

ANTIOXIDANT AND HEPATOPROTECTIVE POTENTIALS OF *LASIMORPHA SENEGALENSIS* SCHOTT LEAF EXTRACT ON CARBON TETRACHLORIDE—TETRACHLORIDE-INDUCED LIVER DAMAGE IN RATS

ABSTRACT

Aims: The present study assessed the antioxidant and hepatoprotective potentials of the methanolic leaf extract of *Lasimorpha senegalensis* – a medicinal plant used by the indigenous people of Nigeria to treat hepatitis and feverish conditions.

Place and Duration of Study: The research work was conducted in the Department of Pharmacognosy and Environmental Medicine and Department of Plant Science and Biotechnology, both in the University of Nigeria, Nsukka, from May to August, 2019.

Methodology: Phytochemicals analyses and acute toxicity study of the sample followed standard procedures. *In vitro* antioxidant assay was by DPPH and H₂O₂ models. A total of 25 male Wistar albino rats (120 – 150 g) were grouped into five, each group with five animals. Hepatotoxicity was induced with carbon tetrachloride (1 ml/kg). The treatment groups (3-5) received extract (200 and 400 mg/kg) and Silymarin (100 mg/kg). Endogenous antioxidants (superoxide dismutase, catalase, glutathione peroxidase), plasma malondialdehyde and liver enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphate) were determined after treatment.

Results: The results showed the leaf extract had appreciable amounts of bioactive phytochemicals and free radical scavenging activity (IC₅₀ of 0.52 mg/ml and 0.71 mg/ml for DPPH and H₂O₂ respectively) with no toxicity at 5000 mg/kg. The extract also elevated the endogenous antioxidants, and significantly ($p \leq .05$) reduced lipid peroxidase and liver enzymes.

Conclusion: This report justifies the local use of this plant in the management of various diseases related to oxidative stress and liver damage.

Keywords: *Lasimorpha senegalensis*, oxidative stress, liver enzyme, lipid peroxidation, CCl₄

1. INTRODUCTION

Oxidative stress is a condition caused by free radicals and highly reactive oxygen species (ROS) which build up in human bodies, if not properly taken care of by the endogenous systems, under normal physiological activities [1]. Free radicals and ROS are toxic to human cells, affect activation of enzymes and in the process damage the tissues. Several

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diseases and conditions are associated with oxidative stress; they include cancer, Parkinson's disease, Alzheimer's disease, ageing and neural and cardiovascular malfunction [1,2]. The liver being the organ where detoxification occurs is most susceptible to disease attack as result of the intake of hepatotoxic drugs, smoking, air pollution and pesticide poisoning. Apart from detoxification, the liver is regarded as the relevant organ in the body because it regulates several metabolic activities in the body – it secretes bile and also produces blood-clotting factors. Common liver diseases include jaundice, hepatitis and cirrhosis [1,3].

Antioxidants, also referred to as free radical scavengers, are substances that can inhibit or delay the damage of cells caused by free radicals and unstable molecules that the body produces as a reaction to environmental and other pressures. Their sources are either natural or artificial. Vitamins A, C and E, lycopene, manganese and beta-carotene are good examples [4]. They are compounds that delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reaction, therefore preventing damage done to the body's cell by reactive oxygen species [5].

Phytochemicals are non-nutritive, bioactive compounds found in plants [6]. Barbosa *et al.* [7] reported that approximately 10,000 phytochemicals have been currently identified which includes flavonoids, alkaloids, saponins, tannins, and terpenoids, etc. Farahmandfaret *al.* [8] referred to phenolic compounds as strong antioxidants due to their ability to scavenge free radicals, singlet oxygen and superoxide radicals. Their radical scavenging action is due to hydroxyl groups replacing the aromatic ring of the phenolic components [9,10].

Lasimorpha senegalensis Schott (swamp arum) belongs to the Arum family (Araceae). The plant is herbaceous, perennial, and has a vigorously stoloniferous short, thick rhizome which produces a clump of leaves, stolons or put forth suckers. The strong development of underground suckers enables *L. senegalensis* to form large populations which can be harvested from the wild for local use as food and medicine. It has no major widespread threats and is widely and abundantly distributed [11]. It has been reported for its enormous role in traditional medicine across West Africa. For instance, the leaves are used in Congo to facilitate child delivery, treat cough, nervousness and agitation [12], while the leaf sap and fruits are used in Côte d'Ivoire and southern Nigeria to treat hiccups, dysentery and gonorrhoea [13]. Among the Igbo tribe in Nigeria, decoction of the leaves is taken used in treating hepatitis and sexually transmitted diseases (Oral Communication). However, literature searches show no available information on the scientific validation of biological activities, apart from a recent publication on the antimicrobial activity of the methanolic leaf extracts [14], hence this present work was embarked on to assess the antioxidant and hepatoprotective potentials of the methanolic leaf extract.

2. MATERIAL AND METHODS

2.1 Collection of plant material

Fresh leaves of *Lasimorpha senegalensis* were collected from Adada River, Nkpologu Nsukka, in Uzo-uwani Local Government Area, Enugu State, Nigeria (6°51'24"N 7°23'45"E). It was properly authenticated and voucher specimen (PCG/UNN/0335) deposited at the Herbarium of the Department of Pharmacognosy and Environmental Medicines, University of Nigeria, Nsukka. Thereafter, they were washed with tap water, drained and allowed to dry under shade at room temperature ($25 \pm 2^\circ\text{C}$). The dried leaves were pulverized with mortar and pestle to a coarse powder.

Comment [CL1]: Indicate duration for drying

2.2 Extraction

A measured quantity (500 g) of the pulverized sample was extracted with 90% methanol (Sigma-Aldrich, analytical grade) using a Soxhlet apparatus for 8 hours. The liquid extract was concentrated using rotary evaporator (Buchi Rotavapor® R-215) under a reduced

pressure to obtain the extract. This was collected in a beaker and stored in the refrigerator until use.

2.3 Phytochemical analysis

The qualitative and quantitative phytochemical analyses were carried out following the standard procedures of AOAC [15]. The concentrations of each of the phytochemical groups was calculated after absorbance was read using a UV/Vis spectrophotometer (Schmadzu UV-1800). Reagents used for each of the tests and their respective wavelength of spectrophotometric reading are shown in Table 1 below.

Table 1. Methods for the determination of phytochemicals

Phytochemical	Reagents	Wavelength (nm)
Tannins	Conc. HCl; FeCl ₃ ; 0.008 (K ₃ Fe(CN) ₆);	720
Flavonoids	HCl; NaOH; FeCl ₃	233
Steroids	Ethylacetate; chloroform; 0.1 M NH ₄ OH;	240
Saponins	Dil. H ₂ O	620
Alkaloids	Phosphate buffer; 5 % trichloroacetic acid; Casein solution;	580
Hydrogen cyanide	Alkaline picrate solution; dil. water	540
Glycosides	15 % lead acetate; chloroform; glacial acetic acid; 5 % Ferric chloride; conc. H ₂ SO ₄	530
Phenols	Folin-Ciocalteu reagent; 20% trioxocarbonate (iv) solution;	560
Soluble carbohydrate	Picric acid; dil. water	540
Reducing sugars	Ethanol; iodine solution (5 g iodine + 1 g potassium iodide); dil. water	480

2.4 In Vitro Antioxidant Screening

2.4.1 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The DPPH scavenging assay was carried out following [method previously described by Mensore *et al.* \[16\]](#). Briefly, different concentrations of the extract and standard ascorbic acid was reacted with 0.5 mM DPPH (in methanol) (2:1 v/v) in a cuvette, incubated for a 30-minute period, and the absorbance of the resulting solution was read at 517 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800). The percentage antioxidant activity was calculated from ascorbic acid standard curve.

2.4.2 Hydrogen peroxide scavenging assay

The method of Bokhari *et al.* [17] was followed to investigate hydrogen peroxide scavenging capacity of the samples. Briefly, hydrogen peroxide (2 mM) solution prepared in phosphate

buffer (50mM, pH 7.4) was reacted with varying concentrations of the test samples and standard ascorbic acid. Their absorbance was read at 230 nm ~~with spectrophotometer (Shimadzu UV-1800).~~ Percent scavenging activity was determined from ascorbic acid standard curve.

2.5 Animal studies

Twenty five healthy white albino Wistar rats (weighing 120 – 150 g), fed with standard animal feed and clean tap water, were used in this study. They were allowed to acclimatize with the working laboratory for a period of 1 week. Ethical approval was obtained from the Research Ethics Committee, University of Nigeria. The animals were divided into 5 groups (A-E), each group with five randomized replicates and were ~~administered-treated~~ as follows: Group A (toxic group) received only CCl₄ (1 ml/kg)

Comment [CL2]: Indicate approval number

Groups B (normal control) received normal saline ~~+CCl₄ (1 ml/kg)~~

Group C received 200 mg/kg extract + CCl₄ (1 ml/kg)

Group D received 400 mg/kg extract + CCl₄ (1 ml/kg)

Comment [CL3]: Duration of extract treatment not properly described

Group E (positive control) received Silymarin (100 mg/kg) + CCl₄ (1 ml/kg)

~~Hepatotoxicity. They were given a was induced with a~~ single dose of CCl₄ (1 ml/kg) ~~to induce hepatotoxicity~~ on Day 7 and the treatment continued for another three days. After 24 hours of last treatment and fasting, blood was collected through the retro-orbital plexus for the determination of biochemical parameters. The animals were then sacrificed and the sliced liver tissues were homogenized, and centrifuged at 1000 rpm for 10 min, and stored for use in the determination of antioxidant enzyme activity [11].

Comment [CL4]: This section is not consistent with results since it was stated that antioxidants were determined in the serum

2.5.1 Acute toxicity study

Lorke's procedure [18] of LD₅₀ determination was used. The experimental mice were divided into three group (n=3) and were orally administered 10, 100 and 1000, and later, 1000, 1600, 2900 and 5000 mg/kg of the extract. The animals were constantly observed for mortality over a period of 24 hours.

2.5.2 In vivo antioxidant assays – determination of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx)

These were carried out spectrophotometrically on the liver homogenates from the animals following the standard procedure and reaction conditions as described by Azarmehr *et al.* [19].

Comment [CL5]: Be specific here. was analyses performed on homogenate or serum

2.5.3 Liver enzymes function tests

The blood serum obtained after centrifuging the blood samples at 2500 rpm for 10 minutes was used for estimation of the biochemical parameters to determine the functional state of the liver. Serum aminotransferase activities including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP) and plasma malondialdehyde (MDA) were assayed using the ~~RANDOX~~ commercial Enzyme kit according to the methods of Reitman and Frankel [20] and Schmidt and Schmidt [21].

2.6 Statistical analysis

All data were expressed as mean ± standard error of mean (S.E.M.). Data were analyzed using one way Analysis of Variance (ANOVA) at 5% level of significance on the Statistical Package for Social Sciences (SPSS) version 20 software. Duncan's multiple range test (DMRT) was used for post hoc analysis to separate the means at p ≤ 0.05.

3. RESULTS AND DISCUSSION

The essence of this work was to determine the phytochemical constituents of the leaves of *Lasimorpha senegalensis* as well as the potentials of its methanolic extract as natural antioxidant and hepatoprotective agent.

3.1 Phytochemical constituents

Table 2 shows the result of phytochemical analysis of the leaf extracts of *Lasimorpha senegalensis*. The analysis showed phenols, flavonoids and tannins in abundant amounts with concentrations (in mg/100 g) of 1087.43 ± 12.32 , 794.24 ± 1.79 and 575.69 ± 3.85 respectively while steroids, hydrogen cyanide and saponins were found in lower amounts with concentrations 0.78 ± 0.06 , 0.22 ± 0.00 and 0.12 ± 0.00 respectively. This shows that the leaf extract contained important phytochemicals that were easily detected by qualitative and quantitative screening. Phytochemicals present include: phenols, alkaloids, tannins, saponins, steroids, terpenoids, hydrogen cyanide, saponins, polyphenols and flavonoids. Phenols, flavonoids and tannins are more in abundant, followed by alkaloids, polyphenols and terpenoids while steroids, hydrogen cyanide and saponins were the least abundant.

Table 2. Phytochemical analysis of the leaf extract of *L. senegalensis*

Phytochemicals	Qualitative screening	Quantitative determination
Tannins (mg/100 g)	+++	575.69 ± 3.85
Flavonoids (mg/100 g)	+++	794.24 ± 1.79
Alkaloids (mg/100 g)	++	375.83 ± 1.59
Hydrogen cyanide (mg/g)	+	0.22 ± 0.00
Glycosides (mg/100 g)	++	155.54 ± 0.01
Phenols (mg/100 g)	+++	1087.43 ± 12.32
Terpenoids (mg/100 g)	++	81.89 ± 1.31
Steroids (mg/100 g)	+	0.78 ± 0.06
Saponins (mg/g)	+	0.12 ± 0.00
Polyphenols (mg/100 g)	++	275.17 ± 0.21

+ = mildly present; ++ = moderately present; +++ = highly present

3.2 In vitro antioxidant activity

Table 3 below shows the result of percentage antioxidant activity of the leaf extract of *L. senegalensis*. For DPPH scavenging activity, the extract compared favorable with ascorbic, especially at the lower concentrations (0.2, 0.4 and 0.6 mg/ml). The inhibition percentage was observed to be dose-dependent with the higher doses exhibiting the highest activity for H₂O₂. However, the ascorbic acid standard had significantly better activity than the extract at

all concentrations. The IC₅₀ of the sample for DPPH and H₂O₂ scavenging activities were 0.52 mg/ml and 0.71 mg/ml respectively (Figure 1).

Table 3. Result of percentage antioxidant activity of the leaf extract of *L. senegalensis*

Conc. (mg/ml)	% inhibition of DPPH		% inhibition of H ₂ O ₂	
	Sample	Ascorbic acid	Sample	Ascorbic acid
0.2	37.10 ± 0.67	37.48 ± 0.93 ^{NS}	24.93 ± 0.68	51.58 ± 0.89*
0.4	46.00 ± 0.90	47.45 ± 0.17 ^{NS}	34.32 ± 1.04	61.34 ± 0.24*
0.6	54.15 ± 0.36	56.96 ± 0.34 ^{NS}	49.86 ± 0.56	66.21 ± 0.55*
0.8	59.64 ± 0.61	70.50 ± 0.11 ^{NS}	57.27 ± 0.48	76.87 ± 0.39*
1	70.56 ± 0.23	82.18 ± 0.11*	60.34 ± 0.33	78.54 ± 0.34*

NS = not significant at $p \leq 0.05$; * = not significant at $p \leq 0.05$; $n = 3$

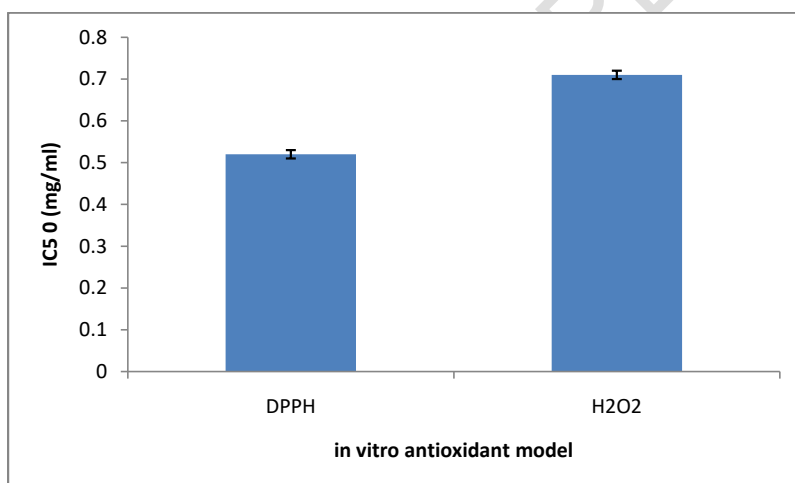


Fig. 1. Comparative IC₅₀ inhibition activity of the leaf extract of *L. senegalensis*

One of the most preferred antioxidant method and accepted mechanism for determining the radical scavenging activity of plant material is DPPH scavenging assay. It is a stable, free radical that possesses a characteristic absorption maximum at 517 nm, which is diminished in the presence of antioxidants capable of reducing it to its hydrazine form by donating hydrogen/electron [22]. Radical scavenging activities play a critical role in preventing the deleterious effect of free radicals in cancer and other diseases. Violet colour DPPH solution is reduced to yellow colour diphenylpicryl hydrazine by the addition of the extract in a concentration dependent manner in DPPH assay. Due to the relatively short time required for analysis, DPPH assay is used extensively to predict antioxidant activities [23].

The result of percentage antioxidant activity (*in-vitro*) of *L. senegalensis* leaf extract expressed as inhibition percentage of free radicals, and the high inhibition percentage corresponds to high antioxidant capacity of the extracts. The results of DPPH radical scavenging activity confirmed the reductive ability of the extract by up to $70.56 \pm 0.23\%$. This is in agreement good antioxidant activities reported in other members of the family. According to Mohammed and Ibrahim [24], the methanolic extract of *Arum maculatum* displayed high DPPH radical scavenging activity of $93.33 \pm 0.5\%$ and while *Arum dioscorides* displayed DPPH radical scavenging activity of $72.643 \pm 1.23\%$ [9]. The DPPH scavenging activity for *L. senegalensis* was lower than that of *Arum dioscorides*.

The removal of H_2O_2 is essential for antioxidant defense. Being an example of reactive oxygen species, H_2O_2 can be toxic to cells by giving rise to hydroxyl radical in the cells, even though itself is not very active [25]. These oxygen species are ubiquitous signaling molecules generated during the regular cellular metabolism [26]. ROS are produced after incomplete reduction of oxygen [27] and become rapidly metabolized with the help of constitutive antioxidative enzymes and nonenzymatic antioxidant scavenging systems such as antioxidant thiols, proteins and vitamins during normal conditions [28]. However, under the influence of environmental stresses such as pathogen attack, cold, drought, salt stress, UV irradiation, etc., reactive oxygen species production increases. Activation of additional defense mechanisms, under that stressed condition, is required to counteract the excessive accumulation of ROS. Failure to scavenge ROS efficiently poses danger to lipids in cellular membranes, proteins and other cellular components leading to dysfunction and ultimately to the appearance of necrotic lesions [29]. Our findings showed that the leaf extract inhibited H_2O_2 by up to $60.34 \pm 0.33\%$ to corroborate with the reported free radical scavenging activity of *Alpinia nigradocumented* by Sahoo *et al.* [30].

3.3 In vivo antioxidant activity

Results obtained from the SOD, CAT, glutathione peroxidase and malondialdehyde activities from the blood serum are shown in Table 4 below. The leaf extract, irrespective of dosage, significantly ($p \leq .05$) reduced MDA concentration of the serum when compared with the normal control. Furthermore, SOD, CAT and GSHPx were all elevated by the leaf extract in a dose dependent manner.

Comment [CL6]: Be specific. was the analyses performed on serum or liver homogenate

Table 4. Effects of the leaf extract on SOD, CAT, GSHPx and MDA concentrations

Treatment	SOD (μ /mg)	CAT (μ /mg)	GSHPx (μ /mg)	MDA
Toxic group	11.93 ± 0.59^d	31.78 ± 0.83^d	12.14 ± 0.55^e	6.01 ± 0.14^a
Control group	13.61 ± 0.78^c	43.11 ± 1.06^c	14.02 ± 0.53^c	5.18 ± 0.18^b
Extract (200 mg/kg)	17.12 ± 0.42^c	49.08 ± 0.78^b	17.29 ± 0.58^c	4.89 ± 0.08^c
Extract (400 mg/kg)	21.71 ± 0.41^b	49.42 ± 1.95^b	22.58 ± 0.41^b	4.13 ± 0.10^c
Silymarin (100 mg/kg)	25.28 ± 0.86^a	54.22 ± 1.07^a	26.23 ± 0.35^a	3.76 ± 0.20^d

Superoxide dismutase (SOD); Catalase (CAT); Glutathione peroxidase (GSHPx); MDA = Plasma malondialdehyde
Means with different letters as superscripts along a column are significantly different at $p \leq 0.05$
Means with same letters as superscripts along a column are not significantly different at $p \leq 0.05$

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase are some of the endogenous antioxidant enzymes found normally in every living cell [4], while malondialdehyde (MDA) is a biomarker of oxidative stress and lipid peroxidation. Our current results showed that the leaf extract of *L. senegalensis* reduced lipid peroxidation by

lowering the concentration of MDA in the experimental animal groups. It also significantly ($p \leq .05$) increased the activity of endogenous antioxidant enzymes such as CAT, SOD and glutathione peroxidase. This is in agreement with the findings of Alireza *et al.* [31] and Adaramoye *et al.* [32]. Gometiet *et al.* [33] also reported reduction of lipid peroxidation and increase in CAT, SOD and glutathione peroxidase in alloxan-induced diabetic rats by some medicinal plants.

3.4 Effects on some Liver Enzymes

The impact of the leaf extracts on some liver enzymes from the tissues homogenates obtained from the animals are summarized in Table 5 below. The leaf extract significantly ($p \leq .05$) reduced the AST, ALT and ALP that were initially elevated by the administration of CCl_4 . This effect was observed to be dose dependent with the higher dose exhibiting greater activity.

Comment [CL7]: Authors should include Bilirubin measurement since it is better measure of liver function

Table 5. Effects of the leaf extract on some liver enzymes

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)
Toxic group	154.00 ± 2.13 ^a	50.22 ± 0.73 ^a	37.24 ± 0.90 ^a
Control group	140.48 ± 1.59 ^b	42.11 ± 1.83 ^b	30.71 ± 0.60 ^b
Extract (200 mg/kg)	138.87 ± 2.23 ^b	38.65 ± 1.73 ^c	29.20 ± 0.38 ^b
Extract (400 mg/kg)	124.59 ± 2.12 ^c	36.83 ± 2.12 ^c	26.29 ± 0.55 ^c
Silymarin (100 mg/kg)	107.66 ± 2.85 ^d	28.30 ± 0.77 ^d	23.05 ± 0.20 ^d

AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphate
 Means with different letters as superscripts across a row are significantly different at $p \leq 0.05$
 Means with same letters as superscripts across a row are not significantly different at $p \leq 0.05$

Liver enzymes are proven indicators of liver damage; hence, they are helpful in toxicological and clinical studies of drug activities. Hepatotoxicity is directly correlated to the elevation of the serum enzymes such as AST, ALT, and ALP. Therefore, high levels of these enzymes determine the extent of liver damage [1,34]. In this present study, we observed a significant increase in AST, ALT and ALP activities across the animals groups as a result of CCl_4 hepatotoxicity. However, the animal groups that received the plant extracts were able to recover from the damages as shown by their lower levels of liver enzymes when compared with the normal control and toxic groups, though this was more significant at a higher dose. The slight decrease in the liver enzymes observed in the animals that received normal saline signifies the ability of the animal tissues to fight toxicity naturally. This implies that the leaves of *L. senegalensis* at a higher dose of 400 mg/kg could ameliorate liver damage. The findings from this study corroborates with Anosike *et al.* [35], Ubohet *et al.* [36] and Omekeet *et al.* [1] who reported hepatoprotective properties of the leaf extracts of *Pyrenacanthastaudtii*, *Psidium guajava* and *Annona senegalensis* in CCl_4 induced liver damage. On the contrary, Etimet *et al.* [37] and some other researchers reported hepatotoxicity of some plant extracts, especially at higher doses.

The antioxidant and hepatoprotective activities of this plant could be attributed its phytochemical constituents, most especially, its high levels of phenolics, flavonoids and tannins [38]. According to Somerville *et al.* [39], it has been found that flavonoids have antibacterial, anti-inflammatory, cytotoxic, anti-tumor and other biological properties and is used in the treatment of neurodegenerative diseases. Sansone *et al.* [40] reported that flavonoids are one of the most important phytochemicals due to their role in the inhibition of

lipid peroxidation, capillary permeability and fragility, and platelet aggregation. Alkaloids and phenolics also exhibit anti-microbial, antioxidant, and anti-inflammatory effects [39,41]. Guclu-Ustundaget *al.* [42] noted that tannins possess anti-mutagenic, anti-carcinogenic, cardio-protective, and anti-viral properties and are used in treating non-insulin dependent diabetes mellitus. Since *L. senegalensis* contain these phytochemicals in appreciable amounts, it has potential to be used for many curative purposes.

4. CONCLUSION

In this present study, we observed that the methanolic leaf extract of *Lasimorpha senegalensis* contain considerable amount of pharmacologically active phytochemicals that could be attributed to responsible for the its antioxidant and hepatoprotective activities. This was evident in the ability of the extract to scavenge free radicals in DPPH and H₂O₂ *in vitro* antioxidant screening models. Moreover, experimental animal model showed that the extract increased the activity of CAT, SOD and glutathione peroxidase while reducing liver function enzymes such as AST, ALT, ALP and MDA. These results therefore justify the local use of this plant in the treatment and management of various diseases related to oxidative stress and liver damage. Further investigations on the particular compounds responsible for these activities are recommended for their possible development and commercialization.

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