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Antioxidant and Hepatoprotective Potentials of Lasimorpha senegalensis Schott Leaf Extract on Carbon Tetrachloride-induced Liver Damage in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors CBC and FIN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EU and CSO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

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Original Research Article

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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: Phytochemical analyses and acute toxicity study of the sample followed standard procedures. *In vitro* antioxidant assay was by DPPH and H_2O_2 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 carbontetrachloride (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 (IC_{50} of 0.52 mg/ml and 0.71 mg/ml for DPPH and H_2O_2 respectively) with no toxicity at 5000 mg/kg. The extract also elevated the endogenous antioxidants and significantly ($p \le .05$) 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 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-clothing 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. Farahmandfar et 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 of Nigeria, decoction of the leaves is 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. MATERIALS 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

 $25 \pm 2^{\circ}\text{C}$ for 10 days. The dried leaves were pulverized with mortar and pestle to a coarse powder.

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 were 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.

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 Mensor 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 30-minutes, and the absorbance of the resulting solution read at 517 nm. 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 (50 mM, pH 7.4) was reacted with varying concentrations of the test samples and standard ascorbic acid. Their absorbance was read at 230 nm. Percentage 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 treated as follows:

Group A (toxic group) received only CCl_4 (1 ml/kg).

Groups B (normal control) received normal saline Group C received 200 mg/kg extract + CCl₄ (1 ml/kg).

Group D received 400 mg/kg extract + CCl_4 (1 ml/kg).

Group E (positive control) received Silymarin (100 mg/kg) + CCl_4 (1 ml/kg).

Hepatotoxicity was induced with a single dose of CCl_4 (1 ml/kg) on Day 7 and the treatment continued for another three days to make a total of 10 days treatment. After 24 hours of last treatment and fasting, blood was collected through the retro-orbital plexus for the determination of biochemical parameters [11].

2.5.1 Acute toxicity study

Lorke's procedure [18] of LD_{50} 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 wereconstantly 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 (GPx)

These were carried out spectrophometrically on the blood serum from the animals following the standard procedure and reaction conditions as described by Azarmehr et al. [19].

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].

Phytochemical	Reagents	Wavelength (nm)
Tannins	Conc. HCl; FeCl _{3;} 0.008 (K ₃ Fe(CN) ₆);	720
Flavonoids	HCl; NaOH; FeCl₃	233
Steroids	Ethylacetate; chloroform; 0.1 M NH ₄ OH;	240
Saponins	Dil. H ₂ O	620
Alkaloids	Phosphate buffer; 5% trichloroacetic acid; Casein	580
	solution;	
Hydrogen cyanide	Alkaline picrate solution; dil. water	540
Glycosides	15% lead acetate; chloroform; glacial acetic acid;	530
	5% Ferric chloride; conc. H ₂ SO ₄	
Phenols	Folin-Ciocalteau reagent; 20% trioxocarbonate (iv)	560
	solution;	
Soluble carbohydrate	Picric acid; dil. water	540
Reducing sugars	Ethanol; iodine solution (5 g iodine + 1 g potassium	480
-	iodide); dil. water	

Table 1. Methods for the determination of phytochemicals

2.6 Statistical Analysis

All data were expressed as mean \pm 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 \leq 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 constituents of the leaf extract of Lasimorpha senegalensis. The analyses showed phenols, flavonoids and tannins to be abundant 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.

3.2 In vitro Antioxidant Activity

Table 3 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 H_2O_2 . However, the ascorbic acid standard had significantly better activity than the extract at all concentrations. The IC₅₀ of the sample for DPPH and H_2O_2 scavenging activities were 0.52 mg/ml and 0.71 mg/ml respectively (Fig. 1).

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].

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

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

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

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

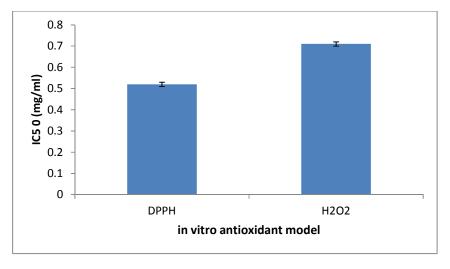


Fig. 1. Comparative IC₅₀ inhibition activity of the leaf extract of *L. senegalensis*

The result of percentage antioxidant activity (*invitro*) 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 $93.33\pm0.5\%$ while *Arum dioscorides* was 72.643 $\pm1.23\%$ [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, 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 nonenzymatic antioxidative enzymes and 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 ± 0.33% to corroborate with the reported free radical scavenging activity of Alpinia nigra documented 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. The leaf extract, irrespective of dosage, significantly ($p \le .05$) reduced MDA concentration of the serum when compared with the normal control.

Chigor et al.; JPRI, 32(21): 70-78, 2020; Article no.JPRI.56218

Furthermore, SOD, CAT and GPx were all elevated by the leaf extract in a dose dependent manner.

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. The 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 ($p \le .05$) 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 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 impacts of the leaf extracts on some liver enzymes from the blood serum obtained from the animals are summarized in Table 5. The leaf extract significantly ($p \le .05$) reduced the AST, ALT and ALP that were initially elevated by the administration of CCl₄. This effect was observed to be dose dependent with the higher dose exhibiting greater activity.

Treatment	SOD (µ/mg)	CAT (µ/mg)	GPx (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	$4.89 \pm 0.08^{\circ}$
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}

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

Superoxide dismutase (SOD); Catalase (CAT); Glutathione peroxidase (GPx); MDA = Plasmamalondialdehyde. Means with different letters as superscripts along a column are significantly different at $p \le 0.05$. Means with same letters as superscripts along a column are not significantly different at $p \le 0.05$

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	$26.29 \pm 0.55^{\circ}$
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 $p \le 0.05$. Means with same letters as superscripts across a row are not significantly different at $p \le 0.05$

Liver enzymes are proven indicators of liver damage; hence, they are helpful in toxicological clinical studies of drug activities. and 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 CCl₄ 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], Uboh et al. [36] and Omeke et al. [1] who reported hepatoprotective properties of the leaf extracts of Pyrenacantha staudtii, Psidium guajava and Annona senegalensis in CCl₄ induced liver damage. On the contrary, Etim 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-tumour 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-Ustundag et al. [42] noted that tannins possess anti-mutagenic, anticarcinogenic, cardio-protective, and anti-viral properties and are used in treating non-insulin dependent diabetes mellitus. contain Since L. senegalensis 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 responsible for the 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 their possible development for and commercialization.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was obtained from the Research Ethics Committee, University of Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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