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# *In vivo* antioxidant assessment of two antimalarial plants-*Allamamda cathartica* and *Bixa orellana*

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PEER REVIEW

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**Comments**

The interesting study provides a metabolites profile of the leaves; demonstrates the *in vivo* antioxidant activities in liver and blood of rats and the protective efficacy of the leaf extracts against $CCl_4$ induced oxidative stress, which justifies the use of the leaves in the antimalarial activities.
(Details on Page 393)

ABSTRACT

**Objective:** To determine the free radical scavenging potentials pytochemical constituents of ethanol leaves extracts of *Allamanda cathartica* (*A. cathartica*) and *Bixa orellana* (*B. orellana*) and thus their effects in antimalarial activities. **Methods:** Both ethanol extracted plant samples were administered at 50 mg/mL, 100 mg/mL and 200 mg/mL to Albino rats and then administered with $CCl_4$ at 1 mL/kg body weight, in liquid paraffin (1:1, v/v) for 2 days (negative control) and compared with 5% Tween 80 (placebo) and vitamin E (positive control) pretreatments. Thiobarbituric acid reactive substances (TBARS), glutathione (GSH) and catalase (CAT) activities in blood and liver tissues were assessed. **Results:** In $CCl_4$ treated rats, TBARS levels significantly increased, while decreased GSH and CAT levels were recorded for both plant extracts. Generally, higher TBARS and GSH values were recorded for blood than for liver homogenates; with reverse trend observed for CAT level. Increased concentrations of *A. cathartica* extract recorded significant antioxidant levels similar to tocopherol (vitamin E). Reducing sugars, saponins, flavonoids were recorded for both species; alkaloids in *A. cathartica* and terpenoids in *B. orellana*. **Conclusions:** *A. cathartica*, possess phytochemicals that recorded significant antioxidative defense activities for blood and liver tissues with increasing concentration. However *B. orellana* did not record similar results.

KEYWORDS

*In vivo*, Anti-malaria, Anti-oxidant, *Allamanda cathartica*, *Bixa orellana*

## 1. Introduction

An estimated 3.3 billion people were at risk of malaria in 2010, with 216 million cases recorded in 2010; 81% of which are in Africa, with six African countries accounting for 60% of the estimated 655 000 death record annually and 86% of these victims are under 5 years of age. People living in the poorest countries are the most vulnerable to malaria and are most dependent on plant resources for malaria management, especially in the population of West Africa[1,2]. However, do all the plants employed by the local population have therapeutic prowess for treating Malaria? This question become pertinent, when it is considered that some of the emerging plants are known for treating other conditions even by the same or other natives. With the increasing resistance of the malaria parasite to a number of currently administered anti-malaria drugs[3], the need to search for new drug materials becomes imperative. And those plants hitherto unemployed for malaria treatment may present promising attributes.

Free radical induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations and affecting a wide range of tissues and organs[4-6,7]. Plant materials

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and herbal extracts have been recorded to protect organs against oxidative stress created by industrial solvents and carbon tetrachloride ($CCl_4$), a potent environmental hepatotoxin through changing the levels of increased lipid peroxidation[8,9], enhancing the decreased activities of antioxidant enzymes, like superoxide dismutase, catalase and glutathione-S-transferase and increasing reduced glutathione level in liver[10,11].

*Allamanda cathartica* L. (*A. cathartica*, golden trumpet) is a widely cultivated ornamental plant of the family Apocynaceae. It has been used as a purgative or emetic, febrifuge, as well as for the treatment of coughs, headaches, jaundice and enlarged spleen resulting from malaria. The milky sap is also known to possess antibacterial and possibly anticancer properties[12,13]. *Bixa orellana* L. (Bixaceae) (*B. orellana*) is a tropical shrub, with a bright red heart shaped, bristly fruit and seeds embedded in orange-red pulp. It could be used to produce a common dye employed for a variety of purpose. It has a long history as a medicinal plant for the treatment of varied conditions such as feverish infections like gonorrhoea, dysentery and hepatitis, as well as for cough, snakebites, intestinal parasites, skin toning and for the treatment of diabetes in traditional medicine systems[14].

Measuring the reactive oxidative species scavenging status of plants has become a good point to commence research on ascertaining their therapeutic potentials. The present study seek to determine the pytochemical constituents and the free radical scavenging potentials of *A. cathartica* and *B. orellana* and hence their relevance in the treatment of malaria.

## 2.Material and methods

### 2.1. Plant and animal samples

Plants samples were collected in Southern Nigeria, during an ethnomedicinal survey, covering 8 states; extending from June-July 2010. Albino rats with average weight of 100-170 g were acclimatized with normal rat feed (Laymore Concentrate) and water *ad libitum*.

### 2.2. Preparation of plant extracts solution

Plant materials were air dried at ambient 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 filtered through Whatmann filter paper No. 42 and then through cotton wool. The extracts were dried and concentrated using a rotary evaporator with the water bath set at 40 °C. 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. The extracts solutions were prepared two days before administration.

### 2.3. Phytochemical screening

Tests were carried out on the ethanol extracts of the plant samples using standard procedures to identify the chemical contents like alkaloids, tannins, cardiac glycosides, reducing sugars, saponins, flavonoids and terpenoids constituents as described in previous studies[15-17].

### 2.4. Determination of antioxidant activity in vivo

Animals were divided into four groups of five rats per group, including test group and three control groups: positive, negative and placebo. Prior to $CCl_4$ (1 mL/kg body weight) intoxication for 2 days, test group animals were orally administered with 50 mg/kg, 100 mg/kg and 200 mg/kg dose concentrations of aqueous extracts of *A. cathartica* and *B. orellana*. The positive control group received vitamin E, and the normal control group was administered with 5% Tween 80 for 7 days. The negative control group received only $CCl_4$ for 2 days. After the final dose of $CCl_4$, the animals were starved overnight and sacrificed under mild anesthesia using chloroform.

### 2.5. Preparation of liver homogenates and blood

Following Manna *et al*. procedure with slight modification; 200 mg of harvested liver tissue were homogenized in 10 volume of 100 mmol/L $KH_2PO_4$ buffer containing 1 mmol/L ethylene diamine tetraacetic acid, pH 7.4 and centrifuged at 3 500 r/min for 4 minutes at 4 °C[18]. Blood were collected through cardiac puncture and stored in biofreezer.

### 2.6. Estimation of lipid peroxidation status (TBARS)-MDA assay

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) was measured using the methods in previous studies[19,20]. A volume of 0.1 mL of liver tissue homogenate and blood samples were treated separately with 2 mL of mixture [1:1:1 TBA-TCA-HCl reagent (thiobarbituric acid 0.37%), 0.25 N HCl, 10% TCA] and incubated for 45 min in a water bath (100 °C), cooled and centrifuged at room temperature for 10 min at 1 000 r/min. The absorbance of clear supernatant was measured against reference blank at 535 nm. TBARS concentrations were calculated using the MDA extinction co-efficient $1.56 \times 10^5$ $M^{-1}cm$.