Antioxidant Potential of the Methanol Extract of *Dalbergia saxatilis* (Hook. F) Leaf

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Abstract

Antioxidants of natural origin have become the target of a great number of researches in finding the sources of potentially safe, effective, and cheap antioxidants. This study investigated the antioxidant potential of the methanol extract *Dalbergia saxatilis* leaf using the cotton pellet-induced granuloma method in Wistar rats. Thirty rats of both sexes weighing 150-200 g were divided into five groups of six rats each. Group one received distilled water 1ml/kg, group two received ascorbic acid 250 mg/kg, while groups three, four, and five received graded doses of the methanol leaf extract of *Dalbergia saxatilis* (250 mg/kg, 500 mg/kg and 1000 mg/kg) respectively daily for 9-days. The rats were sacrificed on the 10th day. Blood samples were collected in plain bottles for evaluation of oxidative stress markers which include superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and malondialdehyde (MDA). Results showed that the extract at the highest dose (1000 mg/kg) significantly ($p < 0.001$) decreased MDA concentration similar to the vitamin C at 250 mg/kg, while the other doses of the extract decreased MDA concentration but was not significant. Increased CAT concentration was observed in the 500 and 1000 mg/kg though not significant. Increased concentrations of SOD and GSH were also observed across all doses of the extract but only significant at highest and lowest doses respectively. These findings suggest that, the methanol leaf extract of *Dalbergia saxatilis* possesses antioxidant properties which may be via its action on MDA, SOD, CAT, and GSH.

Keywords: Antioxidant, *Dalbergia saxatilis*, Malondialdehyde, superoxide dismutase

Introduction

Activities of free radicals can be lowered using antioxidants. Research has revealed

that the most commonly used antioxidants (butylated hydroxyanisole, BHA and butylated hydroxytoluene, BHT) at the present time are suspected of being hepatotoxic and carcinogenic in laboratory animals (Valko *et al*., 2013). Humans are constantly exposed to free radicals obtained from both natural and man -made sources such as electromagnetic radiation, radon, and cosmic radiation. These free radicals when they are in excess produce too much reactive oxygen species (ROS) relative to antioxidant defense (Shankar *et al*., 2014). ROS are oxygen-based chemical intermediates with high reactivity. The balance between production of ROS and systems meant to mitigate ROS is called 'redox state.' Increased production of ROS is a causal feature in the toxicity of many xenobiotics (Shankar *et al*., 2014). Overproduction of these free radicals can cause oxidative damage to biomolecules eventually leading to many chronic diseases such as cancer, diabetes, stroke, shock (Wolf, 2015). Oxidative stress is the major driving factor responsible for the initiation and progression of chronic diseases such cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases and inflammatory diseases among other syndromes (Arika *et al*., 2019). Agents that scavenge these free radicals are therefore essential so as to prevent such diseases and improve the quality of life.

Antioxidants are molecules that inhibit the oxidation of other molecules (Peter *et al*., 2010). Oxidation reactions can produce free radicals that can start chain reactions (Lobo et al., 2010). When the chain reactions occur in a cell, it can cause damage or death to the cell. Antioxidants terminate the chain reactions by removing the free radicals' intermediates and inhibit other oxidation reactions. Insufficient levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress which may damage or kill cells, and can lead to chronic diseases (Shi *et al*., 2019). These antioxidants delay or inhibit cellular damage mainly through their radical scavenging property (Halliwell, 2015). These low molecular weight antioxidants can safely interact with free radicals and terminate the chain before vital molecules are damaged. Some of such antioxidants, including glutathione, ubiquinol and uric acid are produced during normal metabolism in the body (Halliwell, 2015). Other lighter antioxidants are found in diet. Although, there are several enzymes system within the body that scavenge free radicals, the principle micronutrient (vitamins) antioxidant is vitamin E, vitamin C and B- carotene (Shi *et al*., 2019).

Different parts (leaves, bark and roots) of *Dalbergia saxatilis* are used in traditional medicine for various ailments such as cough, small pox, skin lesions, bronchial ailments and toothache (Saha *et al*., 2013). Several studies have been conducted on the plant which include; antiepileptogenic, anticonvulsant, antimicrobial, analgesic, anti-inflammatory, antipyretic, as well as determination of the safety profile of *Dalbergia saxatilis* in animal models (Yemitan *et al*., 2013; Koma and Sani, 2014; Hassan *et al*., 2015; Hassan *et al*., 2016; and Ukwuani-Kwaja *et al*., 2021). This study evaluated the antioxidant potential of the methanol leaf extract of *Dalbergia saxatilis* using the cotton pellet-induced granuloma method in Wistar rats.

Materials and Methods

Drugs, Chemicals, Equipment

Methanol, chloroform, and Formalin (Sigma Aldrich, Germany) distilled water, ascorbic acid (Abcam Inc., Cambridge, MA), streptomycin and penicillin (GSK, Brentford, GB), ketamine (Greenco Biologicals PVT Ltd, Kolkata, India), were used for the experiment.

Experimental Animals

Thirty rats of both sexes were obtained from the Animal house facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. They were kept in clean cages and provided with standard feed and water. The

experiments were carried out following the guidelines for the care and use of Laboratory animals (Clark et al., 1997).

Plant Collection and Preparation

Fresh leaves of *Dalbergia saxatilis* were collected from Giwa Local Government Area of Kaduna State, Nigeria in April, 2021. The plant was authenticated by a taxonomist Umar Gallah from the Herbarium section in the Department of Botany, Kaduna State University (KASU), Kaduna, Nigeria. The plant was issued a voucher specimen number (No KASU/BSH/1608) for future reference. The leaves collected were air-dried under a shade until constant weight was attained. The dried leaves were then crushed into fine powders using mortar and pestle. This was extracted using 70% methanol. Five hundred grams (500g) of the powder was added into a flask with 1000ml of the solvent and kept for 24 hours with intermittent shaking and then filtered. The extract was evaporated to dryness on a water bath.

Induction of Granuloma using Cotton Pellets

The rats were grouped into five (5) groups each containing six (6) rats. Thirty pieces of Cotton pellets weighing 0.1 g each were made and sterilized in an autoclave. Chronic inflammation was induced by subcutaneous implantation of the cotton pellets (which was dipped in a solution of penicillin and streptomycin to prevent infection) into the rodent hind limb under ketamine anesthesia (Winter and Porter, 1957). Group one (1) rats were administered distilled water (1mg/kg) and served as the negative control, while group two (2) rats were administered ascorbic acid (250 mg/kg) and served as the positive control. Groups three (3), four (4), and five (5) rats received the aqueous methanol leaf extract of *Dalbergia saxatilis* (250, 500 and 1000 mg/kg) respectively for nine (9) days. The rats were sacrificed on the tenth (10th) day and blood samples were taken for determination of antioxidant parameters (GSH, SOD, MDA and CAT).

Inhibition (%) of granuloma tissue development was calculated using the relation:

Inhibition (%) = [WC−WT/WC] × 100; where WC = weight of granuloma tissue of control group; WT = weight of granuloma tissue of treated group.

Determination of Superoxide dismutase (SOD) Activity

The determination of the SOD activity was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazon dye. Briefly, 300 μL of mixed substrate was added to 200 μL of diluted hemolysates. The samples were mixed well and 75 μL xanthine oxidase was added to reactions. The absorbance was measured at 505 nm and the SOD activity was then calculated according to the manufacturer's instruction (Ransod®-Randox Lab, Antrim, UK) and expressed as U/mL.

Determination of Reduced Glutathione Level

Determination of the level of reduced glutathione (GSH) was carried out according to the method described by Ellman (1959). In this method thiols react with Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid) or DTNB), cleaving the disulfide bond to give 2-nitro-5-thiobenzoate (TNB^-), which ionizes to the TNB^{2-} dianion in water at neutral and alkaline pH. To evaluate GSH level in samples, 15 μL of hemolysates was mixed with 260 μL assay buffer (0.1 M sodium phosphate and 1 mM EDTA, pH: 8) and 5 μL Ellman reagents. Samples were incubated for 15 min at room temperature and the TNB^{2-} formation was quantified in a spectrophotometer by measuring the absorbance of visible light at 412 nm.

Absorbance values were compared with a standard curve generated from standard curve from known GSH.

Determination of Catalase Activity

Catalase activity was determined spectrophotometrically by the method of Koroliuk *et al.*, (1988). Briefly, 10 μL of sample was incubated with 100 μmol/mL of H_2O_2 in 0.05 mmol/L Tris-HCl buffer pH = 7 for 10 min. The reaction was terminated by rapidly adding 50 μL of 4% ammonium molybdate. Yellow complex of ammonium molybdate and H_2O_2 was measured at 410 nm. One unit of catalase activity was defined as the amount of enzyme required to decompose 1 μmol H_2O_2 per min.

Determination of Malondialdehyde (MDA) Activity

Malondialdehyde levels in samples were measured using the thiobarbituric acid reaction method of Placer *et al.*, (1966). Quantification of the thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. To measure MDA level, a working solution containing 15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N hydrochloric acid was prepared. For each sample, 250 μL serum and 500 μL working solution were mixed and placed in boiling water for 10 min. After cooling the samples were centrifuged at 3000 rpm for 10 min. Finally, 200 μL of each supernatant was transferred to microplates and the optical density of samples was measured at 535 nm. The values of MDA were expressed as μmol/L.

Data analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test using the statistical package for social sciences (SPSS) version 22. Results were considered significant at $p \leq 0.05$. Results obtained were presented as graphs and Tables and expressed as (mean ± SEM).

Results

Percentage Yield

The extract obtained after extraction was dark brown, sticky semi solid substance. The percentage yield of the extract was calculated to be 30.6 %w/w.

Effect of the extract of on Cotton Pellet Induced Granuloma

The Methanol leaf extract of *Dalbergia saxatilis* (250 mg/kg, 500 mg/kg and 1000 mg/kg) decreased granuloma formation in a dose-dependent manner with 2.17%, 4.34% and 7.6% respectively, when compared with control, though not statistically significant. Granuloma formation was evaluated by determining the average weights of the dry pellets and percentage inhibition was calculated.

Table 1: Effect of the extract on Cotton Pellet Induced Granuloma

Treatment	Dose	Mean weight of Dry Cotton Pellet (mg) ± SEM	% Inhibition
Distilled Water	1 ml/kg	153.33 ± 4.22	-
Vitamin C	250 mg/kg	146 ± 4.90	4.78
Extract	250 mg/kg	150 ± 4.47	2.17
Extract	500 mg/kg	146.67 ± 3.33	4.34
Extract	1000 mg/kg	141.67 ± 5.3	7.6

Effect of the extract on Malondialdehyde (MDA) Levels

MDA was significantly reduced in the vitamin c group and the highest dose of the extract when compared with the distilled water group. MDA was reduced in the other doses of the extract tested too but not statistically significant.

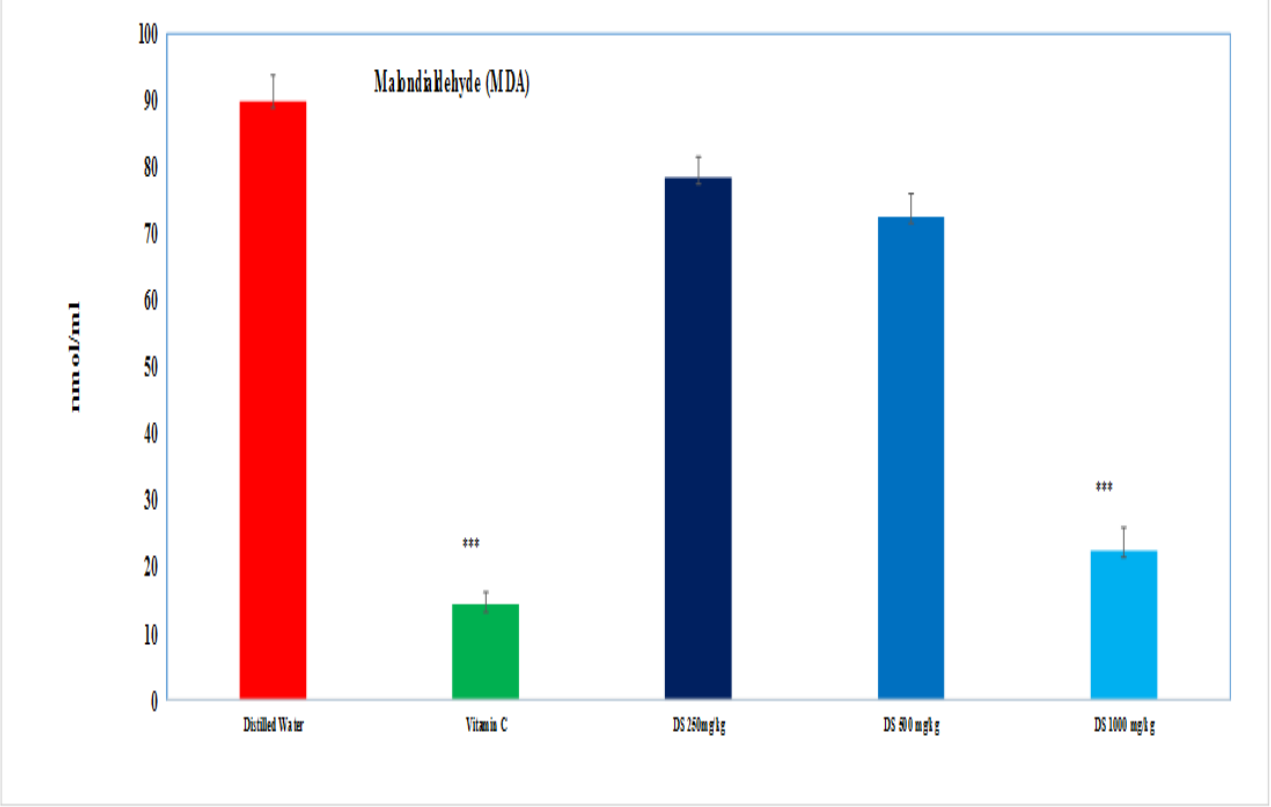

Figure 1: Effect of the extract on Malondialdehyde Levels

Values are mean ± SEM, *** represent *P< 0.001* level of significance. One-way ANOVA followed by Tukey post hoc test, n= 6.

Effect of the extract on Superoxide Dismutase (SOD)

SOD was significantly increased in the vitamin c group and the highest dose of the extract when compared with the distilled water group. SOD was increased in the other doses of the extract tested but not statistically significant.

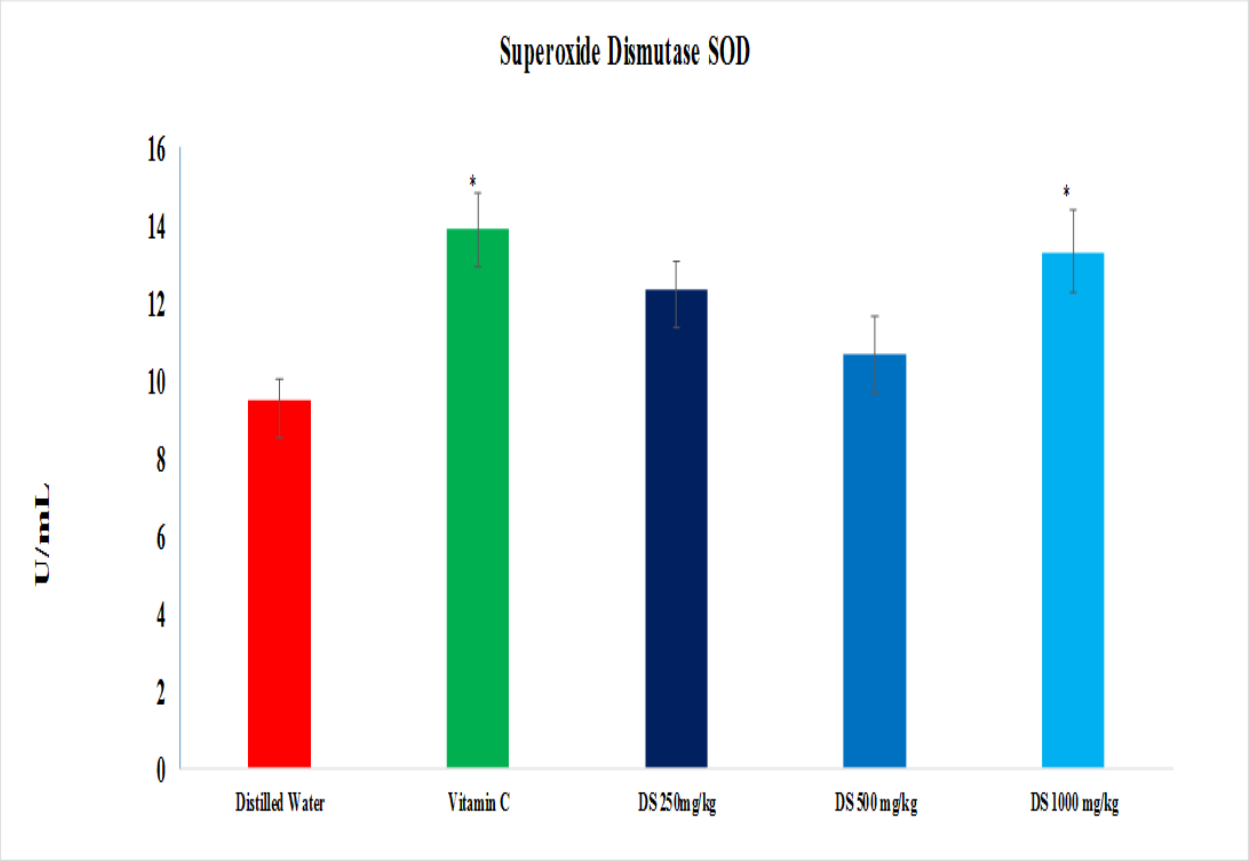

Figure 2: Effect of the extract on Superoxide Dismutase (SOD) Levels
Values are mean ± SEM, * represent *P< 0.05* level of significance. One-way ANOVA followed by Tukey post hoc test, n= 6.

Effect of the extract on Reduced Glutathione (GSH) Levels

Significant increase in GSH was observed when vitamin C group and the doses of the extract tested were compared with distilled water group.

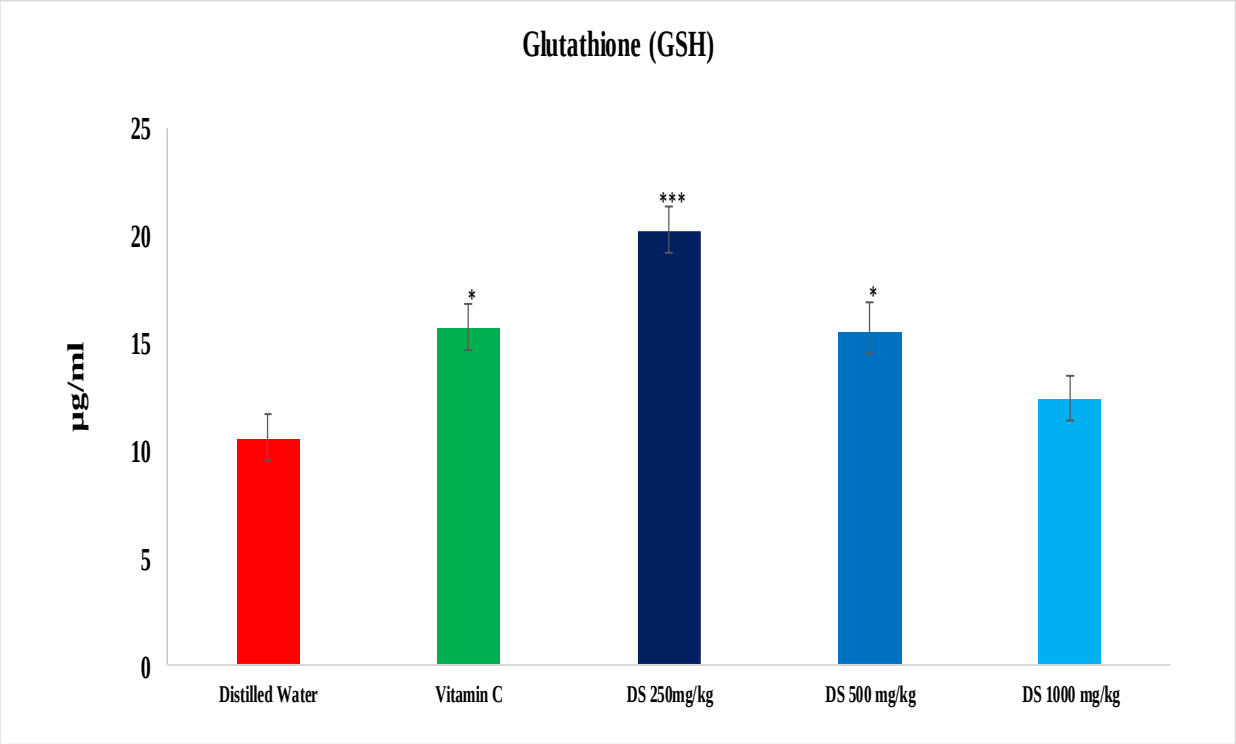

Figure 3: Effect of the extract on Reduced Glutathione (GSH) Levels
Values are mean ± SEM, *, ***represent *P< 0.05* and *P< 0.001* level of significance. One-way ANOVA followed by Tukey post hoc test, n= 6.

Effect of the extract on Catalase (CAT) Levels.
Increase in CAT was observed in the vitamin c and extract tested groups when compared with the distilled water group, though the difference is not of statistical significance.

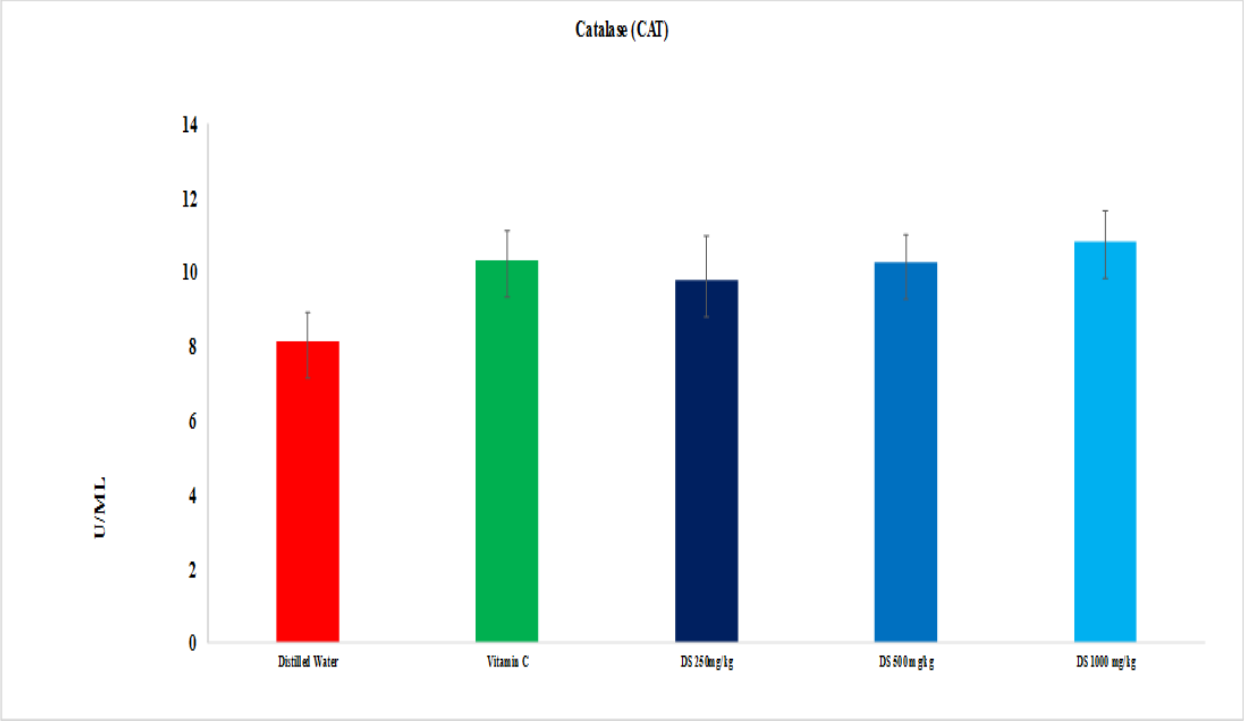

Figure 4: Effect of the extract on Catalase Levels.
Values are mean ± SEM, n= 6.

Discussion

The percentage yield of the extract of was 30.6%w/w. Phytochemical screening of the methanol leaf extract of *Dalbergia saxatilis* revealed the presence of alkaloids, flavonoids, cardiac glycosides, tannins and saponins, while anthraquinones were found absent as reported by (Hassan *et al*., 2015). Phytoconstituents are responsible for the pharmacological activities of plants, for example flavonoids and phenolics are known to improve human health by controlling various cellular pathways and help to provide antioxidant effects (Reza *et al*., 2015). From our studies the methanol leaf extract of *D. saxatilis* possesses antioxidant activity.

Cotton pellet-induced granuloma is a reliable *in vivo* model for studying inflammation and proliferation due to inflammation (Saeed *et al*., 2012). Inflammatory reactions in cotton pellet-induced granuloma model involve the proliferation of macrophages, fibroblasts and granulocyte inflammation (Ma *et al*., 2012). The fluid absorbed by the cotton pellet greatly influences the wet weight of the granuloma, whereas the dry weight correlates with the weight of the granulomatous tissue (Chouhan *et al*., 2011). The findings of this study demonstrated that treatment with the extract inhibited the transudation and proliferation of inflammation to some extent, similar to ascorbic acid therapy. Anti-inflammatory and antioxidant capacity of ascorbic acid (vitamin c) can be attributed to its ability to modulate the DNA binding activity of nuclear factor-kappa B (Carcamo *et al*., 2002). The activation is primarily promoted by oxidative stress and leads to cytokine-induced expression of cell adhesion molecules in the vascular endothelium, and to the TNF-α- and IL-6-induced production of CRP by the liver (Wu *et al*., 2012). Vitamin C can also reduce the plasma levels of inflammatory mediator's TNF-α- and IL-6 via down regulation of hepatic mRNA expression (Jang *et al*., 2014).

The level of Malonaldehyde (MDA) and activities of Superoxide dismutases (SOD), Glutathione (GSH) and Catalase (CAT) were assayed in the rat's plasma to further evaluate the antioxidant activity of the extract. MDA is a known marker of oxidative stress, and it is also one of the final products of polyunsaturated fatty acids metabolism (Stefan *et al*., 2004). An increase in free radicals causes overproduction of MDA. The significant decrease in MDA concentrations in the extract-treated rats with cotton pellet-induced granuloma suggested that the extract has caused a decrease in free radicals and consequently low concentration of MDA.

In addition, SOD constitute a very important antioxidant defense against oxidative stress in the body (Younus, 2018). It acts as a good therapeutic agent against reactive oxygen species-mediated diseases. CAT is one of the crucial antioxidant enzymes that mitigate oxidative stress to a considerable extent by destroying cellular hydrogen peroxide to produce water (Ankita *et al*., 2019). GSH is an antioxidant which has the ability to minimize oxidative stress and the downstream negative effects thought to be associated with oxidative stress (Chad and Darryn, 2005). It is largely known to minimize lipid peroxidation of cellular membranes and other such targets that is known to occur with oxidative stress. Activities of SOD, CAT and GSH increased in the ascorbic acid and extract-treated groups suggesting protection against oxidative stress, whereas these levels decreased in the negative control groups suggesting susceptibility to oxidative stress. These data demonstrate the antioxidant activity of the extract.

The detailed scientific evaluation of the pharmacological properties of methanol leaf extract of the plant (*Dalbergia saxatilis*) in terms of its antioxidant action clearly brought forth its significant therapeutic potential as an antioxidant which was found to be comparable to that of the standard drug.

Conclusion

The results from this study showed that, the methanol leaf extract of *Dalbergia saxatilis* possess antioxidant properties which may be via its action on MDA, SOD, CAT, and GSH concentrations. Further studies should be carried out using lower doses of the plant extract and the effect of the extract on other organs should be ascertained. Also, the extract should be further investigated in order to identify the active ingredients and elucidate its mechanism of action.

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Conflict of Interest

Authors declare no conflict of interest.

References

Ankita L, Yan L. J, Jana C. K, and Das N. (2019). Role of catalase in oxidative stress- and age-associated degenerative diseases. *Oxidative Medicine Cellular Longevity*: 9613090.

Arika Y, Bilbis L. S, Faruk U. Z, and Abbas A. Y. (2019). Toxicity studies of the crude aqueous root extract of albizzia chevalieri harms in albino rats. *Nigerian Journal of Basic and Applied Sciences, 18*(2).

Carcamo J. M, Perez-Cruz I, and Golde D. W. (2002). Vitamin C inhibits FAS-induced apoptosis in monocytes and U937 cells. *Blood*, 102(1), 336-343.

Chad C, and Darryn D. (2005). The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. *Journal of the International Society of Sports Nutrition*, 2(2):1-7.

Chouhan M. M, Islam M. R, Hossain M. Z, Islam M, Saha P. C, and Khan M. A. H. N. A. (2011). Detection of mycobacterial

infection from the lymphnode and lungs of suspected cattle using traditional and molecular technique (Doctoral dissertation, Bangladesh Agricultural University Mymensingh).

Clark J.D., Gebhart G. F., Gonder J. C., Keeling M. E., and Kohn D. F. (1997). The 1996 guide for the care and use of Laboratory Animals. ILAR Journals; 38 (1): 41 – 48.

Ellman G. L. (1959) "Tissue sulfhydryl groups," *Archives of Biochemistry and Biophysics*: 82(1): 70–77.

Halliwel H, Roohbakhsh A, Soltani F, Rezaee R, and Iranshahi M. (2015). Antioxidant and anti-inflammatory properties of the citrus flavonoids' hesperidin and hesperetin: an updated review of their molecular mechanisms and experimental models. *Phytotherapy Research*, *29*(3): 323-331.

Hassan F. I, Umar Z. A, Habib D. U, and Hamza Y. A. (2016). Effect of methanol leaf extract of Dalbergia saxatilis Hook. f (fabaceae) on renal function. *Avicenna Journal of Phytomedicine*, *6*(6): 592.

Hassan F. I, Zezi A. U, Yaro A. H, and Danmalam U. H. (2015). Analgesic, anti-inflammatory and antipyretic activities of the methanol leaf extract of *Dalbergia saxatilis* Hook. F in rats and mice. *Journal of ethnopharmacology*, *166*: 74-78.

Jang I. S, Ko Y. H, Moon Y. S, and Sohn S. H. (2014). Effects of vitamin C or E on the pro-inflammatory cytokines, heat shock protein 70 and antioxidant status in broiler chicks under summer conditions. *Asian-Australasian Journal of Animal Sciences*, 27(5): 749.

Koma O. S, and Sani I. M. (2014). Betulinic acid from antimicrobial root wood extract of *Dalbergia saxatilis* Hook f. (Fabaceae). *European Journal of Medicinal Plants*, 686-694.

Koroliuk M. A, Ivanova L. I, Maiorova I. G, Tokarev V. E. (1988) "A method of determining catalase activity," *Laboratornoe Delo*, 1:16–19.

Lobo V, Patil A,Phatak A, and Chandra N. (2010). Free radicals, antioxidants and functional foods: impact on human health. *Pharmacognosy Reviews*, 4 (8): 118 – 126.

Ma J, Guo C, Pan Y, Lin D, Qiu L, and Wen L. (2012). Antioxidant and anti-inflammatory activities of ethyl acetate extract of Gynura formosana (Kitam) leaves. *Experimental and Therapeutic Medicine*, 14(3), 2303-2309.

Peter R, Li S, Rodriguez-Rocha H, Burns M, and Panayiotidis M. I. (2010). Molecular mechanisms of pesticide-induced neurotoxicity: Relevance to Parkinson's disease. *Chemico-biological interactions*, *188*(2), 289-300.

Placer Z. A, Cushman L. L, and Johnson B. C. (1966) "Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems," *Analytical Biochemistry*, 16 (2): 359–364.

Reza G. R, Afzan A, Karimi E, Sinniah U. R, and Kumara Swamy M. (2015). Phytoconstituents and antioxidant properties among commercial tea (Camellia sinensis L.) clones of Iran. *Electronic Journal of Biotechnology*, 18(6): 433-438.

Saeed R, Biplab D, Devanna N, and Sen S. (2012). Antiinflammatory, antinociceptive and antioxidant activities of Phyllanthus acidus L. extracts. *Asian Pacific Journal of Tropical Biomedicine*, 2(2): S953-S961.

Saha S, Shilpi J. A, Mondal H, Hossain F, Anisuzzman M, Hasan M. M, and Cordell, G. A. (2013). Ethnomedicinal, phytochemical, and pharmacological profile of the genus Dalbergia L. (Fabaceae). *Phytopharmacology*, *4*(2): 291-346.

Shankar R. A, Leamy A. K, Noguchi Y, Shiota M, and Young J. D. (2014).

Palmitate-induced activation of mitochondrial metabolism promotes oxidative stress and apoptosis in H4IIEC3 rat hepatocytes. *Metabolism*, *63*(2): 283-295.

Shi B, Singh S, and Siddiqi N. J. (2019). Biomedical implications of heavy metals induced imbalances in redox systems. *BioMed research international*.

Stefan M, and George S. (2004). An in vitro procedure for evaluation of early stage oxidative stress in an established fish cell line applied to investigation of PHAH and pesticide toxicity. *Marine Environmental Research*, 58(2-5): 631-635.

Ukwuani-Kwaja A. N, Nwaogu J, and Salako M. B. (2021). Contraceptive Effect of Methanol Leaves Extract of Dalbergia saxatilis on Female Albino Rats. *Journal of Infertility and Reproductive Biology*, *9*(1): 35-42.

Valko M, Leibfritz D, Moncol J, Cronin M. T, Mazur M, and Telser J. (2013). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, *39*(1): 44-84.

Winter C. A, and Porter C. C. (1957). Effect of alterations in the side chain upon antiinflammatory and liver glycogen activities of hydrocortisone esters. *Journal of American Pharmaceutical Association of Science Education*, 46: 515-519.

Wolf E. B. (2015). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition journal*, *15*(1): 1-22.

Wu X, Hammarstedt A, Andersson C. X, and Smith U. (2012). Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 27(11), 2276-2283.

Yemitan O. K, and Adeyemi O. O. (2013). Antiepileptogenic and anticonvulsant actions of Dalbergia saxatilis (Hook, F.) in sub-toxic chemical kindling and toxic convulsant models. *European Journal of Medicinal Plants*, 288-296.

Younus H. (2018). Therapeutic potentials of superoxide dismutase. *International Journal of Health Sciences*, *12*(3): 88.