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In vitro Antioxidant, Antihyperglycaemic and Antihyperlipidaemic Activities of Ethanol Extract of *Lawsonia inermis* Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OOO and TO designed the study and wrote the protocol. Author OOO managed the literature searches, performed the statistical analysis, and wrote the first draft of the manuscript. Authors OOI, CM and SA prepared the plant materials and performed the laboratory analyses. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This present study investigated the free radical scavenging activities, antihperglycaemic and antihyperlipidaemic activities of ethanol extract of *Lawsonia inermis* leaves.
Study Design: Twenty male rats were randomly and evenly distributed into four groups, and were subsequently exposed to the following treatments for twenty-one days: Group I (Control): Normal saline; Group II: Untreated Diabetic control; Group III: Diabetic rats treated with glibenclamide (600mg/Kg. b.wt); Group IV: Diabetic rats treated with ethanol extract of *Lawsonia inermis* (400mg/Kg b.wt).
Place and Duration of Study: This work was carried out in the Department of Biochemistry, University of Lagos, Lagos, Nigeria between November 2012 and February 2013.
Methodology: Phytochemical screening and the antioxidant activities of the plant extract

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were carried out. Brine shrimp lethality assay was also carried out on the plant extract for *In vitro* toxicity assay. Blood samples collected from the experimental rats were used for the determination of fasting blood sugar and biochemical profiles following the last oral treatment and an overnight fast.

Results: *Lawsonia inermis* showed maximum inhibition of 77.39%, 50.78% and 83.18 % at 100µg/ml DPPH free radical scavenging activity, in vitro lipid peroxidation and nitric oxide respectively. Following twenty-one days of treatment with oral administration of ethanol extract of *Lawsonia inermis* leaves, fasting blood glucose was significantly reduced (*P<0.001)* compared to the untreated diabetic control. Activities of AST, ALT, and ALP were significantly decreased *(P<0.001)* in the diabetic rats treated with the extract compared to the untreated diabetic control rats. Diabetic rats treated with ethanolic leaf extract of *Lawsonia inermis* showed statistically significant decrease *(P<0.001)* in the levels of total cholesterol, total triglyceride and low density lipoprotein cholesterol when compared with untreated diabetic rats.

Conclusion: The ethanol extract of *Lawsonia inermis* leaves showed antioxidant, antihyperglycaemic and antihyperlipidaemic activities.

Keywords: Brine shrimp lethality assay; free radical scavenging activities; antihyperglycaemic activity; Lawsonia inermis.

1. INTRODUCTION

Lawsonia inermis Linn, popularly known as henna plant, belongs to the family of Lythraceae. It is called Laali among the Yoruba speaking people of Nigeria. As a medicinal plant, different parts of the plant have been used to treat different ailments such as hepatic injury, rheumatoid arthritis, headache, diarrhoea, leprosy, fever, cardiac disease and ulcers [1-3]. Henna plant has been used cosmetically and medicinally worldwide. For instance, henna leaf, when dried and soaked in water is widely used traditionally among the Yoruba and Hausa people of Nigeria for decorating nail, hands and feet with different patterns. Leaves of henna plant are small, greenish brown to dull green, opposite in arrangement along the branches with very short petioles, about 1.5-5cm long and 0.5 to 2cm wide [4,2]. The major component of henna is a colouring agent known as lawsone, 2-hydroxy-1, 4- napthoquinone - $C_{10}H_6O_3$ [4]. It has been established that the hydroalcoholic extract of *L. inermis* possesses antioxidant activities [5]. Meanwhile, inventory of antidiabetic plants in Lagos State, Nigeria revealed that two percent of traditional practitioners have used *L. inermis* leaves in the management of diabetes [6].

Diabetes is a metabolic disorder characterized by hyperglycaemia and defect in insulin secretion or action of endogenous insulin [7]. During diabetes, hyperglycaemia elicits increased production of reactive oxygen species sequel to glucose auto-oxidation and protein glycosylation [8-9]. Generally, oxidative stress results from an imbalance between increased free radical production and reduced activity of antioxidant defences [10]. This may be responsible for increased oxidative stress in the pathogenesis of diabetes and other complications like dyslipidaemia. Hence, this present study investigated the free radical scavenging activities, antihyperglycaemic and antihyperlipidaemic activities of ethanolic leaf extract of L*awsonia inermis*.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Material

Fresh leaves of *L. inermis* were collected from Ilaro, Ogun State, Nigeria in October 2012. The plants were identified and authenticated by a taxonomist in the herbarium, Department of Botany, University of Lagos, Akoka, Lagos. The voucher number was LUH 4874. The leaves of *L. inermis* were air dried in an open space of laboratory. 200g of the pulverized plant material was soaked in 70% ethanol. It was kept in the laboratory at room temperature for 48 hours after which it was filtered using muslin cloth and filter paper. The filtrate was collected in a beaker; the ethanol was allowed to evaporate over a water bath to yield 25.5g extract concentrate.

2.2 Phytochemical Screening of Crude Extract

Chemical constituents of the ethanol extract was screened and identified by the methods described by Sofowara [11], Trease and Evans [12] and Harborne [13].

2.2.1 Test for reducing sugar (Fehling's reagent)

2 mL of the aqueous extract was boiled with equal volumes of fehling's solutions A and B for 15 minutes. A brick red precipitate indicates the presence of reducing sugar. 2 mL of the aqueous extract was boiled with 1mL dilute HCl for 15 minutes, cooled and neutralised with dilute ammonia. It was then boiled with equal volume of fehling's solutions A and B for 15 minutes, a more intensive brick red precipitate indicated the presence of glycosides.

2.2.2 Test for terpenoids (Salkowski test)

5 mL of the extract was mixed in 2 mL of chloroform and 3 mL concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

2.2.3 Test for steroids

2 mL of acetic anhydride was added to 5 mL of extract with 2 mL H_2SO_4. The colour changed from violet to blue or green indicated the presence of steroids.

2.2.4 Test for alkaloid

5 mg of the extract dissolved in 3 mL of acidified ethanol was warmed slightly and then filtered. 1 mL of the filtrate was treated with few drops of Mayer's reagent and another 1 mL with Dragendroff's reagent and turbidity was observed.

2.2.5 Test for tannins

A few drops of 0.1% ferric chloride was added to the extract solution and observed brownish-green or a blue-black coloration which signified the presence of tannins.

2.2.6 Test for flavonoids

The presence of flavonoids in the plant samples was determined as follows:

(a) 5 mL of dilute ammonia solution was added to 5 mL of the extract solution followed by the addition of concentrated tetra-oxo sulphate (VI) acid. A yellow coloration observed in the extract indicated the presence of flavonoids. The yellow coloration disappeared on standing.
(b) Few drops of 1% Aluminum solution were added to 2 mL of extract. A yellow coloration was observed indicating the presence of flavonoids.
(c) 5 mL of the extract solution was in each case heated with 10 mL of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 mL of filtrate was shaken with 1 mL dilute ammonia solution. A yellow coloration was positive for flavonoids.

2.2.7 Test for saponins

5 mL of the extract solution was shaken vigorously for a stable persistent froth. The frothing were mixed with olive oil and shaken vigorously then observed for the formation of emulsion.

2.3 Brine Shrimp Lethality Assay

The Sea water was collected from Lagos Bar Beach and filtered to remove dirt and sand particles.

2.3.1 Hatching of shrimps

Artemia salina Leach (Brine shrimp) eggs were hatched in shallow rectangular container (7× 15 cm) filled to three quarter with the filtered sea water and the eggs were sprinkled into it. The bowl was partly covered to allow light to penetrate through the open space and allowed to stay in an undisturbed environment at room temperature. After 24 hours, the phototropic shrimp larvae (nauplii) moved towards the illuminated side of the container. The newly hatched free-swimming pink-coloured nauplii were harvested from the bottom outlet as the cyst capsules floated on the surface to ensure pure harvest of nauplii. The freshly hatched free-swimming nauplii were used for the bioassay.

2.3.2 Brine shrimp lethality test

100 mg of the plant extract was dissolved in 20 mL of sea water to give a stock solution of 5000 µg/ml from which various concentrations of 10, 100 and 1000 µg/ml solution were prepared. Potassium dichromate in concentration of 10, 100 and 1000 µg/ml was used as a positive control. All doses were calculated by serial dilution technique while sea water served as the negative control. All these concentrations were in triplicate vials making 9 vials of 5 ml each.

In each of the vial, 10 nauplii were transferred and the set up was allowed to remain for 24 hours, under constant illumination. After 24 hours, the survived nauplii in each vial were counted with the aid of hand lens and the average number of survived larvae was determined. The graph of probit against logarithm of dose was plotted and the LC_{50} was determined. The percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ Mortality} = \frac{N_t}{N_0} \times 100$$

Where, N_t = Number of killed nauplii after 24 h of incubation,
N_0 = Number of total nauplii transferred, that is 10.

The LC_{50} (Median lethal concentration) was then determined using Probit analysis.

2.4 Evaluation of Antioxidant Activity of the Extract

2.4.1 Estimation of total phenolic compound

The amount of total phenol content was determined by Folin-Ciocateu reagent method [14] using gallic acid as a standard following the method of Slinkard and Singleton [15]. 0.5 mL of extract and 0.1 mL of Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 minutes. Then, 2.5 mL sodium carbonate (7.5%w/v) was added and further incubated for 30 minutes at room temperature and absorbance measured at 760 nm. The concentration of total phenol was expressed in terms of gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value.

2.4.2 Total flavonoid content estimation

Total soluble flavonoid of the extract was determined with aluminium chloride using quercetin as standard [16]. 1 mL of sample (100µg/mL) was mixed with 3mL of methanol, 0.2mL of 10% Aluminum chloride, 0.2 mL of 1M potassium acetate and 5.6mL of distilled water. Then it was incubated at room temperature for 30 minutes and the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The flavonoid content was then calculated.

2.4.3 Total antioxidant capacity determination

The total antioxidant capacity of the extract was determined by Prieto e*t al.* [17]. 0.3mL extract was mixed with 3mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95ºC for 90 minutes. After the samples had cooled to room temperature, the absorbance of the ethanolic solution of each was measured at 695 nm against blank in a UV spectrophotometer. The blank solution contained 0.3 mL of Methanol in the place of extract and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the sample. The total antioxidant capacity was expressed as equivalent of ascorbic acid (standard).

2.4.4 DPPH radical scavenging activity assay

The free radical scavenging activity of the extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated [18,19]. An aliquot of 0.5 mL of extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/ ml) was mixed with 2.0mL of reagent solution (0.004 g of DPPH in 100 mL methanol). The control contained only DPPH solution instead of sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in

absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm by using UV-Vis spectrophotometer. Gallic acid was used as the standard in this assay. The scavenging effect was calculated using:

$$\% \text{ inhibition} = \frac{[A_0 - A_1]}{A_0} \times 100$$

Where A_0 is the absorption of the blank sample and A_1 is the absorption of the extract

2.4.5 In vitro Lipid peroxidation assay

Lipid peroxidation was induced by Fe^{2+}ascorbate system in liver homogenate and estimated as thiobarbituric acid reacting substances (TBARS) by the method of Buege and Aust [20]. Freshly excised rat liver was sliced and processed to get 10% homogenate in cold 150m*M* KCl-Tris- buffer. The reaction mixture contained liver homogenate, Tris- HCl buffer (20 mM; pH 7.0), $FeCl_2$ (2 mM), Ascorbic acid (10 mM) and 0.5 mL plant extract (25-100 µg/ml)in a final volume of 1mL. The reaction mixture was incubated at 37ºC for 1 hour. Lipid peroxidation was measured as malondialdehyde (MDA) equivalent using trichloroacetic acid (TCA), thiobarbituric acid (TBA) and HCl (TBA-TCA reagent: 0.375% w/v TBA; 15% w/v TCA and 0.25 N HCl). The incubated reaction mixture was mixed with 2mL of TBA-TCA reagent and heated in a boiling water bath for 15 minutes. After cooling, the flocculent precipitate was removed by centrifugation at 10,000 xg for 5 minutes. Finally, malondialdehyde concentration in the supernatant fraction was determined spectrophotometrically at 535 nm. The concentrations of extract that would inhibit by 50%, the production of thiobarbituric acid reactive substances, i.e. IC_{50} values, were calculated. Ascorbic acid was used as standard.

2.4.6 Nitric oxide scavenging activity assay

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO•. Under aerobic condition, NO• reacts with oxygen to produce stable products (nitrate and nitrite), which can be determined using Griess reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride can be immediately read at 550 nm. 4 mL of plant extract or standard solution of different concentrations (25, 50, 75, 100 µg/ml) were taken in different test tubes and 1.0mL of Sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30ºC to complete the reaction. 2mL solution was withdrawn from the mixture and mixed with 1.2 mL of Griess reagent (1% Sulphanilamide, 0.1% naphthylethylenediamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylenediamine was measured against blank using a spectrophotometer at 550 nm [21]. Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation: $[(A_0 – A_1)/A_0] \times 100$. Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract or standard.

2.5 Experimental Animals

Twenty wistar rats, twelve weeks old weighing (120-150g) were obtained from the animal house College of Medicine of the University of Lagos, Idi-Araba. The animals were housed in clean plastic cages, well ventilated (26 ± 2ºC and relative humidity 30-35%) in 12 hours light and 12 hours dark cycle respectively. This research was conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH Publication: 83-23) [22] and in strict compliance to the guidelines on the use and care of animals at the College of Medicine, University of Lagos. The animals were fed with pelleted rat chows, provided with water *ad libitum* and were allowed to acclimatize for two weeks before commencing the experiment.

2.5.1 Induction of diabetes in rats

Fifteen Wistar male rats weighing 139.30 ± 5.69 were induced with alloxan monohydrate ($C_4H_2N_2O_4.H_2O$, 10%) 150mg/kg body weight dissolved in normal saline (0.2 ml, 154mM Nacl) given intraperitoneally (i.p) after 18 hours fasting to induce hyperglycaemia in experimental rats as described by Aruna *et al.*, [23]. The blood glucose level (BGL) was monitored for 72 hours after alloxan administration in blood samples collected by tail tipping method using a Glucometer (ACCU-CHEK, Roche Diagnostics). Animals with blood glucose level greater than 250mg/dl were considered diabetic and selected for this study.

2.5.2 Experimental design

There were four groups of rats in this study, each group comprising five rats. Rats in the control and diabetic groups were treated as shown below:

Group I (Control): Normal saline
Group II: Diabetic control untreated
Group III: Diabetic rats treated with glibenclamide (600mg/Kg. b.w) [24]
Group IV: Diabetic rats treated with*Lawsoniainermis* (400mg/Kg b.w) [24]
After twenty-one days of treatment, blood samples were collected through occular puncture into Lithium heparin bottles for biochemical analysis and the animals sacrificed by cervical dislocation. Blood samples were centrifuged at 3500 rpm for 10 minutes. The plasma was used for the biochemical assay.

2.6 Assessment of Blood Chemistry

Aspartate Aminotransferase-AST and Alanine Aminotransferase-ALT were determined colorimetrically by the method of Reitman and Frankel [25] using Randox Reagent Kit. Alkaline Phosphatase was measured by the method described by Tietz *et al.*, [26] using Teco Reagent kit. Biolabo reagents were used for the determination of Urea [27], Creatinine [28], Triglyceride [29], total Cholesterol [30], and High Density Lipoprotein (HDL) – Cholesterol [31]. LDL-Cholesterol was determined mathematically using the following formula as posited by Lopez *et al.*, [32]:

LDL–Cholesterol = Total Cholesterol – (TG/2.2) – HDL-Cholesterol.

2.7 Statistical Analysis

Data were analyzed by comparing values for different treatment groups with the values for controls. Results were expressed as mean ± SEM. The significant differences among values were analysed using analysis of variance (One-way ANOVA) and Bonferroni for post hoc tests at *P-value* < 0.05. Graph pad prism 5.0 software was used for the analysis.

3. RESULTS

The phytochemical screening of the crude extract of ethanolic leaf extract of *L. inermis* revealed the presence of steroid, terpenoid, tannin, phenolic compounds, and flavonoids. Table 1 showed 46.06 ± 0.04, 49.47 ± 0.02, and 103.94 ± 0.01for the total phenolic content, total flavonoid content, and total antioxidant capacity respectively. In vitro toxicity assay of brine shrimp test shows LC_{50}=1000.0 while the control showed 14.12 (Table 2). *L. Inermis* showed maximum inhibition of 77.39%, 50.78% and 83.18 % at 100µg/ml DPPH free radical scavenging activity, in vitro lipid peroxidation, and nitric oxide respectively. However, the IC_{50} values for DPPH, lipid peroxidation and nitric oxide were 27.5µg/ml, 98.13µg/ml and 24.38µg/ml. The table shows that the percentage inhibition was in a concentration dependent manner (Table 3).

Table 1. Quantitative analysis of the plant extract

TPC (mg/100g)	TFC (mg/100g)	TAC (mg/100g)
46.06 ± 0.04	49.47 ± 0.02	103.94 ± 0.01

TPC: Total Phenolic Content; TFC: Total flavonoid content; TAC: Total antioxidant capacity

Table 2. Brine Shrimp lethality assay

	Control			*Lawsonia inermis*		
Log Dose	**1**	**2**	**3**	**1**	**2**	**3**
% Mortality	40	100	100	14	30	50
Probit	4.75	8.09	8.09	3.92	4.48	5
LC_{50}		14.12			1000.0	

Log of dose concentrations, 10, 100, and 1000 µg/ml solutions are 1, 2, and 3 respectively.

Table 3. Free radical scavenging activities of the plant extract

		25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
DPPH	Extract	49.22 ± 0.01	59.74 ± 0.03	68.07 ± 0.02	77.39 ± 0.02
	Standard	91.96 ± 0.04	95.96 ± 0.02	97.22 ± 0.02	97.28 ± 0.01
LPO	Extract	14.21 ± 0.02	23.06 ± 0.02	33.91 ± 0.01	50.78 ± 0.01
	Standard	40.23 ± 0.02	44.42 ± 0.03	57.95 ± 0.02	60.24 ± 0.02
NO	Extract	51.73 ± 0.03	80.94 ± 0.03	83.66 ± 0.02	96.55 ± 0.02
	Standard	79.01 ± 0.33	76.08 ± 0.01	71.53 ± 0.01	83.18 ± 0.01

Values are Mean ± SEM, (n=5); DPPH: 1,1-diphenyl-2-picrylhydrazyl; LPO: Lipid peroxidation; NO: Nitric oxide

Fasting blood glucose of all the diabetic induced rats (group II-IV) was significantly increased *(P<0.001)* after three days of diabetes induction compared to non-diabetic control rats (group I). Following twenty-one days of treatment with oral administration of ethanolic leaf extract of

L. inermis, fasting blood glucose was significantly reduced *(P<0.001)* just like the rats treated with glibenclamide when compared with the non-treated diabetic control (Table 4).

Biochemical profiles of the experimental rats after twenty-one days of treatment (Table 5) shows that the levels of AST, ALT and ALP were significantly decreased *(P<0.001)* in the diabetic rats treated with the extract of *L. inermis* compared to the non-treated diabetic control rats. Urea and Creatinine levels in the orally administered extract of *L. inermis* also showed a statistically significant decrease *(P<0.001)* compared to the untreated diabetic rats. The levels of total cholesterol, total triglyceride, and Low density lipoprotein cholesterol of untreated diabetic rats were significantly increased while high density lipoprotein cholesterol was significantly decreased when compared to the non-diabetic control rats. Diabetic rats treated with ethanolic leaf extract of *L. inermis* showed statistically significant decrease *(P<0.001)* in the levels of total cholesterol, total triglyceride and Low density lipoprotein cholesterol when compared with untreated diabetic rats. The level of high density lipoprotein cholesterol in diabetic rats treated with extract of *L. inermis* did not show any significant difference (P>0.05) compared to the untreated diabetic rats.

Table 4. Effect of *Lawsonia inermis* extract on blood glucose level (mg/dL) during the 21 days treatment

Parameters	Group I	Group II	Group III	Group IV
Basal Value	83.6 ± 3.5	80.8 ± 5.6	70.6 ± 6.9	86.6 ± 5.6
After 3 days	83.4 ± 3.7	431.8 ± 51.8 [a]	421.8 ± 47.6 [a]	455.0 ± 24.7 [a]
After 21 days	85.0 ± 2.7	389.6 ± 40.2 [a]	120.6 ±5.9 [b]	132.2 ± 6.9 [b]

Values are Mean ± SEM, (n=5). [a, b] Statistical significance at p< 0.001.
a: significant compared to control; b: significant compared to diabetic group. Group I – Control; Group II – Diabetic control; Group III – Glibenclamide; Group IV – Diabetic + L. inermis

Table 5. Biochemical profiles of the experimental rats after 21 days of treatment

Parameters	Group I	Group II	Group III	Group IV
AST (U/L)	110.2 ± 4.09	273.8 ± 19.67[a]	159.0 ± 15.02[ab]	141.8 ± 2.65[b]
ALT (U/L)	24.52 ± 0.72	108.4 ± 4.91[a]	69.7 ± 0.46[ab]	74.60 ± 1.28[ab]
ALP (U/L)	60.50 ± 0.45	151.20 ± 2.17[a]	136.20 ± 1.94[ab]	1.09 ± 0.10[ab]
Urea (mmol/L)	2.81 ± 0.36	12.04 ± 0.41[a]	11.21 ± 0.08[a]	8.92 ± 0.29[ab]
CREA (mmol/L)	15.58 ± 0.16	90.68 ± 0.49[a]	81.88 ± 0.70[ab]	29.99 ± 0.05[ab]
TCHOL(mmol/L)	2.77 ± 0.03	6.49 ± 0.14[a]	4.38 ± 0.09[ab]	3.61 ± 0.03[ab]
TG (mmol/L)	0.72 ± 0.02	2.38 ± 0.03[a]	1.38 ± 0.04[ab]	1.38 ± 0.16[ab]
HDL (mmol/L)	1.53 ± 0.08	0.43 ± 0.01[a]	1.08 ± 0.04[ab]	0.56 ± 0.03[a]
LDL (mmol/L)	0.91 ± 0.01	4.76 ± 0.19[a]	2.64 ± 0.01[ab]	2.39 ± 0.01[ab]

Values are Mean ± SEM, (n=5). [a, b] Statistical significance at p< 0.001.
a: significant compared to control; b: significant compared to diabetic group. Group I – Control; Group II – Diabetic control; Group III – Glibenclamide; Group IV – Diabetic + L. inermis

4. DISCUSSION

Free radicals are generated during normal metabolic processes but there is an imbalance of oxidants/antioxidants in favour of the former in diabetes. Hence, this study investigated the free radical scavenging activities of *L. inermis*, antiglycaemic and antihyperglycaemic activities of ethanol extract of *L. inermis*. Phytochemical analysis of *L. Inermis* leaves has revealed the presence of gallic acid, mannitol, mucilage, alkaloid [4], lawsone (2-hydroxy-1,4-

naphthoquinone), apigenin, p-coumaricacid, 2-methoxy-3-methyl-1,4-naphthoquinone, apigenin-7-apiosyl-glucoside (apiin), luteolin, cosmosiin [33]. Flavonoids alongside with triterpenoids and polyphenols are naturally occurring compounds in plants with great beneficial impact on human's state of well being [34]. They possess antioxidant activities by scavenging excessive free radicals produced during metabolic processes [35]. The presence of flavonoids and total phenolic compounds observed in this study may be responsible for the total antioxidant activities exhibited by the plant extract. These findings supported previous report on *L. inermis* [33].The brine shrimp lethality test for the plant extract showed that the plant is safe and non-toxic.

It is a common practice to use 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) to investigate the free radical scavenging activity of antioxidants [36]. The ethanol extract of *L. inermis* used in this study reduced the stable free radical DPPH to a yellow colour of diphenyl-picrylhydrazine comparable with gallic acid standard due to its scavenging properties. This could in part be explained by the presence of flavonoids and naphthoquinones [37].The result of lipid peroxidation assay in this study further exhibited the efficacy of *L. inermis* leaf extract in inhibiting peroxidation by way of free radical scavenging activities. It has been surmised in a previous study that constituents of methanol extract of *L. inermis* leaves act as free radical scavengers that prevent phospholipid membrane peroxidation and protect immune-compromised cells from free radical damage [37].The plant extract showed a significant nitric oxide scavenging which could be attributed to the presence of phenolic compounds in the extract [38]. This present study revealed the potential of ethanol extract of *L. Inermis* leaves as an antioxidant.

Alloxan monohydrate is one of the chemicals used to induce diabetes in rat models which bring about elevated blood glucose and reduced level of insulin [39]. The diabetic rats treated with oral administration of ethanolic leaf extract of *L. Inermis* showed the anti-hyperglycaemic effect which may be due to potentiation of insulin release from β-cell of islets [40] or as a result of enhanced transport of blood glucose to muscles and adipose tissues [41]. It has been observed that oxidative cellular damage orchestrated by free radicals participate in the development of diabetes [42], it can then be deduced that the antidiabetic activity of the plant extract could be attributed to the free radical scavenging properties of flavonoids and phenolics contained in the extract.

Serum levels of AST, ALT and ALP have been used to evaluate extent of liver damage in alloxan-induced diabetic rats [43]. The significant increase in the liver enzyme activities observed in this study in the diabetic control has also been demonstrated in similar studies which may be as a result of liver damage caused by alloxan [44-45]. The decrease in the activities of AST, ALT and ALP observed in this study showed that the extract was able to repair tissue damage elicited by the diabetic conditions. This agreed with what has been reported that ethanol *extract of Rheum emodi* caused a significant reduction in AST, ALT and ALP activities in alloxan-induced diabetes [46].

Diabetic nephropathy is a common complication of diabetes mellitus with abnormal kidney function parameters [47]. Kang *et al.*, [48] showed increased level of serum urea with no observable change in serum creatinine. However, our results showed increased level of urea and creatinine in agreement with what was observed in Streptozotozin-induced diabetic rats [49].The plant extract administered in this study ameliorated the renal dysfunction resulting from diabetes as reported by Sawiress [50].

Dyslipidaemia is a well-known complication in diabetes [51]. In normal, non-diabetic state, insulin tends to increase receptor-mediated uptake of LDL-cholesterol, hence decreasing activity of insulin but during diabetes, hypercholesterolemia may occur by a way of increased β-oxidation of fatty acids which generate a lot of acetyl-coA and increased cholesterol synthesis [52]. In line with previous studies, uncontrolled diabetes causes increase in total cholesterol, triglycerides and LDL cholesterol associated with decrease in HDL [53-54]. Our present study showed elevated levels of lipid profile in the diabetic condition. Results obtained from this study showed reduction in the levels of total cholesterol, total triglyceride, and Low density lipoprotein cholesterol in support of anti-hyperlipidaemic effect of aqueous leaf extract of *Moringa oleifera* in alloxan induced diabetic rats [55]. The antihyperlipidaemic effect might have resulted from decreased fatty acid concentration in the circulation and reduced cholesterol synthesis possibly due to the flavonoid constituents of the plant extract administered.

5. CONCLUSION

Our study revealed the in vitro antioxidant, antihyperglycaemic, and antihyperlipidaemic activities of ethanol extract of L*awsonia inermis* leaves. These may explain the traditional use of the plant in treating diabetes in Lagos, Nigeria. The effects of *L. inermis* documented in this study might have been made possible through the total phenolic compounds and flavonoid constituents of the plant extract. It will be worthwhile in the future to investigate the mechanism by which ethanolic leaf extract of *Lawsonia inermis* exert these effects.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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