OPEN ACCESS JOURNALS
Indiana Journal of Agriculture and Life Sciences
Abbriviate Tittle- Ind J Agri Life Sci
ISSN *(Online)*- 2583-1623
Journal Homepage Link- https://indianapublications.com/journal/IJALS
DOI- 10.5281/zenodo.7267429

INDIANA PUBLICATIONS
PRODUCTIVE AND QUALITY RESEARCH

Research Article

Volume-02|Issue-05|2022

In Vivo Bioactivity-Guided Fractions and Compounds Isolation of Antidiabetic Activities of *Lawsonia Inermis* Linn Leaves in Streptozocine Induced Diabetic Wistar Rats

Abdulfatai Aremu*[1], Olayinka A. Oridupa[2], Ganiu J. Akorede[1], Afisu Basiru[3], & Akeem O. Ahmed[4]

[1]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria
[2]Department of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[3]Department of Veterinary Physiology and Biochemistry Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria
[4]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, Nigeria

Article History
Received: 14.10.2022
Accepted: 22.10.2022
Published: 31.10.2022

Citation
Aremu, A., Oridupa, O. O., Akorede, G. J., Basiru, A., Ahmed, A. O. (2022). In Vivo Bioactivity-Guided Fractions and Compounds Isolation of Antidiabetic Activities of *Lawsonia Inermis* Linn Leaves in Streptozocine Induced Diabetic Wistar Rats. *Indiana Journal of Agriculture and Life Sciences*, *2*(5), 53-61.

Abstract: Diabetes mellitus (DM) is a chronic metabolic disorder affecting substantial percentage of the world populace. Conventional treatments for DM have many limitations in terms of side effects and high rates of secondary failure. Antidiabetic effect of crude *Lawsonia inermis* Linn. leaves have been reported. Nevertheless, bioactive fraction and compounds accountable for the antidiabetic effect needs to be isolated for possible drug candidate. Thus, this study was designed to isolate the antidiabetic fraction and compounds of *Lawsonia inermis* Linn. leaves (LILL) in streptozocine (STZ) induced diabetic Wistar rats.

Bioactivity-guided fractionation was carried out. Fractions obtained were assayed for their anti-diabetic activities on streptozotocin-induced diabetic rats. Eleven groups of diabetic rats (n=5) were separately administered 5 and 10 mg/kg body weight of each of the fractions. Blood glucose levels were monitored using a glucometer for the first 24-hour and day 1-5 Most potent bioactive fraction-c was subjected to HPLC-MS for compounds isolation and antidiabetic activities. Data were analyzed using ANOVA at $p \leq 0.05$ and 0.01.

All fractions (A-D) decreased blood glucose just as metformin. Three major compounds; gallic acid, tannic acid and 2, hydroxyl 1,4-napthoquinone were isolated from fraction-c. Diabetic rats administered 2-hydroxyl 1, 4, naphthoquinone (Lawsone) had the lowest blood glucose (156.30±50.59 mg/dl**)** compared to metformin (239.30±25.82 mg/dl), gallic acid (241.80±46.59 mg/dl), tannic acid (240.50±58.85 mg/dl) and untreated diabetic control (477.00±29.36 mg/dl).

Fraction C is the bioactive fraction responsible for antidiabetic activities while 2-hydroxyl 1, 4, naphthoquinone is the compound responsible for the anti-diabetic effect.

Keywords: Antidiabetic, Fractions and compounds, Blood glucose level, *Lawsonia inermis* Linn. leaves, Lawsone, Wistar rats.

INTRODUCTION

Diabetes mellitus is one of the oldest diseases (more than 3000 years) ever witnessed by mankind as stated in Egyptian manuscript (Ahmed, 2011). The distinctive difference linking the two types of diabetes (type I and II) was clearly made in 1936 and type II was initially ascribed as metabolic syndrome in 1988 (Ahmed, 2011; & Diabetic care, 2009).

Many countries in the world have seen a surge in number of cases of diabetes which has been associated with lifestyle changes linked to increased cases of obesity. The metabolic outcome of constant high blood glucose and dyslipidemia increased the incidence of atherosclerosis, chronic kidney disease and partial or total blindness (Diabetic care, 2009). This complication will subsequently lead to increase burden on diabetic patients and the entire public health care system. It has been suggested that adequate awareness about pathogenesis and complications of diabetes will help in prevention, management and treatment of the disease so as to meet the challenges in health care delivery system (Rowley *et al.*, 2012). An estimate of about 366 million individuals had diabetes in 2011 which is projected to increase to about 552 million by the year 2030 (Diabetes atlas, 2011). The numerical population living with diabetes is increasing around the world as 80% of people with this ailment are inhabitant of poor and developing countries. Epidemiological survey has shown that diabetes has been linked to about 4.6 million deaths (Diabetes atlas, 2011). Reports have shown that it is imperative to measure the incidence of diabetes and to design the provision of resources towards managing this common disease (Desai & Tandon, 2009).

Medicinal plants remedies are being explored as alternatives to conventional synthetic drugs for management/treatment of various diseases (Yuan *et al.*,

***Corresponding Author:** Abdulfatai Aremu

2016). *Lawsonia inermis* is a medicinal plant successfully used in African Traditional Medicine for management of diabetes mellitus. The leaves are used alone or combination with other herbs (Elhassan *et al.*, 2020).

Lawsonia inermis Linn (Henna) is a small branched tree with spiny and glabrous shrub and range from 200-800 cm in height [8]. *Lawsonia* is the only plant in this genus and *L. inermis* Linn (syn. *L. alba*) is the only representative of the genus. Belongs to Lythraceae (family); mostly seen in many parts of the world such as South-West Asia, North Africa and many parts of Middle-East (Orwa *et al.*, 2009). This plant is attached to a certain believe signifying prosperity, favours and happiness in Southern Asian nations like Pakistan, India, United Arab Emirate and Iran. It is also used for religious and ritualistic ceremonies among the Hindu and Muslim groups (Ayodhya *et al.*, 2010).

Various researches have shown the importance of substituting chemically synthesize drugs with plant-based products going by the evidence of relating diabetes mellitus with higher formation of free radicals and reduced antioxidant activities (Naziroğlu and Butterworth, 2005). Inspite of producing newer drugs and their validation by various scientist, the research still continues all over the world to evaluate the anti-diabetic effect of compounds isolated from natural agents devoid of toxic effects (Shukla *et al.*, 2011).

Reports have shown that methanolic extract of *Lawsonia inermis* Linn leaves possess a significant anti-diabetic activity. This present study was designed to evaluate the bioactivity guided fractionation and compounds isolation for antidiabetic activities of *L. inermis* on Streptozocine induced diabetic Wistar rats.

METHODOLOGY

Collection, preparation of plant material and Sequential extraction of crude methanol extract of *Lawsonia inermis* Linn. leaves

Leaves of *Lawsonia inermis* Linn was harvested from a farm land in Oke-oyi in Ilorin East area council of Kwara state, North Central, Nigeria. Taxonomically, it was both identified and authenticated at University of Ibadan Herbarium and a specimen was deposited and assigned a voucher number **UIH-22460.** The leaves of *Lawsonia inermis* Linn were dried at room temperature (25^{o}C) under shade in a room for four weeks. Two kilograms of powdery leaves of *Lawsonia inermis* was soaked in 5 litre of N-hexane, ethyl acetate and methanol successively for 72 hours. Each mixture was gently decanted and filtered. The filtrate was immediately evaporated at temp 40°C using a rotary evaporator. The concentrate (wet residue from different solvent) was dried and stored 4^{o}C in the refrigerator until use.

Column Chromatographic Separation of Methanol Fraction

The crude methanol extract was subjected to Vaccumn Liquid Chromatography (VLC), Column Chromatography and Higher performance Liquid Chromatography (HPLC) and fractions obtained were pooled together using Thin Layer Chromatography (TLC).

Vaccumn Liquid Chromatography (VLC)

Lawsonia inermis Linn. leaves (Samples) were pre-adsorbed on VLC silica gel to make slurry and loaded to the column in a dry state. The adsorbent is silica gel 60 e.g Merck 40-63 microns (230-400 mesh). It was then eluted with different combinations of increasing polarity. Graded combinations of analytical graded of methanol, N-hexane and ethyl acetate were used for the separation. Samples were collected into labelled test tubes with particular attention to the solvent used for elution. The progress of elution was monitored with Thin Layer Chromatography (TLC).

Thin Layer Chromatography (TLC)

Pre-coated TLC plates (aluminum foil) were used in this study. TLC Fingerprinting of methanol Fraction of *L. inermis* Linn leaves was done following standard method. Briefly, 15 cm long TLC plate was cut and marked cautiously with a marker. 20µl of the extract was dotted onto marked plate using a capillary tube. N-Hexane: Ethyl acetate: Acetone (1ml: 9 ml: 2ml) was used as mobile phase. The plate was kept in a chromatographic compartment containing the individual solvent system and was covered with glass plate to prevent solvent evaporation. The plate was left in the chamber until the solvent travelled a distance of about 10cm. The plate was then viewed with UV-florescence analysis cabinet at both short and long wavelengths.

Visualization of the Thin Layer Chromatography Plate

The TLC finger printing plate was derivatized with anisaldehyde sulphuric acid reagent followed by heating at 100°C till colored bands of various secondary metabolites appeared. The observations were taken before and after derivatization in visible as well as ultraviolet light.

Rf values were calculated as follows:

$$\text{Distance traveled by substance Rf} = \frac{\text{Distance traveled substance}}{\text{Distance travelled by solvent}}$$

Column Chromatography

The pooled samples were dried and tested on TLC plates to ascertain the various compound(s) in them. Fractions with one or two compounds were selected for further purification using Column Chromatography (CC) method.

***Corresponding Author:** Abdulfatai Aremu

Briefly, 10g of dried and purified methanol fraction of *L. inermis* Linn leaf was dissolved in the mobile phase N-Hexane: Ethyl acetate: Acetone (1ml: 9 ml: 2ml). This solvent system was exposed to column chromatography (CC). Glass-52 Column filled with 650g of silica gel was employed for this procedure. Following different color band development within the column, different fractions collected. Fractions gotten were dried using rotary evaporator reduced pressure (Abbot and Andrews, 1979).

Higher Performance Liquid Chromatography (HPLC) and Mass spectroscopy (MS)

HPLC analysis of samples were done on Shimadzu U-HPLC Nexera system brand (Shimadzu, MA, USA). Basic principle of using HPLC is related to sample and solvent (eluent) distribution in both stationary and mobile phase. Molecules are mostly delayed when moving through the stationary phase. The basic interaction within the sample molecule is column that ensure compound separation at varying times.

Method: HPLC with UV Detector (MS)
Description: CHANNEL 1
Column: uBondapak
Carrier: Acetonitrile/Water (70/30)
Data file: *LAWSONIA INERMIS* LINN (HENNA LEAVE).

Ethical Consideration

This study was ethically approved by ACUREC who is the regulatory body in charge of animal use in University of Ibadan. ACUREC issue a full approval with assigned number: **UI-ACUREC/18/0063.** All stress factors such as handling, feeding, housing, environmental conditions etc were adequately provided and the animals were humanly handled.

Experimental Animals

Wistar rats weighing 120-140g, (70 rats) were used for the two phases of the experiment. Normal temperature and humidity were maintained during the course of this study. The rats were fed with standard animal feed and water which were provided *ad libitum*. BGL of all experimental rats were assessed using fine test glucometer (United Kingdom).

Diabetes induction

Experimental diabetes was induced using streptozocine (STZ). STZ was dissolved in distil water and injected intraperitonially at 65mg/kg.

Bioactive antidiabetic activities of the fractions (A-D)

Experimental rats were randomly grouped to eleven of four rats per group and each group was treated for 5days as thus;
Control: Normoglycemic control
Diabetic untreated: Hyperglycaemic control
Diab+LiFr A-5mg: Diabetic and treated at a dosage 5 mg/kg methanol sub-fraction A of *Lawsonia inermis* Linn leaves
Diab+LiFr A-10mg: Diabetic and treated at a dosage 10 mg/kg methanol sub-fraction A of *Lawsonia inermis* Linn leaves
Diab+LiFr B-5mg: Diabetic and treated at a dosage 5 mg/kg methanol sub-fraction B of *Lawsonia inermis* Linn leaves
Diab+LiFr B-10mg: Diabetic and treated at a dosage 10 mg/kg methanol sub-fraction B of *Lawsonia inermis* Linn leaves
Diab+LiFr C-5mg: Diabetic and treated at a dosage 5 mg/kg methanol sub-fraction C of *Lawsonia inermis* Linn leaves
Diab+LiFr C-10mg: Diabetic and treated at a dosage 10 mg/kg methanol sub-fraction C of *Lawsonia inermis* Linn leaves
Diab+LiFr D-5mg: Diabetic and treated at a dosage 5 mg/kg methanol sub-fraction D of *Lawsonia inermis* Linn leaves
Diab+LiFr D-10mg: Diabetic and treated at a dosage 10 mg/kg methanol sub-fraction D of *Lawsonia inermis* Linn leaves
Diab+Metformin: Diabetic and treated at a dosage of 500 mg/kg metformin.

Bioactive Antidiabetic activities of the three major isolated compounds from fraction-c

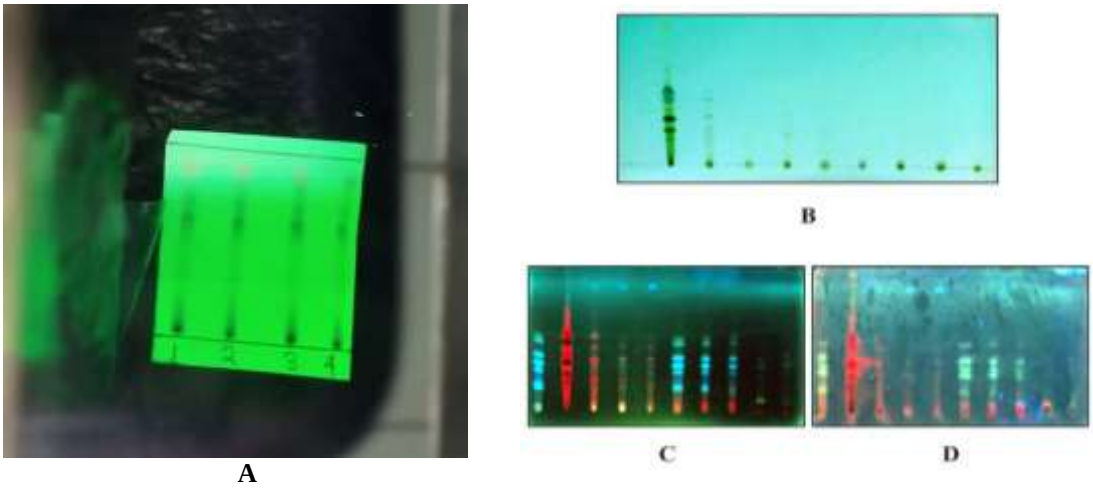
Rats were randomly grouped into six of four rats per group and each group was treated for 5days as thus;
Control: Normoglycemic control
Diabetic untreated: Hyperglycemic untreated control
Diab+Gallic acid: Diabetic and treated at a dosage 1000 μgkg^{-1} of gallic acid
Diab+Tannic acid: Diabetic and treated at a dosage 1000 μgkg^{-1} of tannic acid
Diab+Lawsone: Diabetic and treated at a dosage 1000 μgkg^{-1} of 2-hydroxyl, 1, 4-napthoquinone
Diab+metformin-500 mg: Diabetic and treated at a dosage of 500mg/kg metformin.

RESULT

Result of VLC and TLC

The result of VLC and TLC following solvent combination of chloroform and methanol at increasing ratio showed thirteen different sub-fractions (f1-f13). The TLC result with similar spot and retention factor (Rf) are combined as shown below.

***Corresponding Author:** Abdulfatai Aremu



Thin Layer Chromatography under UV-light

Fractions of *Lawsonia inermis* Linn. leaves

Fraction A (2 spot) = f1(CHCl3 100%) and f2 (CHCl3: MeOH) (97:3)
Fraction B (3 spot) = f3, f4, f5 and f6 (EA and Acetone) (100%, 3:2, 2.5:2.5 and 2:3)
Fraction C (3 spot) = f7, f8, f9 and f10 (EA and Acetone) (2:3, 1:4, and 0.5:4.5)
Fraction D (2 spot) = f11, f12 and f13 (CHCl3: MeOH) (3:2 and 2:3).

The fractions pulled together conclusively showed 10 major spot and the fraction (A-D) were cleanse through running through the column silica gel. The total weight of the dried sample recovered were:
Fraction-A = 102 mg
Fraction -B = 678 mg
Fraction-C = 2.2 g
Fraction-D = 1.7 g

Instrument	:	Shimadzu LC-20ADXR with PDA Detector
Column	:	Supelco Ascentis C-18 (250 mm x 4.6 mm x 5µm)
Mobile phase	:	50:50 (Methanol: Deionized water)
Wavelength	:	334 nm
Flow rate	:	1.0 mL/min
Retention time	:	5.4 mins

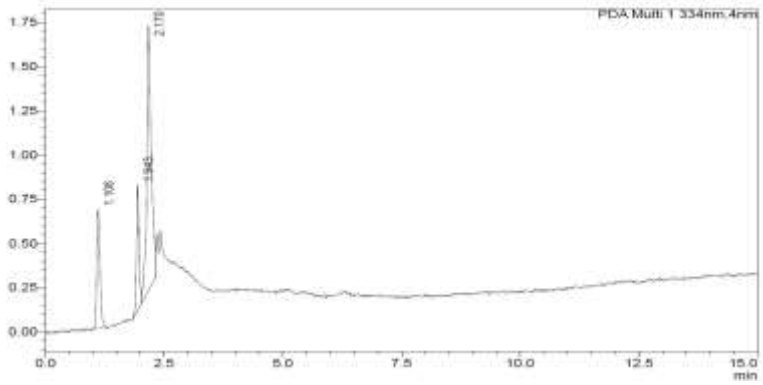


Figure 1: High Performance Liquid Chromatograph of Fraction A from *Lawsonia inermis* Linn leaves

Instrument	:	Shimadzu LC-20ADXR with PDA Detector
Column	:	Supelco Ascentis C-18 (250 mm x 4.6 mm x 5µm)
Mobile phase	:	50:50 (Methanol: Deionized water)
Wavelength	:	334 nm
Flow rate	:	1.0 mL/min
Retention time	:	5.4 mins

***Corresponding Author:** Abdulfatai Aremu

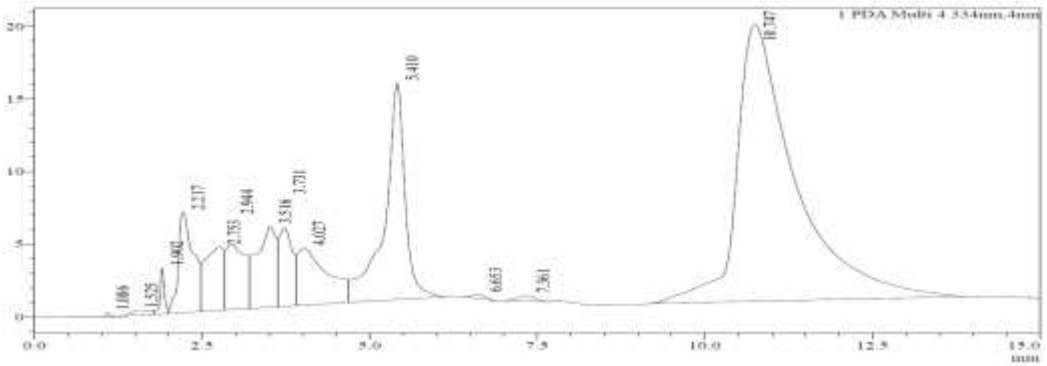


Figure 2: High Performance Liquid Chromatograph of Fraction B *Lawsonia inermis* Linn leaves

Instrument	:	Shimadzu LC-20ADXR with PDA Detector
Column	:	Supelco Ascentis C-18 (250 mm x 4.6 mm x 5µm)
Mobile phase	:	50:50 (Methanol: Deionized water)
Wavelength	:	334 nm
Flow rate	:	1.0 mL/min
Retention time	:	5.4 mins

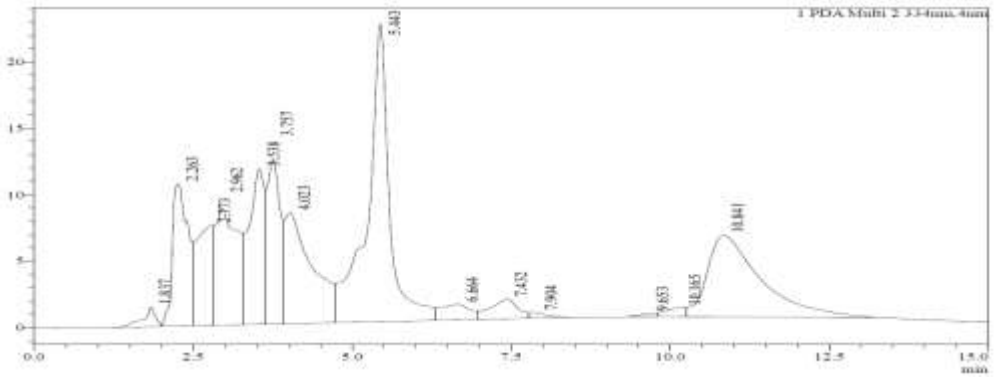


Figure 3: High Performance Liquid Chromatograph of Fraction C from *Lawsonia inermis* Linn leaves

Instrument	:	Shimadzu LC-20ADXR with PDA Detector
Column	:	Supelco Ascentis C-18 (250 mm x 4.6 mm x 5µm)
Mobile phase	:	50:50 (Methanol: Deionized water)
Wavelength	:	334 nm
Flow rate	:	1.0 mL/min
Retention time	:	5.4 mins

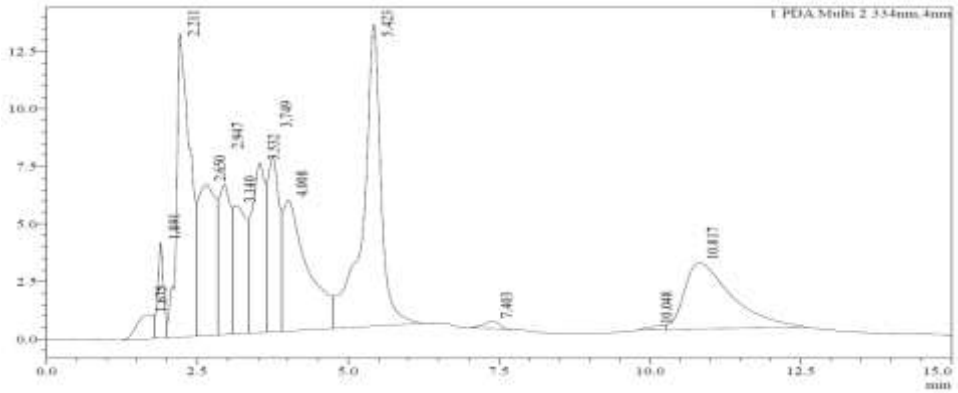


Figure 4: High Performance Liquid Chromatograph of Fraction D from *Lawsonia inermis* Linn leaves

HPLC-MS result of Fraction C

The most active of the four subfractions obtained, Subfraction C, was subjected to HPLC-MS and the three major compounds with highest peak were identified as Tannic acid, gallic acid and 2-hydroxy,1,4-napthoquinones (lawsone) as shown below:

***Corresponding Author:** Abdulfatai Aremu

Figure 5: HPLC-MS of compounds in fraction C obtained from *Lawsonia inermis* leaves

Component	Retention	Area	Height	External	Units
Tannic Acid	1.083	179.9600	19.705	0.0000	
Gallic Acid	1.583	228.6450	14.923	0.0000	
Lawsone	3.133	1477.0080	58.454	0.0000	
		1885.6130		0.0000	

Antihyperglycaemic Bioactivity Guided (A-D) (24 hour)

Antihyperglycaemic activities of bioactive guided fraction of *Lawsonia inermis* Linn leaves over 24 hours in diabetic rats showed non-significant increased blood glucose level (BGL) in both normoglycaemic and hyperglycaemic untreated controls at 1hour post-treatment but all treatment groups showed non-significantly decreased blood glucose except fraction C-10mg/kg (247.20±136.90 mg/dl) that decreased significantly ($p<0.01$) when compared to initial blood glucose (0 hour). At 3 hours post treatment, blood glucose decreased non-significantly in all treatment groups and untreated controls especially Fraction-C-10mg/kg (165.30±71.13 mg/dl) that decreased significantly ($p<0.01$) when compared to 0hour BGL.

6 hours post administration, blood glucose increased non-significantly in all treatment groups and the two untreated controls when compared to 0hour BGL.

12-hours post administration, similar trend was observed just like 6hour showing non-significant increased blood glucose in all treatment groups and normoglycaemic control but hyperglycaemic untreated control (594.80±4.99 mg/dl) increased significantly ($p<0.001$) at 12hours when compared to 0hour BGL.

After 24-hour of treatment, all treatment groups and normoglycaemic control showed significant ($p<0.001$) decreased blood glucose except hyperglycaemic untreated control that increased non-significantly (467.80±27.69 mg/dl) when compared to 0hour BGL.

Sub-fraction B-5mg/kg (100.00±26.77 mg/dl), C-5mg/kg (97.75±7.50mg/kg), D-5mg/kg (105.50±31.82 mg/dl) and 10mg/kg (110.20±10.82) showed significant ($p<0.001$) decreased blood glucose after 24hours when compared to all other treatment groups (Table 1).

All fractions (A-D) reduce blood sugar across 5 days treatment just as metformin. Fraction(c) at 5mg/kg and 10mg/kg have the lowest fasting blood sugar (80.75±15.88 and 124.00±39.60) by day 5 even lower than metformin which is a standard drug (Table 2).

Table 1. 24-hour antihyperglycaemic study of bioactive guided methanol fraction (A-D) of *Lawsonia inermis* Linn leave and metformin in hyperglycemic rats.

Group/Hours	0 Hour	1 Hour	3 Hours	6hours	12 Hours	24 Hours
Control	118.50±10.72	151.00±14.00	116.70±2.52	118.00±3.61	144.10±30.35	84.67±22.74
Diabetic Untreated	562.50±43.49	596.50±5.19	449.50±57.08	565.50±5.45	594.80±4.99[c]	467.80±27.69
Diab+Li Fr A-5mg	410.50±133.20	284.80±77.01	180.50±53.02	219.70±92.20	210.30±81.43	156.50±74.25
Diab+ Li Fr A-10mg	572.50±25.00	409.30±118.00	336.30±121.80	410.30±172.00	500.50±122.10	169.50±63.40
Diab+ Li Fr B-5mg	483.80±131.0	366.30±150.40	241.00±118.90	369.50±227.60	406.80±207.50	150.00±26.77[c]
Diab+ Li Fr B-	548.30±83.90	468.00±113.10	395.80±130.3	480.00±187.4	556.00±70.72	176.50±95.64

***Corresponding Author:** Abdulfatai Aremu

10mg			0	0		[c]
Diab+ Li Fr C-5mg	241.80±92.40	199.50±72.35	166.00±63.36	171.50±90.55	178.50±85.24	97.75±7.50[c]
Diab+ Li Fr C 10mg	568.00±29.03	247.20±136.90[b]	165.30±71.13[c]	201.00±119.00	272.80±138.40	136.50±67.68[c]
Diab+ Li Fr D-5mg	580.00±16.33	397.20±59.57	284.80±47.79	289.50±46.69	566.30±11.09	105.50±31.82[c]
Diab+ Li Fr D-10mg	555.00±65.38	339.70±83.94	240.00±91.65	424.70±243.50	470.00±205.30	110.20±10.82[c]
Diab+Metformin	568.70±15.28	335.30±103.60	340.30±104.51	431.70±44.24	596.70±4.16	158.70±64.67[c]

Results are shown as Mean ±SD: n=4
a b c *Significant ap≤0.05 bp≤0.01 cp≤0.00

Table 2. Blood Glucose Levels (Mg/Dl) Of Wistar Rats Administered Methanol Fractions A-D of Lawsonia Inermis Linn Leaves in Hyperglycemic Rats

Groups/days	Day 1	Day 2	Day 3	Day 5
Control	84.67±22.74	83.67±23.25	106.00±4.58	77.33±10.02
Diabetic untreated	467.80±27.69	452.80±22.23b	424.30±63.69[c]	351.50±87.88[c]
diab+LiFr A-5 Mg	156.50±74.25	146.50±74.25	167.50±45.96	138.50±75.66
diab+LiFr A-10 mg	169.50±63.40	174.50±59.61a	343.30±126.30[c]	140.00±58.85
diab+LiFr B-5 mg	150.00±26.77[c]	140.00±16.77	188.00±75.42	126.00±48.17
diab+Li Fr B-10 mg	176.50±95.64[c]	158.50±46.94	246.50±98.76	192.00±78.25
diab+LiFr C-5 mg	97.75±7.50[c]	91.25±13.65	122.80±18.21	80.75±15.88
diab+LiFr C 10 mg	136.50±67.68[c]	108.80±36.31	111.30±41.05	124.00±39.60
diab+LiFr D-5 mg	105.50±31.82[c]	104.80±30.32	169.00±49.16	143.30±46.60
diab+LiFr D-10 mg	110.20±10.82[c]	84.00±10.82	183.30±53.45	140.00±38.16
diab+Metformin	158.70±64.67[c]	152.60±61.70	292.00±12.17[b]	172.70±24.70

Results are shown as Mean ±SD: n=4
a b c *Significant ap≤0.05 bp≤0.01 cp≤0.001

Anti-Hyperglycemic Study Using the Three Major Compounds from Fraction-C of *Lawsonia Inermis* Linn Leaves

Blood glucose of diabetic treated with major compounds; gallic acid, tannic acid and 2, hydroxyl 1,4-napthoquinone from fraction C of *Lawsonia inermis* Linn over 5days showed non-significant decreased BGL. BGL rises significantly ($p<0.01$) across 5days treatment in diabetic untreated control while all treatment groups presented non-significant ($p>0.05$) decreased BGL compared to hyperglycaemic and normoglycaemic control groups (Table 3).

The three major compounds reduced BGL in the course of the 5-days treatment similar to that observed for rats administered metformin. Rats administered Lawsone (2-hydroxyl 1.4, naphthoquinone) had the lowest blood glucose (156.30±50.59 mg/dl**)** on day 5 which was lower than that observed in rats administered metformin, gallic acid or tannic acid (Table 4).

Table 3. 24-hours Anti-hyperglycaemic study for three major compounds in fraction-c; Tannic acid, Gallic acid and 2-hydroxyl, 1.4-napthoquinones (Lawsone).

Group/Hours	0 Hour	1 Hour	3 Hours	6hours	12 Hours	24 Hours
Control	99.50±21.14	91.00±16.75	96.75±13.52	109.30±15.15	113.50±20.01	108.50±12.82
Diabetic Untreated	575.30±28.58	503.50±54.32	536.30±57.08[c]	600.00±0.00[a]	587.50±9.71	600.00±37.38[c]
Diab+Gallic Acid 1mg/Kg	600.00±0.00	446.80±88.65[b]	435.30±87.66[b]	524.00±5.35	575.50±9.23	591.30±6.19[c]
Diab+Tannic Acid 1mg/Kg	595.80±8.50	447.80±59.22[b]	419.00±64.05[b]	535.50±53.33	551.50±56.98	587.30±4.57
Diab+Lawsone 1mg/Kg	568.00±64.00	361.30±62.76[c]	327.00±84.00[a]	479.50±31.89	464.00±61.30	509.00±78.77
Diab+Metformin 500mg/Kg	566.50±41.10	437.30±38.23[b]	363.80±29.92[c]	562.80±40.14	594.50±9.76	591.50±11.36

Data rep. as Mean ±SD: n=4
a b c Significant a p≤0.05 b p≤0.01 c p≤0.001

***Corresponding Author:** Abdulfatai Aremu

Table 4. Blood glucose of three major compounds in sub-fraction C; Tannic acid, Gallic acid and 2-hydroxyl, 1.4-napthoquinones (Lawsone).

Groups/Days	Day 1	Day 3	Day 5
Control	108.50±12.82	86.50±14.89	76.25±11.44
Diabetic Untreated	600.00±37.38^{c}	575.30±37.38^{c}	477.00±29.36^{c}
Diab+Gallic Acid 1 Mg	591.30±6.19^{c}	351.30±75.07^{c}	241.80±46.59^{c}
Diab+Tannic Acid 1 Mg	587.30±4.57	327.50±74.67^{a}	240.50±58.85^{c}
Diab+Lawsone 1 Mg	509.00±78.77	187.50±58.95	156.30±50.59
Diab+Metformin	591.50±11.36	416.30±115.40^{c}	239.30±25.82^{a}

Results are shown as Mean ±SD: n=4
$^{a\,b\,c}$ Significant ^{a} p≤0.05 ^{b} p≤0.01 ^{c} p≤0.001

DISCUSSION

Report have revealed that there are several medicinal plants that have shown promising therapeutic actions against diabetes (Patel *et al.,* 2012). Many plants have been reported to exhibit significant blood glucose reducing properties through various mechanisms that are related to most synthetic oral hypoglycaemic agents. Most of these plants have not been validated or accepted as therapy of choice in treating diabetes mellitus because they lack scientific validation and evaluation (Campbell-Tofte *et al.,* 2012). In this study, extracts of *Lawsonia inermis* Linn. leaves were purified to obtain bioactive fractions as anti-diabetic agent. TLC fingerprinting of methanol extract of *Lawsonia inermis* Linn. leaves and bands were seen at distinct intervals following TLC derivatization and various compounds were seen on the TLC plates. Among those fractions, methanol fraction-c showed the most potent *in vivo* anti-diabetic activities in diabetic induced rats. Most medicinal bioactive compounds from plants have been use in conferring protection against inflammatory mediators in diabetes (Arulselvan *et al.,* 2014). All the bioactive fractions studied showed a great anti-hyperglycemic activity in all the tested four fractions (A-D) but fraction C showed improved anti-diabetic effect that is closely correlated to non-diabetic control. This observation is in line with report of Kumar *et al.* (2010) that confirmed bioactive fractions of *L. inermis* Linn have anti-hyperglycemic activities. The anti-diabetic potential of various fraction from *L. inermis* Linn leaves is directly linked to facilitation of insulin deliverance from pancreatic beta-cells leading to inhibition in absorption of glucose from the gut leading to stimulation of glycogenesis and subsequent protection of the DNA from damage as a result of oxidative injury (Kabbaoui *et al.,* 2016).

Reports have shown that naphthoquinone-containing medicinal plants have antidiabetic action. This is directly linked to anti-diabetic effect of *Lawsonia inermis* Linn which have 2-hydroxyl,1,4-napthoquinone (Lawsone) as the primary phytoconstituent (Kumar *et al.,* 2010, Kabbaoui *et al.,* 2016). The result of the hyperglycaemic study of the major compound(s) in subfraction C presented non-significant reduction in blood sugar across 5 days treatment just as metformin. 2-hydroxyl,1,4-napthoquinone (Lawsone) showed the lowest BGL at day 5 lower than metformin, gallic acid and tannic acid. The blood glucose of untreated diabetic rats rises significantly and this follow the report of various observation that confirmed increased blood sugar in STZ-induced diabetic rats (Kumar *et al.,* 2010).

Gallic acid and its derivative are reported to have high antioxidant natural product thereby leading to promising compound for new drug development (Bharti *et al.,* 2015). It is one of the phenolic compounds that have antidiabetic activity but its use is reported to be unsatisfactory because of its fast degradation during the absorption process (Bharti *et al.,* 2015**).** It was reported that gallic acid is beneficial in treating myocardial damage associated with insulin-dependent form of diabetes (Sujit *et al.,* 2011). Tannic acid stimulates GLUT and inhibit fatty cell differentiation (Liu *et al.,* 2005). Reports have shown that tannic acid significantly decreased body weight, blood glucose and creatinine level but increased insulin and glycogen in STZ-induced diabetic rats thereby concluding that it possesses anti hyperglycaemic action (Bharathi *et al.,* 2017). Oboh *et al.* (2019) reported an additive action between gallic and tannic acids at ratio mixture (1:1) leading to non-significant decreased blood glucose in diabetic rabbits.

CONCLUSION

The results obtained from this present study showed that Fraction C (Fc) is the bioactive fraction responsible for its antidiabetic activities observed in *Lawsonia inermis* Linn leaves while 2-hydroxyl 1, 4, naphthoquinone is the compound responsible for the anti-diabetic effect of the plant. The possible antidiabetic mechanisms are through the facilitation of insulin deliverance from pancreatic beta-cells leading to stimulation of glycogenesis and protection of DNA from damage as result of oxidative injury.

Acknowledgement
We wish to acknowledge the contribution of Shera Bint Bashar who conducted the HPLC and MS of the various fractions and compounds.

Competing interest

***Corresponding Author:** Abdulfatai Aremu

There was no any conflicting interest in the course of this research

Authors Contribution

Abdulfatai A and Olayinka A Oridupa: Conceptualization and designs of the work and manuscript preparation

Akorede G.J, Olatunji A.O and Basiru A. Dosing of experimental rats with the fraction and the compound

Abdulfatai A, Akeem O. Ahmed: Data analysis and manuscript review

Funding: No fund was received.

Data availability statement

Data that support the finding in this study are openly available in the stated reference.

REFERENCES

1. Abbot, D., & Andrews, R. S. (1979). *An introduction to chromatography*. Longman Press, London.
2. Ahmed, A. M. (2011). Historical approach of diabetes mellitus. *Saudi Medical Journal*, *23*(4), 373-378.
3. Arulselvan, P., Ghofar, H. A. A., Karthivashan, G., Halim, M. F. A., Ghafar, M. S. A., & Fakurazi, S. (2014). Antidiabetic therapeutics from natural source: A systematic review. *Biomedicine & Preventive Nutrition*, *4*(4), 607-617.
4. Ayodhya, S., Kusum. S., & Anjali, S. (2010). Hypoglycaemic effect of different extracts of various medicinal plants seen in Ayurveda. *International journal of research pharmaceutical sciences*, *1*(1), 212–224.
5. Bharathi, T. R., & Prakash, H. S. (2017). Comparative evaluation of antidiabetic and antioxidant potency of different extracts obtained from *Memecylon species.* International journal of Pharmacy and Pharmaceutical science, 9,187-91.
6. Bharti, B., Neha, S., Rita, K. (2015). Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications. *ASC advances journal*, *4*(2), 340-452
7. Campbell-Tofte, J. I., Mølgaard, P., & Winther, K. (2012). Harnessing the potential clinical use of medicinal plants as anti-diabetic agents. *Botanics: targets and therapy*, *2*, 7-19.
8. Desai, A., & Tandon, N. (2009). Challenges in prevention and management of diabetes mellitus and metabolic syndrome in India. *Current Science*, 356-366.
9. American Diabetes Association. (2009). Diagnosis and classification of diabetes mellitus. *Diabetes care*, *32*(Supplement_1), S62-S67.
10. Diabetes mellitus Atlas (2011). *Merck Veterinary Manual* (9th Ed., & online version): Archived from the original on 27-09-2011. Retrieved 23-10-2011
11. Idm'hand, E., Msanda, F., & Cherifi, K. (2020). Ethnopharmacological review of medicinal plants used to manage diabetes in Morocco. *Clinical Phytoscience*, *6*(1), 1-32.
12. Kabbaoui, M., Chda, A. L. A. E., Mejrhit, N. A. J. L. A. E., Azdad, O. U. A. R. D. A., Farah, A. B. D. E. L. L. A. H., Aarab, L. O. T. F. I., ... & Tazi, A. B. D. E. L. A. L. I. (2016). Antidiabetic effect of Thymus satureioides aqueous extract in streptozotocin-induced diabetic rats. *Int J Pharm Pharm Sci*, *8*(9), 140-145.
13. Shiv, K., & Alagawadi, K. R. (2010). Hypoglycemic effect of Argyreia nervosa root extract in normal and streptozotocin-diabetic rats. *Der Pharmacia Lettre*, *2*(2), 333-337.
14. Liu, H. A., Li, S. H., & McNeill, J. H. (2005). In vivo activity of *vanadium* on glucose transporter (GLUT4) translocation in cardiac tissue of STZ-induce hyperglycemic rat. *Molecular and cell biochemistry*, 217, 121-129.
15. Naziroğlu, M., Butterworth, P. J. (2005). Protective effects of moderate exercise with dietary. *Indian journal of experimental biology*, *40*(6), 900–902.
16. Oboh, G., Ogunsuyi, O. B., Adegbola, D. O., Ademiluyi, A. O., Oladun, F. L., 2019 Influence of gallic and tannic acid on therapeutic properties of acarbose *in vitro* and *in vivo* in *Drosophila melanogaster*. *Biomed journal*, 42(5), 317–327.
17. Orwa, C., Mutua, A. R., Kindt, J. R., & Simons, A. (2009). *Agro-forest tree database: Tree reference and selection guide, 2009*. World Agro-forestry Centre, Kenya. http://www.worldagroforestry.org/output/agroforestree-database.
18. Patel, D., Prasad, S., Kumar, R., & Hemalatha, S. (2012). An overview on antidiabetic medicinal plants with insulin mimetic ability. *Asian Pacific journal of tropical biomedicine*, 2, 320–330.
19. Rowley, W. R., & Bezold, C. (2012). Creating public awareness: state 2025 diabetes forecasts. *Population health management*, *15*(4), 194-200.
20. Shukla, A., Bukhariya, V., Mehta, J., Bajaj, J., Charde, R., ……&Charde, M. (2011). Vitamin C and E on blood antioxidative defense mechanism in rats with streptozotocin. *India journal of Pharmacology*, *3*(2), 123-156.
21. Sujit, T. K., Savita, R. K., Sohrab, A. S. A., & Manikrao, M. (2011). Bactericidal and bacteriostatic activity of isolated naphthoquinone from *Lawsonia inermis* Linn and synthetic Lawsone on *Staphylococcus spp* of epidermis. *Pharmacology online*, *2*, 156–163
22. Yuan, H., Ma, Q., Ye, L., & Piao, G. (2016). The traditional medicine and modern medicine from natural products. *Molecules*, *21*(5), 559.

***Corresponding Author:** Abdulfatai Aremu