its anti-leishmanial, antidiabetic, anticonvulsant, anti-inflammatory, antimicrobial, hypolipidemic, antioxidant, antiulcer, anxiolytic and hypotensive activities.

#### Innovations & breakthroughs

Majority of anti-malaria medications have been from

plant sources and such plant species have good reactive oxidative species (ROS) scavenging status. The present study seeks to determine the phytochemical profile and ascertain the antioxidant potentials, and thus ascertain the plants efficacy on the management of malaria and inclusion in the preparation of the anti-malaria concoction “Agbo”.

### Applications

Widely distributed in all animal tissues including RBC and liver is the enzymatic antioxidant CAT, which decomposes hydrogen peroxide and helps protect tissues from highly reactive hydroxyl radicals. Like MDA levels, CAT measured in tissues is indicative of the degree of damages the tissues are undergoing or the degree of protection offered by the protective enzymatic agents against ROS. 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 200 mg/kg pre-treatment recorded higher CAT levels, showing improved protective potential with increased concentration of the plants extracts.

### Peer review

This is a good study in which the authors evaluated the phytochemical profile and potential anti-oxidant properties of *D. edulis* and *F. exasperata*. The oxidative stress suppressive effects of the plants extracts are comparable to vitamin E even at the lower concentration of 50 mg/kg body weight, showing the plants materials possess effective restoring prowess against CCl<sub>4</sub> induced intoxication in blood and liver tissues.

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