

PHYTOCHEMICAL SCREENING AND *IN VIVO* ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF CAESALPINIA BONDOC (L.) ROXB**Ogunlana O.O.^{1*}, Ogunlana O.E.², Ntube C.A.¹, Olagunju J.A.³ and Akindahunsi A.A.⁴**¹Department of Biological Sciences, College of Science and Technology, Covenant University, P.M.B. 1023, Ota, Ogun State, Nigeria;²Department of Biochemistry, Crawford University, Igbesa, Ogun State, Nigeria,; ³Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Medicine, Lagos State University, Ikeja, Lagos State, Nigeria, ⁴Department of Biochemistry, Federal University of Technology, Akure, Nigeria.**Correspondence to:** Ogunlana O.O., Department of Biological Sciences, College of Science and Technology, Covenant University, P.M.B. 1023, Ota, Ogun State, Nigeria.**Email id:** kellybee2001@yahoo.com**Received date:** 26-7-12**Revised date:** 00-00-00**Accepted date:** 24-8-12**ABSTRACT**

Phytochemical screening, antioxidant activity *in vivo* and lipid peroxidation of 75 % ethanolic extract of young twigs and leaves of *Caesalpinia bonduc* were carried out by chemical test, and assessment of catalase and peroxidase activities and lipid peroxidation in Wistar rats after oral administration of different concentrations of the plant extract for ten days. Phytochemical screening of the extract revealed the presence of all major classes of phytochemicals: tannins, flavonoids, saponin, steroids, terpenoids, cardiac glycosides, glycosides, except phlobatannins. There were significant ($p < 0.05$) graded increase in catalase and peroxidase activities and decrease in TBARS concentrations in the extract tested rats in comparison with normal control, Vitamin C and amodiaquine tested rats. The various pharmacological activities of *C. bonduc* may be due to its antioxidant activity.

Key words: *Caesalpinia bonduc*, Catalase, Peroxidases, Antioxidant activity.**INTRODUCTION**

Oxidation is essential to many living organisms for the production of energy to fuel biological processes, however reactive oxygen species (ROS) are often generated from these processes. Cell death and tissue damage often result from the continued production of ROS in the living organism. The importance of the reactive oxygen species (ROS) has attracted increasing attention over the last decade. Reactive oxygen species includes free radicals such as superoxide anion radicals ($O_2^{\cdot-}$), hydroxyl radicals (OH^{\cdot}), non free radicals such as H_2O_2 and singlet oxygen (1O_2), along with various forms of activated oxygen. They have been implicated in the pathology of several conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias¹. Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules² thereby, protecting the body from damage caused by free radical induced oxidative stress. Antioxidants have also been defined as radical scavengers with ability to protect human body against free radicals².

Human body has multiple enzymatic and non enzymatic antioxidant systems to protect the cellular molecules against reactive oxygen species induced damage³. However, the innate defense may not be enough for continued

oxidative stress. As a result, certain amounts of exogenous antioxidant are constantly required to maintain an adequate level of antioxidant in order to balance the ROS in the human body. Several studies have reported that plants phytoconstituents such as flavonoids and flavones are widely distributed secondary metabolites with potent antioxidant and antiradical properties⁴. Synthetic antioxidant, butyl hydroxyl anisol (BHA), and butyl hydroxyl toluene (BHT), are commonly used antioxidant but have been suspected to cause liver damage⁵. Hence, compounds especially from natural sources capable of protecting against ROS mediated damage may have potential application in preventing and/or curing many diseases.

Caesalpinia bonduc, (family: *Caesalpinaceae*, genus *Fabaceae*), commonly known as Gray Nicker nut (English) and Ayòó (Yoruba, Nigeria), is a prickly shrub with grey, hard, globular shaped seeds and a smooth shining surface⁶. It is a medicinal plant predominantly distributed in the tropical and subtropical regions of Africa, Asia and the Caribbean⁷. The pharmacological study of the plant reveals that the seed and bark extract show antihelminthic, anticancer, antimalarial, antihyperglycemic, anti-inflammatory, antirheumatic and antipyretic activities^{8; 9}, antidiuretic and

antibacterial¹⁰, anticonvulsant¹¹, anti-anaphylactic, antidiarrheal and antiviral¹², anti-asthmatic¹³, antiameobic and anti-estrogenic activities¹⁴, nematocidal¹⁵ and abortifacient¹⁶ activities. However, there is paucity of report on the antioxidant activity of the leaves and young twigs of *C. bonduc* in literatures. Hence, the purpose of the present study was to phytochemically screen and evaluate the *in vivo* antioxidant activity of the ethanolic extract of *C. bonduc*.

MATERIALS AND METHODS

Plant material: Young twigs and leaves of *C. bonduc* (Linn) Roxb. were collected from Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state, Nigeria. Plant identification was done by Dr. Conrad Omonhinmi, Biological Sciences, College of Science and Technology, Covenant University, Ota, Ogun state, Nigeria. Authentication and voucher referencing were carried out at FRIN with voucher specimen SHI108408 deposited in their Herbarium.

Preparation of plant extract: The leaves and young twigs of the plant were air-dried at room temperature and powdered. Three hundred grams of the plant was extracted with 75 % v/v ethanol by maceration using three successive cold extractions for 72 hours. The total filtrate was concentrated to dryness using rotary evaporator at 50°C and the percentage (%) yield of the dry residue calculated¹⁷. The extract was re-suspended in 0.25 % sodium carboxymethyl cellulose and used for the *in vivo* assays.

Phytochemical screening of the leaves and twigs of *C. bonduc*: Chemical tests were carried out on the aqueous extract of the powdered twigs and leaves of *C. bonduc*. Standard procedures as described by^{18, 19 & 20} were used to identify its various phyto-constituents.

Animals: Healthy adult female albino Wistar rats (42), purchased from the National Institute of Medical Research (NIMR), Yaba, Lagos, Nigeria, weighing between 60 - 100 g were used for the experiments. The rats were housed in standard cages in the animal house of Covenant University, Ota, Ogun State. They were allowed to acclimatize for two weeks and were given adequate supply of food and water. The cages were cleaned daily and the animals were treated according to standard ethical guidelines as adopted by Covenant University Experimental Animal Ethical Committee. The rats were weighed before dosage administration commenced. Each group contained 6 rats and extract dosage was based on the body weight of the rats. The groups were assigned based on dosage as follows: group I to VII with 50 mg/kg bwt of extract, 100 mg/kg bwt of extract, 150 mg/kg bwt of extract, 200 mg/kg bwt of extract, 10 mg/kg bwt of amodiaquine (negative control), 10 mg/kg bwt of vitamin C (positive control) and 2ml/kg bwt of distilled water (normal control) respectively²¹. After 10 days of dosing, the rats were sacrificed after overnight fast and blood samples were collected by cardiac puncture into heparin tubes.

Determination of the catalase activity: Catalase activity was determined using the method of^{22 & 23}, in which the disappearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm. One unit enzyme activity is equal to one micromole of hydrogen peroxide decomposed per minute under specified condition at 25°C. The reaction mixture consisted of 0.5 ml of substrate (30 % H₂O₂ in phosphate buffer) and to this was added 1 ml of diluted sample and decrease in absorbance was monitored every 10 seconds for 70 seconds at 240 nm. The catalase activity was calculated using molar extinction coefficient for H₂O₂ at 240nm (43.6 M⁻¹cm⁻¹).

Determination of the peroxidase activity: Peroxidase activity was determined as reported by²⁴. The reaction mixture consisted of 3.0 ml of pyrogallol in 0.1 M phosphate buffer of pH 7.0, and 0.5 ml of 1 % (v/v) H₂O₂. To this was added 0.1ml of enzyme sample and change in absorbance at 430 nm was monitored for 2 min at 30 sec interval. The peroxidase activity was calculated using molar extinction coefficient of oxidized pyrogallol (4.5 M⁻¹cm⁻¹).

Determination of thiobarbituric reactive substances concentration: Blood concentration of thiobarbituric acid reactive substances (TBARS) is an index of lipid peroxidation and oxidative stress. Thiobarbituric acid reactive substances (TBARS) were determined by the method of²⁵. 0.1ml of blood in 0.04M Tri-HCl (pH 8.3) buffer was treated with 2.0 ml of TBA-TCA-HCL, 1:1:1 reagent (thiobarbituric acid (TBA) 0.37%, 0.25N HCl and 15% (w/v) TCA) and incubated at 95°C for 15 min. TBARS content was determined using the extinction coefficient of 155nM⁻¹cm⁻¹ at 535 nm.

RESULTS

Phytochemical constituents: Table 1 shows the result of the qualitative phytochemical assessment of *C. bonduc*. The leaves and twigs of *C. bonduc* contain all tested phytochemicals except phlobatannins.

Table 1: Phytochemical screening of *C bonduc*

Phytochemicals	Results
Tannins	+
Flavonoids	+
Saponin	+
Steroids	+
Phlobatannins	-
Terpenoids	+
Cardiac Glycosides	+
Glycosides	+

+ represents a positive result and - represents a negative result

In vivo antioxidant activity of the crude extract of *Caesalpinia bonduc*

Figure 1 shows that there was significant (P <0.05) increase in peroxidase activity of the extract treated rats in comparison with control. These increases were highly significant at the

extract treated doses of 150 and 200 mg/kg bwt compared with control and vitamin C treated rats. Figure 2 shows that there was significant ($P < 0.05$) increase in catalase activity of the extract treated rats in comparison with control. These increases were highly significant at the extract treated doses of 100 to 200 mg/kg bwt in comparison with control and vitamin C administered rats. Figure 3 depicts the concentration of thiobarbituric acid reactive substances (TBARS) as a measurement of lipid peroxidation profile of the test material. TBARS was significantly increased in amodiaquine treated rats and decrease in graded doses in extract treated rats except in group treated with 50 mg/kg bwt when compared with normal control. The concentration of TBARS was significantly reduced in extract treated doses of 150 and 200 mg/kg bwt when compared with rats treated with vitamin C (Figure 3).

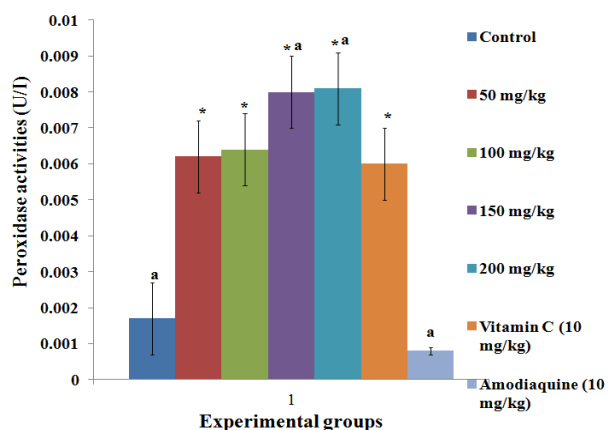


Figure 1: Bar Chart Showing the Effect of the Ethanolic Extract of *C. bonduc* on the Peroxidase Activity in Experimental Rats. * means that the difference between the control and the treated groups is significant at $p < 0.05$. ^a means that the difference between vitamin C and the other treated groups is significant at $p < 0.05$. Values are presented as mean \pm SEM of six replicates.

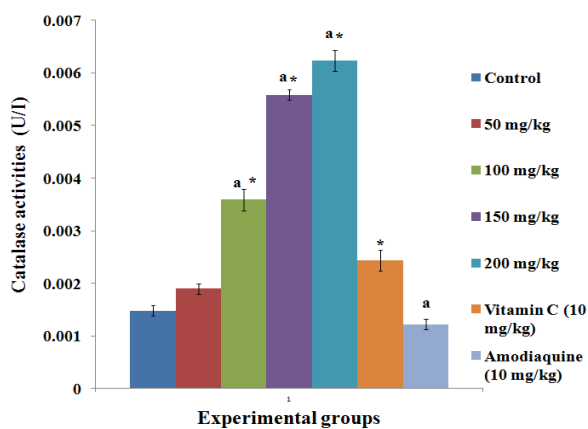


Figure 2: Bar Chart Showing the Effect of the Ethanolic Extract of *C. bonduc* on the Catalase Activity of the Experimental Rats. * means that the difference between the control and the treated groups is significant at $p < 0.05$. ^a means that the difference between vitamin C and the other treated groups is significant at $p < 0.05$. Values are presented as mean \pm SEM of six replicates.

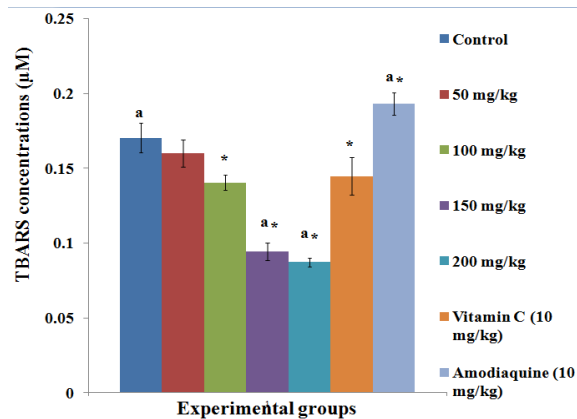


Figure 3: Bar Chart Showing the Effect of the Ethanolic Extract of *Caesalpinia bonduc* on TBARS Concentration of the Experimental Rats. * means that the difference between the control and the treated groups is significant at $p < 0.05$. ^a means that the difference between vitamin C and the other treated groups is significant at $p < 0.05$. Values are presented as mean \pm SEM of six replicates

DISCUSSION

This study and that reported by²⁶ revealed the presence of alkaloids, flavonoids, glycosides, saponin, tannins and terpenoids in the phytochemical analysis of *C. bonduc*. Flavonoids are phenolic compounds with potent metal chelating and free radical scavenging activities²⁷. They provide protection against free radicals by acting as antioxidant involved in scavenging reactive oxygen species along with other natural antioxidant, vitamins and enzymes²⁸. Previous studies have reported the presence of triterpenoid lupeol, present in *C. bonduc* with ability to protect cells and tissues from oxidative stress by increasing the activity of catalase²⁹. *In vitro* antioxidant activity of the methanolic extract of the root of *C. digyna* is highly comparable with reference antioxidants³⁰ and this correlated with its estimation of the total phenolic compounds.

The antioxidant activity of the *C. bonduc* seeds by measure of its DPPH, hydroxyl, nitric oxide and Super oxide radicals' scavenging activities has a significant linear correlation with its total phenolic content³¹. The high antioxidant activity of *C. bonduc* (Figure 1 & 2) might be due to the presence of phenolic components such as flavonoids in them. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups³². The phenolic compounds may contribute directly to antioxidative action³³. The phenolic compounds of *C. bonduc* leaves and twigs extract (Table i) could be major contributors to its antioxidant activity.

The role of the production of free radicals has been associated with various physiological and pathological events such as inflammation, aging, mutagenicity and carcinogenicity³⁴. Antioxidants are vital substances with the ability to protect the body from damage caused by free radical-induced oxidative stress³⁵. Natural antioxidants such

as polyphenols, present in medicinal and dietary plants have been implicated in the prevention of oxidative damage³⁶. The observed increases in catalase and peroxidase activities and decrease in concentration of TBARS in rats treated with high doses of *C. bonduc* (Figure 1, 2 & 3) in comparison with vitamin C may suggest that the antioxidant activity of the plant is comparable with reference antioxidant drugs. Significant antioxidant activity of methanolic extract of the leaves of *C. bonduc* in EAC (Ehrlich ascites carcinoma) bearing mice has been reported⁹. Significant *in vitro* antioxidant activity of protosappanin A, protosappanin B and brazilein, phenolic compounds, isolated from *C. sappan* has also been reported³⁷.

CONCLUSIONS

In conclusion, this study shows that the ethanolic extract of the leaves and twigs of *C. bonduc* has significant catalase and peroxidase activity. It is therefore suggested that it might have antioxidant properties comparable to known standards. *C. bonduc* possesses certain phytochemicals that have been linked to be effective in the prevention of several degenerative diseases. Hence, its use as a medicinal plant in parts of Nigeria is justified. The mechanism(s) of antioxidant action of *C. bonduc* remain(s) open for investigation. Further studies need to be carried out on the specific chemical constituents in the extract responsible for its antioxidant activities.

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