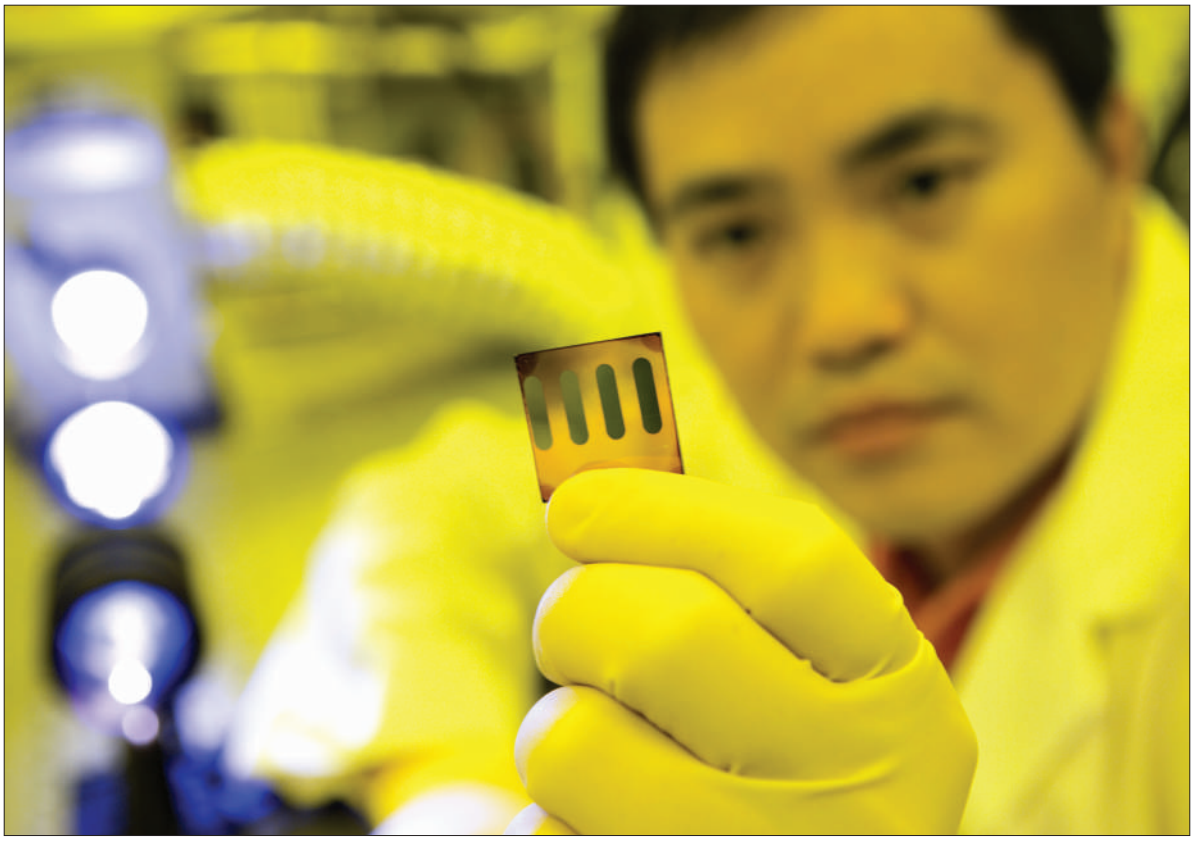

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Research Article
In vitro and *in vivo* Antioxidant Activity of Crude Methanolic Leaves Extract of *Acacia nilotica* (Linn.)

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Abstract

Background and Objective: Many synthetic antioxidants were reported to have several side effects, such as the risk of liver damage and carcinogenesis in laboratory animals. This has led to the search for more effective, less toxic and cost-effective natural antioxidants, particularly from plants. This research aimed to evaluate the *in vitro* and *in vivo* antioxidant potentials of crude methanolic leaves extract of *Acacia nilotica*. **Materials and Methods:** The extract was evaluated for its antioxidant compounds, *in vitro* and *in vivo* antioxidant activities. The levels of flavonoids, total phenolic compounds, ascorbic acid and α-tocopherol were determined. **Results:** The DDPH radical scavenging activity and the ferric reducing the power of the extract were found to be high and comparable to that of standard ascorbic acid. The result of the *in vivo* antioxidant activity showed that CCl_4 treatment caused a significant ($p < 0.05$) decrease in the plasma GSH level compared to that of the normal group treatment. The 150, 300 and 600 mg kg^{-1} b.wt. of extracts pre-treatments for 7 days significantly ($p<0.05$) enhanced the level of GSH when compared to the toxic (CCl_4) control groups, but lower when compared to positive (ascorbic acid) and normal control groups. Also, elevated MDA levels were observed for the CCl_4 treated group (toxic control) than for other control samples, with the ascorbic acid pre-treatment showing the least MDA levels. Test samples (150-600 mg kg^{-1} b.wt.) showed a significantly ($p<0.05$) lower MDA levels in the liver tissues compared to the CCl_4 control group. **Conclusion:** The results obtained in the present study indicate that the leaves of *A. nilotica* are a potential source of natural antioxidants, which may provide leads in the ongoing search for natural antioxidants from plants.

Key words: *Acacia nilotica,* DPPH, GSH, ascorbic acid, antioxidants, *in vitro* and *in vivo,* potential source

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Free radicals are generated when living cells make use of oxygen for various normal physiological processes. The by-products of such reactions are generally Reactive Oxygen Species (ROS) such as superoxide anion (O_2^-), hypochlorous acid (HClO), hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) that result from the cellular redox process. At relatively low concentrations, ROS exert beneficial effects on cellular functions and immune response and are crucial for life but at elevated levels, ROS leads to oxidative stress capable of damaging cell structures and functions, including macromolecules such as lipids, proteins, sugars and DNA[1]. Oxidative stress plays a crucial role in the development of chronic and degenerative ailments such as cancer, skin diseases, autoimmune disorders, rheumatoid arthritis, osteoarthritis, cataract, aging, coronary heart disease and various neurodegenerative diseases[2-5]. Antioxidants, which are either naturally produced within the cell, or externally supplied neutralize oxidative stress in biological systems. These antioxidants capable of scavenging the free radicals act by preventing and repairing damages caused by ROS and therefore can enhance the immune defense, cellular response and lower the risk of cancer and other degenerative diseases[1].

Although there are many synthetic antioxidants in use. It was however reported that they have several side effects, such as risk of liver damage and carcinogenesis in laboratory animals[6]. Medicinal plants are an important source of antioxidants[7]. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of diseases such as cancer, heart diseases and stroke[8]. Secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and stem bark[9].

Acacia nilotica (L.) commonly called Babul, Gum Arabic tree, Egyptian thorn, or Prickly Acacia is a multipurpose tree with nitrogen-fixing ability. It grows from sea level to over 200 m and can withstand high temperatures (above 50°C) and dry humidity[10]. The tree is commonly found in tropical and subtropical regions from Egypt to Mauritania southwards to South Africa and in Asia eastwards to Pakistan and India[11]. *Acacia nilotica* is a single-stemmed plant, grows to 15-18 m in height and 2-3 m in diameter. The *A. nilotica* is one of the most important trees and almost all its parts are used traditionally in medicine, including leaves, bark, root, flower, pods, gum, etc.[12,13].

There is therefore a need for more effective, less toxic and cost-effective antioxidants. Medicinal plants appear to have these desired comparative advantages, hence the growing interest in natural antioxidants from plants. This research aimed to evaluate the *in vitro* and *in vivo* antioxidant potentials of crude methanolic extract of *Acacia nilotica*.

MATERIALS AND METHODS

Location and duration of the research: The research was carried out at Pharmacognosy and Pharmacology Laboratories of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The research was conducted in 14 months, from July 2016 to November 2017.

Plant collection and authentication: Fresh disease-free leaves of the plant used were separately collected from Bodinga, Sokoto State, Nigeria and was identified and authenticated by a Botanist at the Biological Sciences Department, Usmanu Danfodiyo University, Sokoto, Nigeria. The plant was identified as *Acacia nilotica* (Linn.) with voucher number UDUH/ANS/0247.The samples were shed-dried, ground and kept in air-tight containers until further use.

Preparation of plant extract: The methanolic crude extract was prepared by soaking a sample (100g) of the powdered plant part material in 90% methanol (600 mL) for 72 hrs. The extracts were filtered using clean cloth and Whatman No. 1 filter paper. The filtrate was concentrated in a vacuum at 30°C and stored in sterile sample containers at 4°C until further use.

Estimation of antioxidant compounds

Determination of total phenolics: The Total Phenolic Content (TPC) of the crude methanolic extract of *Acacia nilotica* was analyzed using the method of Folin-Ciocalteu reaction with gallic acid as standard[8]. 100 µL of the extract was added to 500 µL of (50%) Folin-Ciocalteu reagent followed by the addition of 1 mL of 20% Na_2CO_3 solution. The mixture was incubated at room temperature for 20 min. and the absorbance was measured at 730 nm. The total phenolic content is to be expressed as Gallic Acid Equivalents (GAE) in milligrams per gram samples.

Determination of vitamin C: The Vitamin C content of the crude methanolic extract of *Acacia nilotica* was determined using Rutkowski's method[14]. About 1 mL of the extract was added to 1 mL of Phosphotungstate Reagent (PR) in a centrifugal tube and left at room temperature for 30 min. The mixture was centrifuged at 7000 g for 10 min. The absorbance (Ax) of the test sample (supernatant) was measured at 700 nm. The standard sample was prepared as

above (using 1 mL of the standard solution instead of the extract) and the absorbance (As) taken at 700 nm.

Vitamin C concentration was calculated using the formula:

$$Cx = \frac{Ax}{As} \times Cs$$

where, Cs is concentration of the standard solution

Determination of vitamin E: The Vitamin E content of the crude methanolic extract of *Acacia nilotica* was determined using the previous method[15]. To 0.5 mL of the extract, 0.5 mL anhydrous ethanol was added, plugged and shaken. Three milliliter of xylene was then added and shaken. The mixture was then centrifuged at 15000 g for 10 min. About 1.5 mL of the supernatant was then pipetted into another test-tube containing 0.25 mL solution of bathophenanthroline. This was followed by the addition of 0.25 mL $FeCl_3$ solution. The absorbance of the test sample (Ax) was then taken at 539 nm against the blank. The standard sample was also prepared as above using α-tocopherol instead of extract. The absorbance of the standard (As) was also taken at 539 nm. The concentration of vitamin E in the extract (Cx) was calculated as follows:

$$Cx = \frac{Ax}{As} \times Cs$$

where, Cs is concentration of the standard solution

Determination of flavonoid content: The flavonoid content of the crude methanolic extract of *Acacia nilotica* was determined using precipitation method[16]. About 0.5 g of the extract was boiled in 100 mL of the hydrochloric acid solution for about 35 min. The hydrolysate was filtered to recover the filtrate. The filtrate was treated with ethylacetate dropwise twice until in excess. The precipitated flavonoid was recovered by filtration using a weighed filter paper after drying in the oven at 100°C for 30 min, it was cooled in a desiccator and reweighed. The difference in weight gave the weight of the flavonoid which was expressed as a percentage of the weight of the sample analyzed:

$$\text{Flavonoid } (\%) = \frac{W_2 - W_1}{W_3} \times 100$$

where, W_1 is weight of empty filter paper -1.32 g, W_2 is weight of filter paper+flavonoid, W_3 is weight of sample = 0.5 g.

***In vitro* antioxidant potential**

Ferric Reducing Antioxidant Power (FRAP) assay: The ferric reducing antioxidant power of the crude methanolic extract of *Acacia nilotica* was determined using potassium ferrocyanide-ferric chloride method[17]. Two millimeters of the extract was added to 2.5 mL of potassium ferrocyanide, then, incubated at 50°C for 20 min. About 2.5 mL of trichloroacetic acid was added to the mixture which was then centrifuged at 650x g for 10 min. To the supernatant (2.5 mL), distilled water (2.5 mL) and 0.5 mL ferric chloride was added. The absorbance was then read at 700 nm. Higher absorbance indicates greater reducing capacity which is calculated as follows:

$$\text{Reducing power} = \frac{AM}{AC} \times 100$$

where, AM is absorbance of reaction mixture, AC is absorbance of control mixture (Distilled water)

DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay: The free radical scavenging activity of the extract was analyzed by the DPPH assay following a standard spectroscopic method[18]. The extract (2 mL) at varying concentrations ranging of 0.2, 0.4, 0.6, 0.8 and 1.0 mg mL^{-1} each was mixed with 1 mL of 0.5 mM DPPH (in methanol) in a cuvette. The absorbance at 517 mm was taken after 30 min of incubation in the dark at room temperature. The percentage of antioxidant activity was calculated as follows:

$$\text{Antioxidant activity } (AA)\ (\%) = \frac{Abs_{sample} - Abs_{blank}}{Abs_{control}} \times 100$$

One milliliter of methanol plus 2 mL of the extract was used as a blank, while, 1 mL of the 0.5 mM DPPH solution plus 2 mL of methanol was used as a negative control. Ascorbic acid (vitamin C) was used as reference standards.

***In vivo* antioxidant potentials**

Experimental animals: Female albino rats, weighing 160-200 g, were used in this toxicity study. They were obtained from the Animal Research Centre (ARC) of the Ahmadu Bello University (ABU), Zaria, Nigeria. The rats were kept in the Animal House of the Department of Pharmacology, Faculty of Pharmacy, Usmanu Danfodiyo University, Sokoto, Nigeria, where they were acclimatized to standard laboratory conditions for 7 days. The rats were maintained on 12 hrs light:dark cycle, with standard pellet diet and water *ad libitum.* Approval was obtained from the Animal Ethics Committee,

Usmanu Danfodiyo University, Sokoto, Nigeria. The institutional animal ethical guidelines were strictly observed.
Experimental Design

Twenty four rats were used in this study. The rats were divided into 6 groups of 4 rats each for the *in vivo* antioxidant study as follows:

Group 1: Received distilled water (10 mL kg^{-1} b.wt.) and served as the normal control
Group 2: Received CCl_4 (2.5 mL kg^{-1} b.wt.) and served as the negative control
Group 3: Received vitamin C (25 mg kg^{-1} b.wt.) and served as the positive control
Group 4: Received 150 mg kg^{-1} b.wt. of crude methanolic extract of *Acacia nilotica*
Group 5: Received 300 mg kg^{-1} b.wt. of crude methanolic extract of *Acacia nilotica*
Group 6: Received 600 mg kg^{-1} b.wt. of crude methanolic extract of *Acacia nilotica*

After the experimental period, the animals were sacrificed by cervical decapitation after anesthetizing with chloroform. The liver from each rat was collected, weighed and tissue homogenate was prepared by weighing 1 g of tissue, minced with fine sterile laboratory standard homogenized with 10 mL of 10 mM ice-cold phosphate buffer (pH 7.4). The resultant mixture was centrifuged at 8000 rpm for 10 min.

Biochemical analyses: After homogenates were cold centrifuged as mentioned above, the supernatants (10%) were used for the various biochemical analyses.

Determination of lipid peroxidation (MDA): Malondialdehyde (MDA), as an index of lipid peroxidation, was determined by the thiobarbituric acid reactive substances (TBARS) assay method[19]. About 0.1 mL of liver homogenate (10% w/v) was treated with 2 mL of (1:1:1 ratio) TBA-TCA-HCl reagent (thiobarbituric acid 0.7%, 15% trichloroacetic acid and 0.25 N HCl). All the tubes were placed in a boiling water bath for 30 min and cooled. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 532nm against reference blank. The concentration of MDA was calculated using the equation:

$$MDA = \frac{A}{E} \times L$$

where, A is the absorbance of the sample, E is the extinction coefficient (1.56×105 M^{-1} cm^{-1}) and L is the length of the light path.

Estimation of Reduced Glutathione (GSH): The principle[20] is based on the fact that glutathione reacts with an excess of alloxan to produce a substance with an absorption peak at 305 nm.

Procedure:

- 1.0 mL oxalate sample and 7.0 mL water were mixed with 2.0 mL of 25% metaphosphoric acid and the mixture centrifuged at 3000 rpm for 10 min
- The test tubes were set up as follows:
- Reagent blank, 2 tubes -b and bo
  - b = 1.0 mL of 5% metaphosphoric acid+1.0 mL 0.1 M alloxan
  - b_o = 1.0 mL of 5% metaphosphoric acid+1.0 mL H_2O

Standard, 2 tubes-S and So

- S = 1.0 mL of 50 mg dL^{-1} glutathione standard+1.0 mL alloxan
- S_o = 1.0 mL standard+1.0 mL H_2O

Sample, 2 tubes-X and Xo

- X = 1.0 mL filtrate+1.0 mL alloxan
- X_o = 1.0 mL filtrate+1.0 mL H_2O

- All the tubes were arranged in order and 1.0 mL of 0.5 M phosphate buffer was added to all the tubes followed immediately by 1 N NaOH solution and mixed.
- Six min later, 1.0 mL of 1 N NaOH was again added across each tube to stop the reaction and stabilizes the product
- At 305 nm, b was read against bo, S against So and X against Xo

The concentration (mg dL^{-1}) of glutathione was calculated from:

$$GSH = \frac{Ax - Ab}{As - Ab} \times 50\left(mg\,dL^{-1}\right)$$

RESULTS

Levels of non-enzymatic antioxidant compounds: The levels of non-enzymatic antioxidants analyzed in the leaf methanolic extract of *Acacia nilotica* are presented in Table 1. Ascorbic acid was found to be 76.56 ± 1.24 μM L^{-1}, α-tocopherol was

found to be 39.73±0.5 mg dL^{-1}, flavonoid content was found to be 12.00±4.00% and total phenolic content was found to be 2.95±0.01 μM L^{-1}.

In vitro antioxidant activity

DPPH radical scavenging activity: The result of the DPPH radical scavenging activity of the extract is shown in Table 2. It shows that the extract exhibited high antioxidant activity in comparison to ascorbic acid standard. The highest inhibition observed for the extract was 89%, while that of the standard ascorbic acid was 95.2%. The IC_{50} of the extract was found to be 0.45 mg mL^{-1} as compared to that of standard ascorbic acid of 0.37 mg mL^{-1}.

Ferric reducing power: The result of the ferric reducing power of the extract and standard ascorbic acid is presented in Table 3. It shows that the extract exhibited high antioxidant activity in comparison to ascorbic acid standard. The highest Ferric reducing the power of the extract and standard ascorbic acid was 75.6 and 92.6%, respectively.

***In vivo* antioxidant activity:** The *in vivo* antioxidant activity of the extract is shown in Table 4. Effects of the plant extract on GSH levels for all experimental groups as shown in Table 4 indicates that CCl_4 treatment caused a decrease of GSH level in blood plasma when compared to that of the normal group treatment. The 150, 300 and 600 mg kg^{-1} b.wt. of extracts pre-treatments for 7 days significantly ($p<0.05$) enhanced the levels of GSH when compared to the toxic (CCl_4) control group, but lower when compared to positive (ascorbic acid) and normal control groups.

In addition, the MDA levels of the liver samples of all experimental groups are shown in Table 4. Elevated MDA levels were observed for the CCl_4 treated group (toxic control) than for other control samples, with the ascorbic acid pre-treatment showing the least MDA levels. Test samples (150-600 mg kg^{-1} b.wt.) showed a significantly ($p<0.05$) lower MDA levels in the liver tissues compared to the CCl_4 control group.

Table 1: Levels of non-enzymatic antioxidant compounds of the extract

Parameters	Amount
Flavonoids (%)	12.00±4.00
Total Phenolic Compounds (μM L^{-1})	2.95±0.01
Ascorbic acid (μM L^{-1})	76.56±1.24
α-tocopherol (mg dL^{-1})	39.73±0.39

Data presented as Mean±SD (n = 3)

Table 2: DPPH radical scavenging activity of the crude extract and standard ascorbic acid

Concentration (mg mL^{-1})	Absorbance		Inhibition (%)		IC_{50} (mg mL^{-1})	
	Extract	Ascorbic acid	Extract	Ascorbic acid	Extract	Ascorbic acid
0.2	0.20±0.01	0.23±0.01	27.3	31.9	0.45	0.37
0.4	0.33±0.01	0.37±0.01	47.4	53.6		
0.6	0.42±0.01	0.47±0.02	61.3	69.0		
0.8	0.52±0.01	0.55±0.02	76.7	81.3		
1.0	0.60±0.02	0.64±0.01	89.0	95.2		

Data presented as Mean±SD (n = 3)

Table 3: Reducing power of extract and standard ascorbic acid

Concentration (mg mL^{-1})	Absorbance		Inhibition (%)	
	Extract	Ascorbic acid	Extract	Ascorbic acid
0.2	0.15±0.02	0.23±0.01	23.2	35.5
0.4	0.19±0.01	0.32±0.01	29.3	49.4
0.6	0.21±0.01	0.37±0.02	32.4	57.1
0.8	0.34±0.01	0.44±0.01	52.5	67.9
1.0	0.49±0.02	0.60±0.01	75.6	92.6

Data presented as Mean±SD (n = 3)

Table 4: *In vivo* antioxidant activity of the extract on experimental animals

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
GSH (mg dL^{-1})	155±6.16*	42.1±5.06	155±11.9*	122±9.07*	132±3.86*	137±3.69*
MDA (nm L^{-1})	7.55±0.23*	28.0±0.62	6.46±0.50*	12.7±0.66*	12.6±0.78*	7.07±0.16*

Data presented as Mean±SD (n = 4),*Significantly different from Toxic CCl_4 (Group 2) control group (p 0.05), GSH: Reduced glutathione, MDA: Malondialdehyde, Group 1: Normal control, Group 2: Toxic (CCl_4) control, Group 3: Positive (ascorbic acid) control, group 4: 150 mg kg^{-1} b.wt., group 5: 300 mg kg^{-1} b.wt. and group 6: 600 mg kg^{-1} b.wt. of crude methanolic leaves extract of *Acacia nilotica*

DISCUSSION

The levels of non-enzymatic antioxidants analyzed in the leaf methanolic extract of *Acacia nilotica* are presented in Table 1. The extract was found to be a good source of nonenzymatic antioxidants. Ascorbic acid was found to be 76.56±1.24 μM L^{-1}. Ascorbic acid is a water-soluble vitamin and is widely required in the metabolic processes of living organisms. Ascorbic acid is used medicinally and also used industrially in the conservation of food products[21].

The level of α-tocopherol in the extract was found to be 39.73±0.5 mg dL^{-1}. Vitamin E, a major lipid-soluble antioxidant belonging to tocopherols, is the most effective chain-breaking antioxidant within cell membranes. It can repair oxidizing radicals directly by preventing the chain propagation step during lipid peroxidation[22].

Flavonoids are phenolic substances which are the largest group of phenols. They are plant constituents with antibacterial and antifungal properties[23]. The flavonoid content of the extract was found to be 12.00±4.00%.

Phenols are the most widespread secondary metabolites in the plant kingdom. These diverse groups of compounds have received much attention as a potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and metal chelators. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donating ability and singlet oxygen quenching[23]. In the present study, the total phenolic content of the extract was found to be 2.95±0.0 1 μM L^{-1}.

Phenols are very important plant constituents with multiple biological functions including antioxidant activity owing to their radical scavenging ability primarily due to the presence of OH groups. Previous studies showed a very strong correlation between phenol and antioxidant activity[24-26].

DPPH (1,1- diphenyl-2- picrylhydrazyl) Free Radical Scavenging Assay is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts[27]. Scavenging activity for free radicals of DPPH has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources. The result of the present study showed that the methanolic extract of *Acacia nilotica* possessed a certain amount of phenolic compounds and exhibited high antioxidant activity. The high scavenging activity of the methanolic extract *in vitro* may be due to hydroxyl groups existing in the phenolic compounds, a chemical structure that can provide the necessary component as a radical scavenger[26]. The results obtained above are in consonance with other researches which have also shown a strong correlation between phenolic contents and antioxidant activity[28].

Ferric Reducing Antioxidant Power (FRAP) Assay was used to evaluate the *in vitro* reducing the power of the methanolic extract of *Acacia nilotica.* Antioxidants have the ability to scavenge free radicals *in vitro* by reducing Fe^{3+} to Fe^{2+} through electron transfer ability[29]. This serves as an indicator of antioxidant activity. The findings showed that the extract possesses *in vitro* ferric reducing activity though not as high as that of ascorbic acid. FRAP assays are widely used to determine the efficiency of antioxidant compounds in plants to compete with the FRAP reagent and reduce iron from ferric to the ferrous state. Antioxidant compounds that are able to function in this approach are categorized as secondary antioxidants where they suppress the radical formation and prevent oxidative damage[30].

The primary defense 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[31,32]. MDA is the major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of lipid peroxidation and ultimately tissue damage by a series of chain reactions.

CCl_4 administration resulted in a marked elevation in MDA (lipid peroxidation) levels in the liver. The increased MDA content might have resulted from an increase of Reactive Oxygen Species (ROS) as a result of stress due to CCl_4 intoxication. Increased MDA content is an important indicator of lipid peroxidation[33]. Seven days oral feeding of 150, 300 and 600 mg kg^{-1} b.wt. doses of the extract of *Acacia nilotica* resulted in a significant decrease ($p<0.05$) in MDA levels in liver over CCl_4 control (Table 4). This is comparable to the significant decrease ($p<0.05$) in MDA levels in liver of the ascorbic acid control group over CCl_4 control.

Cellular GSH levels are maintained by glutathione reductase and NADH activities. 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 machinery of the liver are upheld[34]. In the present study, CCl_4 administration markedly decreased the levels of GSH in the liver demonstrating oxidative stress.

Previous studies on the mechanism of CCl_4 hepatotoxicity have shown that GSH plays a key role in detoxifying the reactive toxic metabolites of CCl_4. Liver necrosis begins when the GSH levels are markedly depleted[35]. GSH plays a crucial

protective role as a scavenger of free radicals that combine with non-protein thiols at the GSH reactive center to abolish free radical toxicity[34,35]. In this study, GSH levels in liver were significantly increased following 7 days oral administration of 150, 300 and 600 mg kg^{-1} b.wt. doses of the extract over CCl_4 control indicating its hepatoprotective activity (Table 4). This was also observed in the ascorbic acid control group.

CONCLUSION

The result of the present study signifies the potential of *A. nilotica* leaves as a source of therapeutic agents, which may provide leads in the ongoing search for natural antioxidants from plants. Further, the antioxidant activity observed in this study might be attributed to the presence of phenolic and flavonoid compounds present in the extract.

SIGNIFICANCE STATEMENT

This study discovered the levels of antioxidant compounds, *in vitro* and *in vivo* antioxidant activities of the methanolic root extract of *Acacia nilotica* that can be used as a source antioxidant compounds in the management of chronic and degenerative diseases. The study will help researchers to uncover the critical areas of natural product research in determining the antioxidant activities of plants and other natural products as an important step towards the development of alternative therapeutic agents that many researchers were not able to explore. Thus, a new theory on the search for better, safe and effective therapeutic agents with antioxidant effects may be arrived at.

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