



## ***In vivo* and *In vitro* Antioxidant Activities of Aqueous and Ethanol Stem Bark Extracts of *Vitex doniana* on Doxorubicin-induced Oxidative Stress in Rats**

Mohammed Aliyu Sulaiman<sup>1\*</sup>, Daniel Dahiru<sup>1</sup>, Mohammed Auwal Ibrahim<sup>2</sup>  
and Ahmed Ibrahim Hayatu<sup>3</sup>

<sup>1</sup>Department of Biochemistry, Modibbo Adama University of Technology P. M. B. 2076 Yola, Adamawa State, Nigeria.

<sup>2</sup>Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria.

<sup>3</sup>Central Laboratory Complex, Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria.

### **Authors' contributions**

This work was carried out in collaboration among all authors. Authors MAS designed the study, provided equipment and reagents, conducted the statistical analysis and wrote the first draft of the manuscript; author AIH performed the experiments; author DD managed the analyses of the study and literature searches; author MAI made manuscript revisions. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Background:** Oxidative stress is involved in the pathogenesis of hypertension, myocardial ischemia-reperfusion injury, atherosclerosis, muscular dystrophy, aging and other associated diseases. *Vitex doniana* is used in Adamawa, northern Nigeria to treat oxidative stress associated diseases. However, the antioxidative effects of the plant have not been scientifically examined in oxidative stress experimental animal models. The aim of this study is to investigate the *in vitro* and *in vivo* antioxidant activities of aqueous and ethanol stem bark extracts of *Vitex doniana* in

\*Corresponding author: E-mail: mohammedzee10@hotmail.com, moh'd.aliyusulaiman@mautech.edu.ng, mohammedmail10@gmail.com

oxidative stress model of rats.

**Methods:** The study used 35 adult albino rats weighing  $175 \pm 25$  g, of which 30 were induced with oxidative stress by intraperitoneal injection of doxorubicin (10 mg/kg) for three consecutive days. Animals were treated by oral administration of silymarin (100 mg/kg) and *Vitex doniana* aqueous or ethanol extract (100 mg/kg and 200 mg/kg) for 14 consecutive days before they were sacrificed on the 15th day and blood was analyzed for biochemical indices of oxidative stress.

**Results:** The results of the phytochemistry showed the presence of alkaloids, tannins, flavonoids, steroids, phenols, saponins, terpenoids, glycosides: and total flavonoids ( $52.70 \pm 1.60$  mg/ml and  $75.40 \pm 0.80$  mg/ml), total phenols ( $21.45 \pm 1.54$  mg/ml and  $26.50 \pm 1.22$  mg/ml) for aqueous and ethanol stem bark extracts respectively. The extracts scavenged DPPH radical, reduced  $Fe^{3+}$  and inhibited lipid peroxidation. Doxorubicin significantly ( $p < 0.05$ ) lowered the levels of SOD, CAT, GR and TAS and significantly ( $p < 0.05$ ) but, increased the level of LPO. Oral treatment with *Vitex doniana* extracts significantly ( $p < 0.05$ ) increased the activities of CAT, GR, SOD and TAS while LPO was significantly ( $p < 0.05$ ) lowered. *Vitex doniana* stem bark extracts significantly ( $p < 0.05$ ) improved the biochemical derangements observed in the induced untreated animals in comparable manner to that of Silymarin.

**Conclusion:** The present study provides the scientific rationale for the use of *Vitex doniana* stem bark in traditional medicine and has a viable antioxidative capacity both *in vitro* and *in vivo*.

**Keywords:** Oxidative stress; *Vitex doniana*; doxorubicin; antioxidant activity; silymarin.

## ABBREVIATIONS

CAT : Catalase;  
 DNA : Deoxyribonucleic acid;  
 DOX : Doxorubicin;  
 DPPH : 2, 2'-diphenyl-1-picrylhydrazyl;  
 ELISA : Enzyme-linked immunosorbent assay;  
 FRAP : Ferric reducing antioxidant power;  
 GLP : Good laboratory practice;  
 GR : Glutathione reductase;  
 LPO : Lipid peroxidation;  
 ROS : Reactive oxygen species;  
 SOD : Superoxide dismutase;  
 TAS : Total antioxidant status;  
 TBARS : Thiobarbituric acid reactive substances.

## 1. INTRODUCTION

Oxidative stress refers to the pathological state of reactive oxygen species (ROS) accumulation caused by the excessive production of oxygen radicals or a compromised intracellular antioxidant defense system [1]. ROS are a group of small reactive molecules that play critical roles in the regulation of various cell functions and biological processes. Although essential for vascular homeostasis, uncontrolled production of ROS is implicated in vascular injury. Endogenous antioxidants function as checkpoints to avoid these untoward consequences of ROS, and an imbalance in the oxidant/antioxidant mechanisms leads to a state of oxidative stress[2]. Increased and uncontrolled oxidative stress causes lipid peroxidation, protein and enzyme denaturation

and deoxyribonucleic acid (DNA) damage which causes severe functional damage to endothelial cells [3,4,5]. Oxidative stress is involved in the pathogenesis of hypertension, myocardial ischemia-reperfusion injury, atherosclerosis, muscular dystrophy, aging and other associated diseases by regulating inflammation and stimulating vascular smooth muscle proliferation [6,7,8].

Antioxidant activity is a parameter which recently has been often used to characterize the health promoting properties of various products. It is connected with a common opinion on antioxidants' crucial role in the prevention of oxidative stress related diseases and on the reduction of total mortality associated with diets rich in plant foods, particularly fruits and vegetables [9]. Antioxidant compounds are present in high content in plants. They have shown protective effect against diseases without reducing their therapeutic efficacy. Moreover, there is a growing interest in the usage of natural antioxidants as a protective strategy against cardiovascular related problems [10].

Doxorubicin (DOX) is an anthracycline antibiotic derived from *Streptomyces*, that is most commonly used as an effective chemotherapeutic drug for a wide range of cancers, such as leukemia, solid tumors, soft-tissue sarcomas, and breast cancer [11,12]. DOX intercalates between DNA bases and inhibits the action of topoisomerase II, resulting in the cessation of cell replication and induces ROS

production, which additionally damage cancer cells. However, ROS are one of the major causes of DOX toxicity as well, which limits the therapeutic use of doxorubicin [13].

*Vitex doniana* (Verbenaceae), commonly called black plum, is a widely distributed plant in the eastern and western parts of Nigeria. Traditional medicine practitioners in Nigeria use various parts of the plant in the treatment and management of oxidative stress associated diseases including, rheumatism, hypertension, cancer, and inflammatory diseases [14]. Presently there is little information about the antioxidant capacity of the different parts of the plant in different solvent systems and more so, type of solvent and its polarity used in extraction affect the nature, amount and bioavailability of secondary metabolites extracted [15].

However, the antioxidative effects of the parts of the plant, which are actually used for the traditional management of diseases associated with oxidative stress, have not yet been scientifically examined either in humans or in experimental animal models. Hence, the present study was conducted to comprehensively investigate the *in vitro* and *in vivo* antioxidant activities of the aqueous and ethanol stem bark extracts of *Vitex doniana* in oxidative stress model of rats.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

Thirty five (35) young, Wister strain, male rats were purchased from the National Veterinary Research Institute Vom, Plateau State, Nigeria with an initial mean body weight of  $105.45 \pm 10.74$  g. The animals were housed and maintained in plastic laboratory rat cages in temperature and humidity controlled room (temperature:  $25 \pm 2^\circ\text{C}$ , humidity:  $60 \pm 5\%$ , 12-hour light/dark cycle). Moreover, all the animals were fed with a commercial rat diet (Vital Feeds, Jos, Nigeria) and drinking water *ad libitum*, were allowed to acclimatize for two weeks, and attained a weight of  $175 \pm 25$  g before they were used for the experiment. A standard protocol according to the guidelines of the Good Laboratory Practice (GLP) regulations of world health organization (WHO) as well as the rules and regulations of experimental animal ethics committee of Modibbo Adama University of Technology (M. A. U. Tech), Yola were strictly followed. Ethical clearance for the use of

experimental animals for all procedures was obtained from the Ethics Committee M. A. U. Tech. Yola, Nigeria.

### 2.2 Drugs and Chemicals

Silymarin was purchased from Micro Labs Limited, (India)., Doxorubicin from Zuvius Life Sciences Pvt Limited (India)., Picric acid, Ammonia solution, Total antioxidant status (TAS), Superoxide Dismutase (SOD) and Glutathione reductase (GR) kits were purchased from Fortress diagnostics Ltd. (U.K). All other chemicals used were of analytical grade.

### 2.3 Preparation of Plant Sample

The matured stem bark of *Vitex doniana* was collected in the dry season from Yolde pate ( $9^\circ 12' 0''$  N and  $12^\circ 27' 0''$  E) in Yola South Local Government area of Adamawa State. The stem bark was identified and authenticated at the Herbarium, Department of Plant Science, M. A. U. Tech. Yola, Adamawa State, Nigeria. The stem bark of *Vitex doniana* was washed and air-dried under a shade for a period of two weeks after which they were cut into small pieces and pulverized into fine powder using an electric grinding machine.

### 2.4 Preparation of Aqueous Extract

Aqueous extract of the stem bark of *Vitex doniana* was prepared as described by Oluduro and Aderiye [16]. Exactly 100 g of the powdered stem bark of *Vitex doniana* was soaked in 600 ml distilled water at ambient temperature for three days and filtered using Whatman filter paper No. 1. The bulk filtrate was reduced in vacuum at  $14^\circ\text{C}$ . The solid residue was stored at low temperature until it is needed.

### 2.5 Preparation of Ethanol Extract

The ethanol extract of the stem bark of *Vitex doniana* was prepared according to the method described by Chivapat [17]. Exactly 100 g of the pulverized stem bark powder was macerated in 600 ml of 70 % ethanol at  $40^\circ\text{C}$  for 48 hours. The extract solution was filtered using Whatman filter paper No.1 and evaporated using rotary evaporator under reduced pressure and then the concentrated extract was dried and stored at low temperature until it is required.

### 2.6 Qualitative Phytochemical Analysis

The aqueous and ethanol stem bark extracts of *Vitex doniana* were subjected to qualitative

analysis for various phytoconstituents by observing characteristics color change using standard procedures described by Trease and Evans, and Sofowora [18,14].

## 2.7 Quantitative Determination of Phenols and Flavonoids

### 2.7.1 Determination of total phenols

The total phenolic content of the extracts was determined using the Folin-Colcalteu method as modified by Dewanto [19]. Briefly, 0.5 ml of deionized water and 125  $\mu$ l of Folin- Colcalteu reagent were added to 125  $\mu$ l of the suitably diluted sample extract. The mixture was allowed to stand for 6 minutes before adding 1.25 ml of 7% aqueous Na<sub>2</sub>CO<sub>3</sub> solution. The mixture was then allowed to stand for additional 90 minutes before taking the absorbance at 760 nm. The amount of total phenolics was expressed as gallic acid equivalents (GAE, mg gallic acid/g sample) through the calibration curve of gallic acid.

### 2.7.2 Determination of total flavonoids

The total flavonoid content was determined using a colorimetric method described by Dewanto<sup>19</sup>. To 0.25 ml of the suitably diluted sample, 75  $\mu$ l of 5% NaNO<sub>2</sub> solution, 0.150 ml of freshly prepared 10% AlCl<sub>3</sub> solution, and 0.5 ml of 1 M NaOH solution were added. The final volume was then adjusted to 2.5 ml with deionized water. The mixture was allowed to stand for 5 minutes and the absorption measured at 510 nm against the same mixture, without the sample, as blank. The amount of total flavonoids was expressed as quercetin equivalents (QE, mg Quercetin/g sample) through the calibration curve of quercetin.

## 2.8 Antioxidant Activity Assays

### 2.8.1 DPPH (2, 2'-diphenyl-1-picrylhydrazyl) assay

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging assay as described by Sutharsingh [20] with little modifications. DPPH solution (0.04% w/v) was prepared in 95% ethanol and stock solutions of the extracts and standard ascorbic acid were prepared in the concentrations of 10 mg/ml. Exactly 2 ml, 4 ml, 6 ml, 8 ml and 10 ml of these solutions was taken into five test tubes respectively with same solvent making the final

volume of each test tube up to 10 ml making the concentrations to 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml respectively. Exactly 2 ml of freshly prepared DPPH solution (0.04% w/v) was added to each of these test tubes. The reaction mixture was incubated in the dark for 15 minutes thereafter the optical density was recorded against the blank at 523 nm. For the blank, 2 ml of DPPH solution in ethanol was mixed with 10 ml of ethanol and the optical density of the solution was recorded after 30 minutes. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical [20]. The antioxidant capacity was determined from three replicates and IC<sub>50</sub> values were determined.

### 2.8.2 FRAP (Ferric reducing antioxidant power) assay

The antioxidant activity of the plant extracts was estimated using the FRAP assay as described by Sutharsingh [20] with little modifications. Exactly 1 ml of test sample of both extracts in different concentrations were mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide in separate test tubes. The reaction mixtures was incubated in a temperature-controlled water bath at 50° C for 20 minutes followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 minutes at room temperature. The supernatant obtained (1 ml) was mixed with 1 ml of distilled water and 0.2 ml of 0.1% FeCl<sub>3</sub>. The blank was prepared in the same manner as the samples except that the sample was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power is expressed as an increase in A<sub>700</sub> after blank subtraction [20]. The antioxidant capacity was determined from three replicates and IC<sub>50</sub> values were determined.

### 2.8.3 TBARS (Thiobarbituric acid reactive substance) assay

Thiobarbituric acid reactive substances (TBARS) assay was performed by method described by Aazza<sup>21</sup>. Egg yolk homogenates were used as a lipid-rich medium obtained as described by Dorman<sup>22</sup>. Briefly, 100  $\mu$ l of egg yolk [(10% w/v) in KCl (1.15%)] was added into test tubes labeled 'sample' and 'standard', 50  $\mu$ l of extract and standard vitamin E in ethanol (10 - 1000  $\mu$ g/ml) were added to the test tubes respectively. Then,

300 µl of 20% acetic acid (pH 3.5) and 300 µl of 0.8% (w/v) TBA in 1.1% (w/v) sodium dodecyl sulphate (SDS) were added. The resulting mixture was vortexed and heated at 95°C for 60 minutes. After cooling, at room temperature, 750 µl of butan-1-ol was added to each tube; the contents of the tubes were stirred and centrifuged at 3000 rpm for 10 minutes. The upper organic layer was transferred to 96-well microtitre plate and absorbance was measured at 532 nm using an ELISA plate reader. All of the values were based on the percentage antioxidant index (AI%), whereby the control was completely peroxidized and each sample demonstrated a degree of change; the percentage inhibition was calculated using the formula  $(1 - T/C) \times 100$ , where C is the absorbance value of the fully oxidized control and T is the absorbance of the test sample. The antioxidant capacity was determined from three replicates and IC<sub>50</sub> values were determined. Same amount of deionized water was used as the control [21,22].

## 2.9 Animal Grouping and Induction of Oxidative Stress

Animals were randomly divided into seven groups of five rats each, namely: Normal Control (NC), Oxidative Stress Control (OC), Oxidative Stress + 100 mg/kg BW of Silymarin (Micro Labs Ltd., India) (OSC), Oxidative Stress + low dose (100 mg/kg BW) of *Vitex doniana* aqueous extract (VDAE), (OVAL), Oxidative Stress + high dose (200 mg/kg BW) of VDAE (OVAH), Oxidative Stress + low dose (100 mg/kg BW) of *Vitex doniana* ethanol extract (VDEE), (OVEL), Oxidative Stress + high dose (200 mg/kg BW) of VDEE (OVEH). After two weeks acclimatization period, animals in OC, OSC, OVAL, OVAH, OVEL and OVEH were given intraperitoneal injection of 10 mg/kg BW doxorubicin (Zuvius Life Sciences Pvt, Ltd., India) for three consecutive days to induce oxidative stress. While animals in NC were only supplied with feed (Vital Feeds, Jos, Nigeria) and water only throughout the fourteen days experimental period.

At the end of the experimental period, animals were euthanized by halothane anesthesia and the whole blood of each animal was collected via cardiac puncture using a 10 ml syringe and 25 gauge needle after 24 hours of last treatment. The blood was collected in Vacutainer tubes and centrifuged at 3000 rpm for 15 minutes, plasma and serum were separated and preserved for further analysis. The serum, plasma and whole

blood were used for various biochemical analysis. The biochemical parameters determined were catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD), total antioxidant status (TAS) and lipid peroxidation (LPO).

Blood was collected via cardiac puncture. plasma was obtained after centrifugation at 3 000 r/min for 10 min at 4°C.

## 2.10 Catalase (CAT) Assay

Catalase activity was assayed by the method described by Sinha<sup>23</sup>. The enzyme extract (0.5 ml) was added to the reaction mixture containing 1 ml of 0.01M phosphate buffer (pH 7.0), 0.5 ml of 0.2M H<sub>2</sub>O<sub>2</sub>, 0.4 ml H<sub>2</sub>O and incubated for different time period. The reaction was terminated by the addition of 2 ml of acid reagent (dichromate/acetic acid mixture) which was prepared by mixing 5% potassium dichromate with glacial acetic acid, (1:3 by volume). To the control, the enzyme was added after the addition of acid reagent. All the tubes were heated for 10 minutes and the absorbance was read at 610 nm. Catalase activity is expressed in terms of µmoles of H<sub>2</sub>O<sub>2</sub> consumed/min/mg protein [23].

## 2.11 Glutathione reductase (GR) Assay

Glutathione reductase was determined by method as described by Thomas [24].

Test tubes labelled sample and blank were used. Into the test tube labelled sample, 40 µl of serum was added followed by addition of 1000 µl GSSG reagent to both test tubes and 40 µl of distilled water was added to the test tube labelled blank. The test tubes were mixed well and then 200 µl of NADPH was added to both test tubes. The test tubes were again mixed well and the initial absorbance was taken and the absorbance was read after 1, 2, 3 and 4 minutes respectively.

Glutathione reductase concentration was calculated as  
 U/l of sample =  $4983 \times \Delta A_{340 \text{ nm/min}} \times \text{dilution factor} (20)$

## 2.12 Superoxide dismutase (SOD) Assay

Superoxide dismutase was determined photometrically by method described by Arthur and Boyne [25]. The test principle was based on the role of superoxide dismutase to accelerate the dismutation of toxic superoxide radical (O<sub>2</sub>)

produced during oxidative energy process to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and molecular oxygen.

Into the test tube labelled diluted sample, 50 µl of sample was added while into the test tube labelled standard 50 µl of the standard was added and to the test tube labelled sample diluent 50 µl of sample diluent was added. To all test tubes 1.7 ml each of mixed substrate was then added. The test tubes were then shaken well for proper mix followed by addition of 250 µl of xanthine oxidase to each of the labelled test tubes. The test tubes were well mixed and incubated at 37°C for 30 seconds. The first reading of the absorbance was taken immediately and subsequently at 1, 2 and 3 minutes respectively [26].

The value of superoxide dismutase was calculated using the formula

$$\frac{A_2 - A_1}{3} = \Delta A/\text{min of standard or sample}$$

Sample diluent rate (S<sub>1</sub> rate) = rate of uninhibited reaction = 100%. All standard rates and diluted rates must be converted into percentage of the sample diluent and subtracted from 100% to give a percentage inhibition.

$$100 - \frac{A_{\text{std/min}}}{A_{\text{sml/min}}} = \% \text{ inhibition}$$

### 2.13 Determination of Total Antioxidant Status (TAS)

Total antioxidant status was determined by enzymatic colorimetric method as described by Koracevic [26].

Test tubes labelled reagent blank and sample were used and into each tube 800 µl of TAS buffer was added followed by addition of 50 µl of sample to the test tube labelled sample. The tubes were mixed and initial absorbance was taken as A<sub>1</sub>. Then 125 µl of TAS chromogen was added to each test tube, the tubes were mixed thoroughly and incubated for 5 min at 37°C and the absorbance was taken as A<sub>2</sub>.

TAS was calculated using the formula

$$A_2 - A_1 = \Delta \text{Absorbance of sample/calibrator}$$

$$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{calibrator}}} \times \text{Calibrator concentration}$$

### 2.14 Determination of Lipid Peroxidation (LPO)

Lipid peroxidation levels were measured by the thiobarbituric acid (TBA) reaction using the

method of Ohkawa [27]. Exactly 50 µl of erythrocyte supernatant was added to test tubes containing 2 µl of butylated hydroxytoluene (BHT) in methanol. Then, 50 µl each of acid reagent (1 M phosphoric acid) and TBA solution were added, the tubes were mixed vigorously and incubated for 60 min at 60°C. The mixture was centrifuged at 10,000-rpm × g for 3 min. The supernatant was put into wells on a micro plate in aliquots of 75 µl, and absorbance was measured. TBARS levels were expressed as nmol/g hemoglobin in erythrocyte hemolysates.

### 2.15 Statistical Analysis

The experiment results are expressed as mean ± SEM. The statistical analysis of data was done using one – way ANOVA (Analysis of variance) and the difference between two means determined using student t - test with level of statistical significance taken as p<0.05, with the aid of SPSS software, version 20. (SPSS Inc., Chicago, USA)

## 3. Results

The qualitative phytochemical analysis of aqueous and ethanol stem bark extracts of *Vitex doniana* showed the presence of tannins, flavonoids, alkaloids, steroids and total phenols in both extracts. Additionally, the aqueous extract gave positive results to saponins, terpenoids and glycosides, which were found to be negative in the ethanol extract (Table 1). The ethanol stem bark extract had significantly (P>0.05) higher phenols and flavonoids content compared to the aqueous extract (Table 2).

The aqueous and ethanol stem bark extracts of *Vitex doniana* inhibited DPPH radical, indicating their antioxidant capacity. Both extracts possessed significantly (P>0.05) different DPPH radical scavenging activity compared to vitamin C. The inhibition produced by the aqueous extract was significantly (P>0.05) higher than that of the ethanol extract. The aqueous extract showed concentration-dependent inhibition while the ethanol extract did not (Table 3).

The antioxidant potentials of the aqueous and ethanol stem bark extracts of *Vitex doniana* were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II). The FRAP values for the aqueous extract were significantly (p < 0.05) higher compared to the ethanol extract (Table 4). Both extracts exhibited an increase in chelating activity with increase in drug

concentration; however, both extracts exhibited significantly ( $p < 0.05$ ) lower activity when compared with ascorbic acid.

The aqueous and ethanol stem bark extracts of *Vitex doniana* possess antioxidant capacity and are capable of inhibiting lipid peroxidation in dose dependent manner. Moreover, the ethanol extract was observed to have significantly ( $P < 0.05$ ) better TBARS inhibitory potential compared to the aqueous extract (Table 5).

The effects of aqueous and ethanol stem bark extracts of *Vitex doniana* on activities of antioxidant enzymes (CAT, GR, and SOD) and lipid peroxidation and total antioxidant status are shown in Table 6. The levels of CAT, GR, SOD and TAS were lowered significantly ( $P < 0.05$ ) and the level of lipid peroxidation (TBARS) was

increased significantly ( $P < 0.05$ ) owing to the oxidative stress induced by doxorubicin in rats when compared with rats in NC. However, oral treatment with both extracts significantly ( $P < 0.05$ ) increased the levels of CAT, GR, SOD and TAS and significantly ( $P < 0.05$ ), but lowered lipid peroxidation when compared with animals in NC.

#### 4. DISCUSSION

This study was able to detect some important phytochemical compounds of *Vitex doniana* that are of relevance in phytomedicine. They include alkaloids, flavonoids, phenols and tannins, which have biological and medicinal values such as anti-inflammatory, anti-diabetic, anti-microbial and anti-atherosclerotic properties [28].

**Table 1. Phytochemical composition of aqueous and ethanol stem bark extracts of *Vitex doniana***

Phytochemical constituent	Aqueous	Ethanol
Alkaloids	+	+
Saponins	+	-
Tannins	+	+
Terpenoids	+	-
Flavonoids	+	+
Glycosides	+	-
Steroids	+	+
Phenols	+	+

Key: +: Present, -: Absent

**Table 2. Quantitative determination of total phenols and flavonoids of *Vitex doniana* stem bark extracts**

Phytochemical	Aqueous	Ethanol
Total phenols	21.45 $\pm$ 1.54	26.50 $\pm$ 1.22**
Total flavonoids <sup>#</sup>	52.70 $\pm$ 1.60	75.40 $\pm$ 0.80**

Values are expressed as mean  $\pm$  SEM of triplicate determinations. \*\* = significantly ( $P < 0.05$ ) higher compared to aqueous extract.

\* = mg gallic acid equivalent/g extract powder <sup>#</sup> = mg quercetin equivalent/g extract powder

**Table 3. DPPH radical scavenging activity (% inhibition) and half of maximal inhibitory concentration (IC<sub>50</sub>) of aqueous and ethanol stem bark extracts of *Vitex doniana***

Concentration ( $\mu$ g/ml)	Aqueous extract	Ethanol extract	Standard
20	35.38 $\pm$ 0.99	37.07 $\pm$ 1.22	9.74 $\pm$ 0.31 <sup>#</sup>
40	59.59 $\pm$ 0.75*	50.24 $\pm$ 1.02 <sup>#</sup>	15.32 $\pm$ 0.56 <sup>#</sup>
60	69.76 $\pm$ 0.18*	59.39 $\pm$ 0.78 <sup>#</sup>	29.72 $\pm$ 0.18 <sup>#</sup>
80	76.42 $\pm$ 0.24*	70.77 $\pm$ 0.97 <sup>#</sup>	40.90 $\pm$ 1.38 <sup>#</sup>
100	85.73 $\pm$ 0.05*	82.18 $\pm$ 0.25 <sup>#</sup>	44.04 $\pm$ 0.39 <sup>#</sup>
IC <sub>50</sub> ( $\mu$ g/ $\mu$ L)	109.90	147.84	39.65

Values are mean  $\pm$  SEM for 3 determinations

\* = Significantly ( $p < 0.05$ ) higher compared to standard

<sup>#</sup> = Significantly ( $p < 0.05$ ) lower compared to aqueous extract

**Table 4. Ferric reducing antioxidant power (frap) and (ic<sub>50</sub>) of aqueous and ethanol stem bark extracts of *Vitex doniana***

Concentration (mg/ml)	Aqueous extract	Ethanol extract	Standard
20	72.19 ± 0.69*	33.00 ± 1.54	76.32 ± 0.40
40	82.99 ± 0.95*	38.17 ± 0.38	84.66 ± 1.22
60	87.38 ± 0.52*	43.48 ± 0.42	91.28 ± 0.62
80	88.22 ± 0.59*	61.53 ± 0.73	95.42 ± 0.33
100	88.66 ± 0.24*	65.57 ± 0.67	94.89 ± 1.21
IC <sub>50</sub>	42.42	67.59	38.57

Values are expressed as mean ± SEM of triplicate determinations

\*Significantly ( $p < 0.05$ ) higher compared to Ethanol extract

**Table 5. Percentage (%) inhibition of thiobarbituric acid reactive substances (tbars) and ic<sub>50</sub> of aqueous and ethanol stem bark extracts of *Vitex doniana***

Concentration (mg/ml)	Aqueous extract	Ethanol extract	Standard
20	41.31 ± 0.46	54.38 ± 0.48*	68.68 ± 0.16
40	59.29 ± 0.41	66.35 ± 0.64*	77.24 ± 0.18
60	69.61 ± 0.63	78.81 ± 0.41*	81.80 ± 0.10
80	75.96 ± 0.32	79.76 ± 0.46*	85.31 ± 0.42
100	79.95 ± 0.07	83.16 ± 0.39*	87.52 ± 0.26
IC <sub>50</sub>	51.29	47.48	44.09

Values are expressed as mean ± SEM of triplicate determinations

\*Significantly ( $p < 0.05$ ) higher compared to aqueous extract

**Table 6. Effects of aqueous and ethanol stem bark extracts of *Vitex doniana* on enzymic and non – enzymic indices of oxidative stress (superoxide dismutase, catalase, glutathione reductase, total antioxidant status and lipid peroxidation)**

z	SOD	CAT	GR	TAS	LPO
	(U/ml)	(U/mg)	(U/L)	(mmol/L)	(mg/dl)
NC	180.62 ± 4.06	24.67 ± 0.24	96.44 ± 0.64	1.92 ± 0.05	37.44 ± 0.47
OC	99.73 ± 0.54 <sup>d</sup>	8.43 ± 0.19 <sup>d</sup>	48.28 ± 0.90 <sup>d</sup>	0.36 ± 0.02 <sup>d</sup>	181.96 ± 5.21 <sup>ac</sup>
OSC	200.25 ± 0.37 <sup>ab</sup>	20.13 ± 0.32 <sup>b</sup>	52.06 ± 0.82	2.01 ± 0.02 <sup>ab</sup>	49.12 ± 1.89 <sup>ae</sup>
OVAL	178.67 ± 1.03 <sup>b</sup>	11.11 ± 0.43	51.08 ± 0.75	2.03 ± 0.03 <sup>ab</sup>	72.57 ± 1.83 <sup>ace</sup>
OVAH	182.32 ± 0.73 <sup>b</sup>	20.62 ± 0.50 <sup>b</sup>	54.17 ± 0.53 <sup>b</sup>	2.15 ± 0.05 <sup>abc</sup>	42.57 ± 1.33 <sup>ae</sup>
OVEL	185.09 ± 1.60 <sup>ab</sup>	10.38 ± 0.26	59.42 ± 0.66 <sup>bc</sup>	2.54 ± 0.09 <sup>abc</sup>	93.58 ± 5.22 <sup>ace</sup>
OVEH	188.79 ± 0.33 <sup>ab</sup>	10.09 ± 0.28	66.77 ± 0.68 <sup>bc</sup>	2.68 ± 0.09 <sup>abc</sup>	136.52 ± 7.57 <sup>ace</sup>

Values are expressed as mean ± SEM, (n = 5). a = significantly ( $P < 0.05$ ) higher compared to normal control, b = significantly ( $P < 0.05$ ) higher compared to negative control, c = significantly ( $P < 0.05$ ) higher compared to positive control, d = significantly ( $P < 0.05$ ) lower compared to normal control, e = significantly ( $P < 0.05$ ) lower compared to negative control

Oxidative stress-related disorders can be prevented or managed by antioxidants and the therapeutic effects of many medicinal plants have been hypothesized to result from the free radical scavenging and antioxidant activity of the constituent antioxidant phytochemicals notably phenols, flavonoids and tannins [29]. The presence of these antioxidant phytoconstituents in plants extracts may be responsible for the inhibition of iron-induced lipid peroxidation in rat heart [30].

This study was able to quantitatively analyze total phenols and flavonoids as presented in Table 2. Observation from the present study suggests that the phenolics and flavonoids content is high in the ethanol extract as compared to the aqueous extract. The extractive capability of phenolic components from herb material is considerably dependent on the type of solvent [31]. The present study showed lower flavonoids content in the aqueous stem bark extract of *Vitex doniana* when compared with the ethanol stem bark extract; this is in agreement



with previous report [32], because polar flavonoids such as flavones and isoflavones incline to be more soluble in non-polar solvents.

Phenolic compounds are natural antioxidants that originates from several fruits of both trees and cereals [33]; they are strong antioxidants and hinders the influence of free radicals and ROS, which are the basis of several chronic human diseases [34]. The present study showed the presence of a rich amount of phenolic compounds in the stem bark extracts of *Vitex doniana*, this will encourage the utilization of the stem bark for various purposes.

The aqueous and ethanol stem bark extracts of *Vitex doniana* significantly scavenged DPPH radical in a dose dependent manner (10 - 100 mg/ml). The aqueous extract showed a better antioxidant capacity compared to ethanol extract and ascorbic acid with IC<sub>50</sub> value of 59.90 mg/ml compared to the ethanol extract and ascorbic acid having IC<sub>50</sub> values of 167.84 mg/ml and 89.65 mg/ml respectively (Table 3). The results of this study also correlates to that obtained in a recent study on DPPH scavenging ability of some vegetables [35]. This significant scavenging ability in the *Vitex doniana* stem bark extracts could be attributed to the phenols and flavonoids content of the extracts. Thus, the stem bark of *Vitex doniana* can be used as a cheap source of antioxidants.

The ferric reducing powers of aqueous and ethanol stem bark extracts of *Vitex doniana* increased with increase in their concentrations (10 - 100 mg/ml). The aqueous extract have shown better activity than the ethanol extract having IC<sub>50</sub> value of 42.42 mg/ml whereas the ethanol extract required 67.59 mg/ml to achieve 50% inhibition (Table 4). Iron (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant action of phenolics [36].

The results of the present study showed that both aqueous and ethanol stem bark extracts of *Vitex doniana* significantly ( $P < 0.05$ ) inhibited TBARS comparable with ascorbic acid in a dose dependent manner (Table 5). Lipid oxidation is problematic as many oxidative chemical reactions that are not controlled and constrained by enzymes, may show exponential reaction rates. Some of the products of the attack are highly reactive species that modify proteins and DNA [37].

This study was able to induce oxidative stress in rats by intraperitoneal administration of 10 mg/kg BW doxorubicin for three consecutive days, evidence is the significant ( $p < 0.05$ ) decreased in activities of the antioxidant enzymes CAT, GR, SOD and increased level of lipid peroxidation (TBARs) observed in the doxorubicin induced animals compared to animals in NC group. This is in agreement with previous report that antioxidant enzymes; SOD, CAT, GR, and non - enzymatic antioxidants vitamin C, vitamin E and reduced glutathione are easily inactivated by lipid peroxides or ROS, which results in decreased activities of these enzymes in doxorubicin induced rats [38].

Pharmacological augmentation of endogenous myocardial antioxidants has been identified as promising therapeutic approach in diseases associated with increased oxidative stress. In this study, the doxorubicin induced oxidative stressed animals have shown significant ( $p < 0.05$ ) decrease in activities of SOD and CAT. SOD plays a vital role in protecting the myocytes from oxidative damage by converting superoxide radicals into hydrogen peroxide, which is further metabolized by CAT to molecular oxygen and water. The decrease in the activities of these antioxidant enzymes might be due to myocardial cell damage [39]. Superoxide radicals generated at the site of damage modulates SOD and CAT resulting in the decreased activities of these enzymes and accumulation of superoxide anion, which also damages the myocardium [40]. Oral administration of the stem bark extracts of *Vitex doniana* for 14 days significantly ( $p < 0.05$ ) increased the activities of SOD and CAT in doxorubicin induced rats showing the positive effect of *Vitex doniana* stem bark extracts in the regulation of antioxidative capacity.

Malondialdehyde level is widely used as marker of free radical mediated lipid peroxidation injury. In this study, doxorubicin-induced untreated group (OC) showed significantly ( $P < 0.05$ ) increased lipid peroxidation (TBARs) level and significantly ( $P < 0.05$ ) decreased level of total antioxidant status (TAS) when compared with the NC group. Oral administration of *Vitex doniana* stem bark extracts for 14 days significantly ( $P < 0.05$ ) decreased TBARs and significantly ( $P < 0.05$ ) increased TAS in a dose dependent manner which confirms that concomitant administration of stem bark extracts of *Vitex doniana* extracts could effectively protect against doxorubicin induced oxidative stress. This study agree with earlier reports, that lipid peroxidation

has been implicated in the pathogenesis of increased membrane rigidity, osmotic fragility, reduced erythrocyte survival and perturbations in lipid fluidity [41,42].

Antioxidants play a vital role in scavenging reactive oxygen species and protecting cells from oxidative damage. The protective effect of aqueous and ethanol stem bark extracts of *Vitex doniana* against oxidative stress was further supported by the observed increased myocardial enzymatic and non - enzymatic antioxidant activity. Oral administration of *Vitex doniana* stem bark extracts significantly ( $p < 0.05$ ) elevated the levels of the enzymatic antioxidants CAT, SOD and GR and the total antioxidant status and significantly ( $p < 0.05$ ) but, inhibited TBARs in doxorubicin induced oxidative stressed rats. These findings suggests that these effects are via anti-oxidative by inhibiting free radicals, decreased oxidative stress, and increased antioxidant enzyme activity.

## 5. CONCLUSION

The two varying extracts displayed antioxidant activity *in vitro* and *in vivo* in doxorubicin-induced oxidative stress rat model. However, this study has further validated the claims reported about the antioxidant activity of *Vitex doniana* stem bark extract. These findings agree with the current use of the plant extracts by folk medicine practitioners as antioxidative agent and these findings may be useful to scientists in the field of pharmacology to develop evidence-based alternative medicine in the treatment of oxidative stress related diseases and disorders.

## DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no competing interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. The research was also not funded by the producing company rather, it was funded by personal efforts of the authors.

## AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

## CONSENT

It is not applicable

## ETHICAL APPROVAL

The animal study was conducted in strict compliance with the Animal Research Ethical Committee guide of the Modibbo Adama University of Technology Yola, Nigeria.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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