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Comprehensive Evaluation Of Ehretia Laevis Leaf Extract: In-Vitro Enzyme Inhibition, Anti-Oxidant Activities, And In-Vivo Anti-Diabetic Effects In Stz-Induced Diabetic Rats

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ABSTRACT
In this study, we have aimed to detail evaluation for in-vitro and in–vivo enzyme inhibitory, antioxidant potential of leaf extracts of *Ehretia laevis* for the development anti-diabetic drug. In-vitro activity was tested using standards protocols for ant diabetic studies especially alpha-amylase and alpha-glucosidase inhibitory activities**.** DPPH assay for Antioxidant Activity STZ-induced diabetic albino rats were used to evaluate in-vivo anti-diabetic efficacy. Animals were divided into 7 groups, of which normal control group (NC), diabetic control group(DC) and treated with metformin as standard drug; test animals L1 in hexane fraction at three doses 100 mg/kg/day bwt, 200 &300mg/kg/d after confirmation was given for the period of approx seven days to study its toxic effect. This include blood sugar levels, serum lipid profile, liver function (SGOT &SGPT) and kidney function test. Inhibitory potential of L2 (higher potency) in α-amylase & α-glucosidase were significant as compare to extracts from L1 Table 7. The antioxidant potential test has been shown to have a significant DPPH scavenging activity. Hypolipidemic, hypoglycemic and anti-acetylcholinesterase activities also were demonstrated in extract treated groups which was accompanied by a significant reduction of blood glucose level; lipid profile improvement including liver enzymes normalization detected on the study using in-vivo. HPTLC analysis proved the presence of β-sitosterol and gallic acid. Leaves extracts of E. laevis have a marked antihyperglycemic, enzymes inhibitory and antioxidant activity that could be worthy for management of diabetes mellitus complications in the public health service arenas are feasible as natural therapeutic agents due to popular use with minimal side effects on patients.

Introduction

One of the chronic conditions that impairs glucose metabolism in humans is diabetes mellitus immunologically, this is when there are less quantity of insulin produced by beta cells with lack/absent activity on other body tissues [1]. This situation is more prevalent in obesity and involves altered metabolism of protein as well as fat [2]. It has been termed a driver of predication 2 diabetes and there are varied epidemiological studies to bear witness this claim [3]. Thus, disruption of glucose pathways is not likely to be well tolerated and will result in a catastrophic derangement of blood sugar homeostasis – hyperglycemia [4]. Secondary complications while hyperglycemia Insulin mechanism is disturbed, during the same time Hyperlipidemic and Oxidative stress pathways leading to diabetic retinopathy, neuropathy, cardiovascular diseases, ulcers & amputation [5-6]. Increased production of ROS may disturb cellular physiology, energy generation and signal transduction in normal cells [7], as well leading to damages on proteins, lipids or DNA due to dysfunctioning enzymes[8]. These processes can ultimately lead to significant organ dysfunction such the eyes and kidneys [9] and DM is also associated with various clinical conditions like ischemic heart diseases [10], delayed wound healing. It shortens life expectancy of patients having diabetes [11,12]. Hyperlipidemia is a known risk factor for diabetic-induced, premature atherosclerosis as evidenced by increased histologic analysis of early-onset and progressed lesions [10-13]. Plants with hypolipidamic and antidiabetic activity comprising of antioxidants are used to treat hyperlipidaemic conditions [11]. Even with the availability of wide range of medicines for combating obesity, diabetes and hyperlipidemia conditions in modern medical science, there are certain treatments offers side effects. Hence, there is a slow tendency towards the antiquated herbal medicinal methodologies owing to prevent such complications [14,15]. Hyperlipidemia and its complications are treated with conventional diabetes, which causes many side effects such as gastrointestinal disorders [16], weight gain, and cardiovascular diseases. These very realistic side effects interfere with the life of patients and could lead them not to comply with treatment. Therefore, individuals are turning to alternative therapies that will control the disease with little side effects [17]. Various classes of polysaccharides are the constituents of different Herbal drugs and which has been shown to be helpful in anti-diabetic medications [18]. These natural remedies are also considered to be more powerful, and less toxic than their synthetic counterparts. Herbal medicines are believed to function by multiple pathways which include enhanced insulin response at the receptor site, regeneration of beta cells in pancreas islet, reduction in oxidative stress levels and modulation of lipid profile [19]. Hence, they are considered as lucrative candidates for diabetes control. The interest of using herbs as remedies has been into increase in the number of research works to authenticate these natural medicines [19]. Scientific researches in the field of plants

have shown that more than 90% of plants contain biologically active compounds and many can be used to manage diabetes according chemical base consists polyphenols, flavonoids, alkaloids and terpenoids which could effect on BC-DECM [20]. In fact, these compounds could potentially obstruct the activities of certain enzymes vital for carbohydrate digestion as well as bring about an important release of insulin while offering antioxidant effects hence serving like a natural way to control blood sugar and metabolism [20] The medicinal plant Ehretia laevis, a shrub used in the traditional medicine of several countries for treatment and/or alleviation of various ailments; recently been shown to possess an anti-diabetic effect [21]. Various parts of the plant such as leaves, roots, barks and stems have been utilized for treatment of various ailments due to phytochemicals content [22]. It contains flavonoids, tannins, alkaloids and saponin (some of which have been reported to exhibit different pharmacological properties). In recent years, research oriented towards scientific confirmation of the anti-diabetic property(s) of *Ehretia laevis* has been initiated [23]. I will get a little more into the research on how bananas leaves have been found to help with blood sugar regulation and metabolic disorder in that context. From the ancient studies, hypoglycaemic effect of aqueous extract from *Ehretia laevis* (Stem) on some metabolites in diabetes mellitus is mentioned [24] and others has demonstrated that use of leaf extracts are potential for management of general diabetic conditions. The anti-diabetic effect of *Ehretia laevis* appears to be mediated by an extract on some enzymes involved in carbohydrate metabolism [25]. Such plant extracts have, for example shown α- amylase and α-glucosidase inhibitory capacity [26], since both are enzymes that hydrolyze dietary carbohydrates into glucose. *Ehretia laevis* is used to inhibit these enzymes in order to avoid the uptake of glucose in blood and hence prevent postprandial hyperglycemia [27]. Its antioxidant activity is also very high which are important in the management of diabetes and its complications as well. Diabetic patients present with high blood glucose levels, which initiate ROS generation and oxidative stress that can damage the cellular structures [28]. Due to the oxidative stress it can result in cell damage, but this effect is neutralized by antioxidants present in *Ehretia laevis* since they are able to scavenge ROS produced in vivo. This antioxidant activity also affects the regulation of glucose level and prevents secondary disorders such as diabetic retinopathy, neuropathy and cardiovascular diseases [29]. Hence, the specific objective of this study was to evaluate phytochemical and pharmacological activities of E. laevis leaves with special reference to diabetes. This is carried out by assessing α-amylase and α-glucosidase inhibition, DPPH radical scavenging activity as well its effect on diabetic rats through blood glucose level lipid profile liver function tests kidney function test [30]. These will form the scientific basis of investigating Enantia laevis in diabetes management and its complications as such; a step towards compilation evidence for rational use of herbal agents in diabetic treatment [31].

Material and Methods

Collection and pre-treatment of E. laevis leaves

The aerial parts of E. laevis, harvested from a plant of moderate size growing in the Wardha district of Maharashtra, India, were used in this study and these were the mature leaves. These leaves were then air-dried under aseptic conditions for 6-7 days in order to get a coarse powder [32]. After that, the dried plant materials were stored in airtight containers at the room temperature condition. The plant was identified and got an authentication and certification from the CSIR-NIScPR, Delhi [33].

Defatting of Sample: The finely powdered sample was placed in a thimble, which was then inserted into the Soxhlet apparatus chamber. Defatting was carried out using the non-polar solvent hexane [34].

Extraction of E. laevis Leaves: After the complete removal of hexane, the dried powder was reduced to a particle size smaller than 0.5mm to increase the surface area [35]. This finely powdered sample was placed in a thimble and inserted into the Soxhlet chamber. Ethanol was boiled in the flask to vaporize, and the vapors were then condensed in the condenser. When the solvent reached the siphon arm, it dripped back into the Soxhlet flask, continuing the process until all the solvent from the flask was condensed. The solvent was then evaporated from the extract at room temperature [36].

Fractionation of Extract: The hydroalcoholic fraction was transferred into a separation funnel, shaken, and allowed to settle. Then, 50mL of n-hexane was added to the funnel, shaken, and allowed to settle. The aqueous layer was separated from the funnel, and the remaining n-hexane fraction was poured into a clean glass petri dish. The process was repeated with 50mL of n-hexane until no extract appeared in the n-hexane fraction [37].

HPTLC Analysis: Preparation of Standards Solution (β-Sitosterol and Gallic Acid Standards): β-sitosterol (β-s) and Gallic acid (G.A) was treated as standard solution. Approx 10mg of β-s and G.A were weighed and were dissolved in 10ml of methanol to make up the volume [38]. Then 1 ml of stock solution was transferred to 10 ml of volumetric flask and methanol was added to make up the volume (0.1mg/ml).From these, standard solutions was further prepared via transfer of aliquots (0.1, 0.2,0.3,0.4,0.5 and 0.6 ml) corresponding to (1, 2, 3, 4, 5, and 6 µg/ml) of the stock solution to 10ml Toluene: ethyl acetate (7:3 v/v) was used to elute the compounds in the TLC system. The TLC analysis was performed with pre-coated silica-gel aluminum plates 60 F254 (10x20 cm) with 0.2 mm layer of thickness [39-41].

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Method

In the case of samples, a pre-coated silica-gel aluminum plates F254 were touched gently with the tip of a Hamilton syringe (100µl) equipped with automatic sample applicator Camag Linomat-5 and 8µl of the test solution were applied. Each sample was spread in 6 mm length bands and it was placed 15 mm from both the bottom and left edge of the plate and the bands were placed 10 mm apart[42]. The positive controls, β-Sitosterol and Gallic acid standards, was likewise applied in a manner similar to the sample plugs, in 6 mm length bands. The plate was prepared by using a mobile phase of toluene and acetate buffer in the ratio of 7:3 v/v. Linear ascending development was carried out in a 20 x 20 cm twin trough glass cell which was pre-saturated with 20 ml of the mobile phase for 30 minutes at room temperature [43]. The chromatogram was allowed to develop up to a distance of eighty-five centimeters. After this the thin layer chromatography plate was left to dry at room temperature for some time. The peak areas of both the samples and standards were photographed using a Camag Reprostar 3 photo documentation system and the densitometric scanning was done using Scanner 4. Standard calibration curves were constructed using peak areas of the standard marker compounds; β-Sitosterol and Gallic acid [44].

In-vitro Activity

α-Amylase activity: The extract at 10 µg/mL was tested for alpha-amylase activity. A 500 µl aliquot of the extract was incubated with an equal volume using Hog pancreatic α-amylase at a concentration of 0·5 mg/ml [45]. The mixture was kept at 25°C for 10 min during the enzymatic reaction. To reaction mixture 500 µl of a 1% starch solution in 0.02mol/L sodium phosphate buffer was added and incubated at 25°C for another 10 minutes. Among them, 1 ml of DNSA reagent was used to stop the reaction. The solution obtained was heated in n-hexane at 100°C for 5 minutes and cooled to 25°C using a water bath, diluted with distilled water up to volume (10 ml) from which an absorbance were measured at 540 nm using Biotron Fluostar plate reader. The absorbance readings were scored for the measuring of alpha-amylase inhibitory activity in the test extracts [46].

alpha-Amylase inhibitory activity was determined as;
% Inhibition = [(Abs Untreated - Abs Samples) / Abs Untreated] × 100

α-Glucosidase Activity: 10 µg/ml of test extract was incubated with 100 µl of alpha-glucosidase solution for 10 min at 25^0C. After incubation, 50 µl of 5 mmol/L p-N-D glucopyranoside solution in 0.1 mol/L phosphate buffer (pH 6.9) was added. The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance was measured at 405 nm using a plate reader (Biotron Fluostar, Germany)[47].

Inhibition of alpha-Glucosidase activity was determined by percentage inhibition = ((Absorbance of untreated - Absorbance of samples) / Absorbance of untreated) × 100

Antioxidant Activity:

DPPH Assay: The antioxidant activity was thus determined on the basis of the amount of DPPH radical which has been reduced and the decrease in absorbance. Daily, a new working solution of DPPH at a concentration of 6×10^{-5}M in methanol was prepared before the UV analysis. Hundred micrograms per milliliter of the test extracts were mixed with 3 ml of this solution. The samples were left at RT and protected from light for 15min before analysis. Fluorescence intensity was measured at 515 nm using a BMG Fluostar, Germany while percentage inhibition was computed using MARS software (MGF, Germany)[48,49].

In-vivo Activity: Wister albino rats of either sex in the weight range of 150-200 grams were placed in polypropylene cages with paddy husk as bedding at a temperature of 24 ± 2°C and relative humidity of 30-70%. Lighting regime was kept at 12/12; that is, for twelve hours, the light was on and then off for twelve hours. Water was given to the rats in Burlington glass ad libitum and the rats were fed standard commercial pelleted rat chow procured from M/s. All the mentioned procedures were done according to CPCSEA and were accepted by the institutional animal ethical committee [50].

Acute Toxicity Study: OECD guidelines 423 were followed for acute toxicity study**.** The weights of the male non-pregnant rats were recorded; a single dose of the test substance was administered orally to the rats with the help of a rat oral gavage needle. Food was withdrawn for 2 hours after administration but water was not limited. The patients were also monitored individually for at least 30 min, during the first 24 h with more intensive monitoring during the first 4 h, and then daily for 14 days[51].

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Diabetes Induction: STZ injection was used for the induction of DM which was prepared by using 0.1 M citrate buffer (pH4.5). The dose was administered via i.p. as per 50mg/kg body weight [52]. After one week of injection DM was confirmed. Citrate buffer solution (pH 4.5) was given to control group. Blood was withdrawn via ocular puncture after 7 days of treatment and blood glucose level was measured. In this study rats with diabetic disoreders were used and their glucose level with fasting plasma found to be 280-350mg/dl [52].

Experimental Design: 30 Albino rats were taken in a random way into 6 groups of each:

- Group 1: It contains normal control
- Group 2: This group has Diabetic control
- Group 3: This group received Metformin of 200mg/kg
- Group 4: This group received hexane fraction L1 (200mg/kg)
- Group 5: This group received hexane fraction L1 (400mg/kg)
- Group 6: This group received alcoholic fraction L2 (200mg/kg)
- Group 7: This group received alcoholic fraction L2 (400mg/kg)

Blood and organ samples collection with measurement of blood glucose level

These treatments were taken, depending on the dosage, once a day for twenty one days. Systolic blood pressure was measured at least once per day using a tail cuff method and blood glucose was determined at least once per day by using Accu-Chek glucometer after the blood sample was obtained by the nicking the lateral tail vein with a scalpel blade. All the observations were made in duplicate in order to minimize variations [53]. Last but not the least, the rats were sacrified under a slight anesthetic effect on the last day of the treatment after fasting for 12 hours. Aseptically 10 ml of blood sample was taken in an EDTA vial and allowed to clot for 15-20 minutes and then centrifuged at 3000 r.p.m. for 10 minutes and the separated serum was taken and stored for biochemical analysis. The pancreas and liver were immediately excised in ice-cold isotonic saline solution, fixed in 10% formal saline for the subsequent tests and stored at -4°C in phosphate buffer saline (1. 0X) [53].

VLDL and LDL cholesterol were calculated as per Friedevald's equation:
VLDL-cholesterol = Serum triglyceride/5; LDL-cholesterol = Serum total cholesterol - VLDL-cholesterol - HDL-cholesterol.
Outcome measures were reported in mg/dl

SGPT: SGPT speed up the conversion of L- Alanine and α-ketoglutarate to pyruvate and glutamate. The pyruvate formed reduces 2, 4-dinitrophenylhydrazine to from a brown coloured complex and the concentration of the complex is determined. Briefly, 0. 3 ml of the substrate reagent, 0. 2 ml of pyruvate standard; 0. And 1 ml of distilled water, and 0. 05 ml of DNPH reagent were also mixed and allowed to stand for 20 minutes at room temperature. OD was also occasionally checked and used in Calibration as well. Then, 0 To 5 ml substrate reagent, which was warmed at 37°C for 3 minutes, to this, 1 ml of the serum sample was added and the mixture was allowed to interact at 37°C for half an hour. To the above mixture 0. Of DNPH reagent, 5 ml was added, shaken and left to stand at the laboratory temperature for 20 minutes. To this, NaOH reagent (5 ml) was added and the contents mixed and allowed to stand for 10 minutes for warm temperature or 15 minutes for cold temperature. Next, absorbance of OD was measured at 505 nm [54].

SGOT: SGOT is involved in the transfer of an amino group from L-Aspartate to α-ketoglutarate, to produce oxaloacetate and glutamate. The produced oxaloacetate reacts with NADH in presence of malate dehydrogenase enzyme and converts it to NAD. The increase in absorbance of NADH to NAD is monitored and this is directly proportional to the activity of SGOT present in the sample. In brief, enzyme (1 ml) and start reagent (5 ml) were mixed to obtain the working reagent. Working reagent of 100 µl was added to 20 µl serum sample and the OD was noted at 340 nm at 25-30°C at 2 min and 3 min. Following this, the difference in OD was obtained and this value was then multiplied with the factor 952. The readings were expressed in U/L [55].

Results

HPTLC Analysis: From the HPTLC analysis of the ethanolic and hexane fractions of sample L1, it was found out that the percentage composition of the sample was 0. 35% β-sitosterol while sample L2 contained 1. 72% Gallic acid. This result also pointed to the existence of several other chemicals in the two samples as well.

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Table 1: HPTLC analysis of E. Laevis leaf Extract

Sr. No.	Test Parameter	Units	Results
1.	β-Sitosterol	%	0.35
2.	Gallic Acid	%	1.75

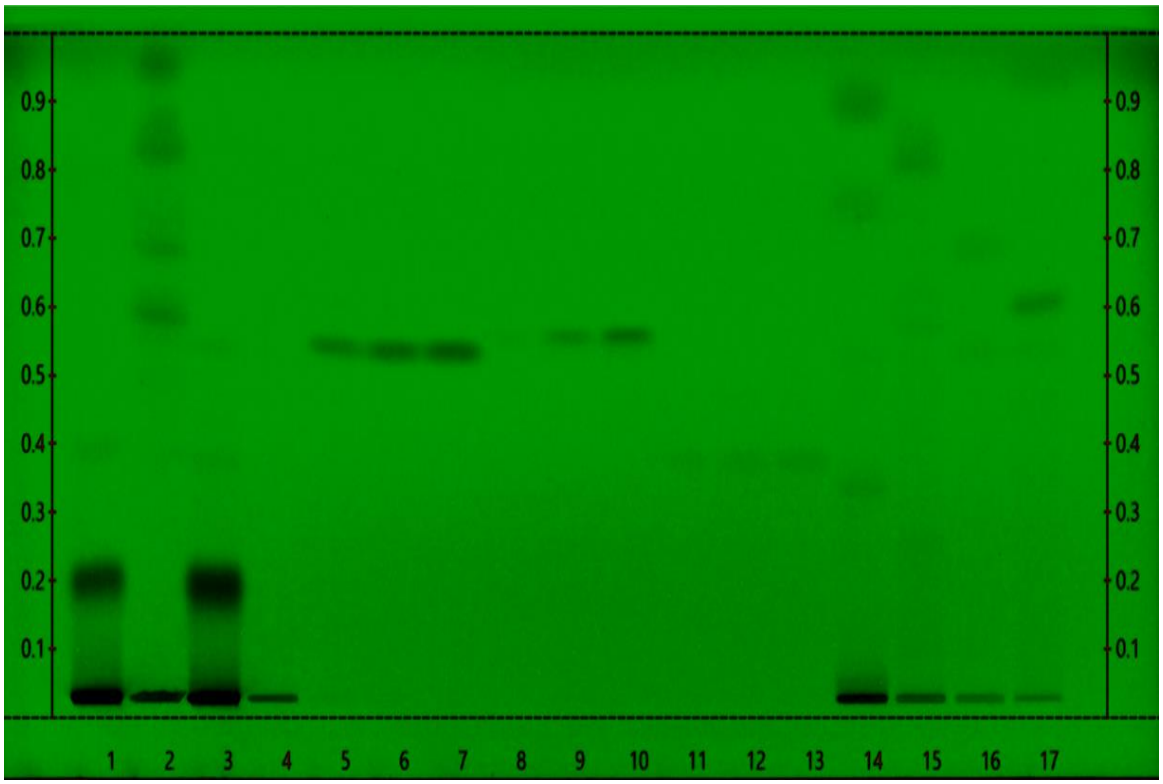

Figure 1: Graph of HPTC analysis of E. Laevis Leaf Extract

Abbreviation- Track-1: Sample L1; Track-3: Sample L1; Track-5: Gallic acid standard; Track-6: Gallic acid standard; Track-7: Gallic acid standard; Track-8: Gallic acid standard; Track-9: Gallic acid standard; Track-10: Gallic acid standard; Track-11: Betasitosterol standard; Track-12: Betasitosterol standard; Track-13: Betasitosterol; Track-15: Sample L2; Track-16: Sample L2; Track-17: Sample L2.

Table 2: RF values of HPTLC fingerprints profile of test samples at 254nm before derivatization

RF values ⟶	RF1	RF2
Sample L1	0.40 (black)	
Betasitosterol Standard Marker	0.40 (black)	
Gallic Acid Standard Marker	-	0.58 (black)
Sample L2	-	0.58 (black)

Table 3: Effect of samples L1 & L2 on α amylase

Concentration µg/ml	L1(%inhibition)	L2 (% inhibition)	Acarbose (%inhibition)
10	41.26	54.31	77.42
1	23.61	18.63	62.51
0.1	18.53	15.94	41.35
0.01	12.39	12.33	25.22
0.001	3.57	6.64	16.50
IC50 Value µg/ml	>10	>9	1

According to table both samples show % inhibition of α amylase of which sample L2 has better % inhibition than sample L1.

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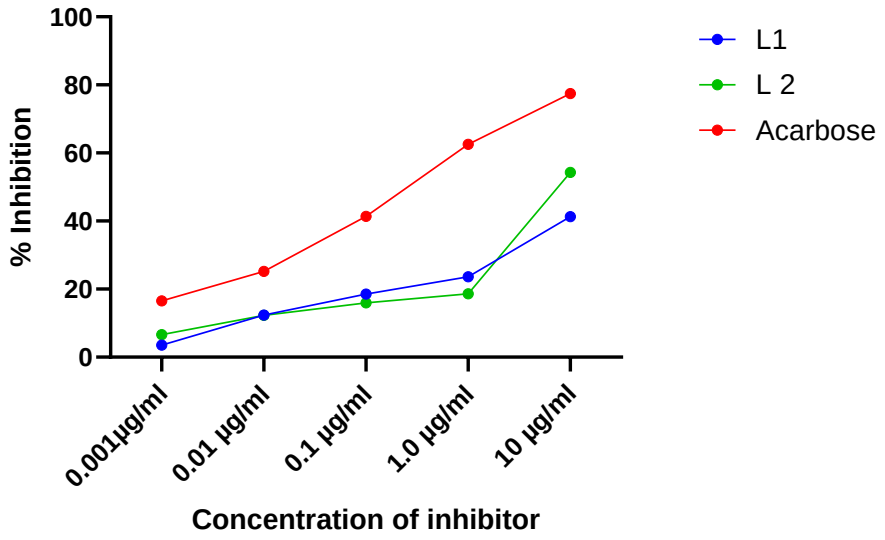

Figure 2: A graph for Comparison of α-Amylase Inhibitory Activity of Hexane (L1) and Ethanolic (L2) Extracts of E. laevis Leaves

Table 4: Effect of samples L1 & L2 with Acarbose as standard on α-Glucosidase

Concentration µg/ml	L1 (% inhibition)	L2 (% inhibition)	Acarbose
10	53.28	51.4	72.2
1	33.51	17.5	41.50
0.1	13.25	2.54	16.66
0.01	4.28	2.15	11.6
0.001	2.16	1.14	8.4
IC50 Value µg/ml	**>9**	**10**	**>7**

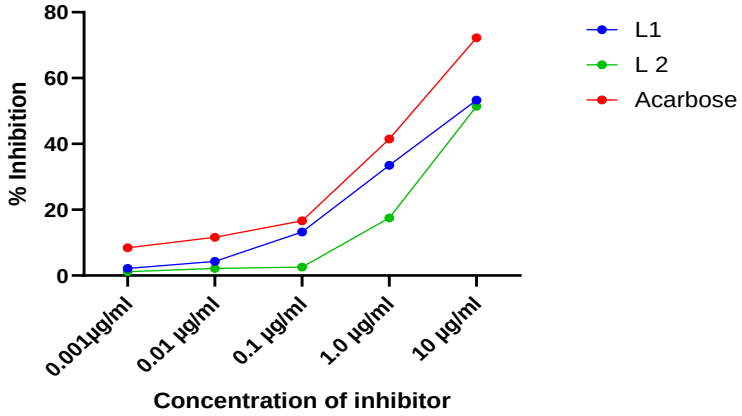

Figure 3: A graph for Comparison of α-Glucosidase Inhibitory Activity of Hexane (L1) and Ethanolic (L2) Extracts of E. laevis Leaves

DPPH Assay:

Table 5: DPPH Radical Scavenging Activity of Hexane (L1) and Ethanolic (L2) Extracts of E. laevis Leaves Compared to Ascorbic Acid

Concentration	L1	L2	Ascorbic Acid
10	52.14	56.57	68.26
1	25.61	34.53	42.38
0.1	12.51	13.62	26.48
0.01	8.24	2.17	14.59
0.001	3.67	1.46	3.61
IC50 Value µg/ml	**10**	**10**	**>4**

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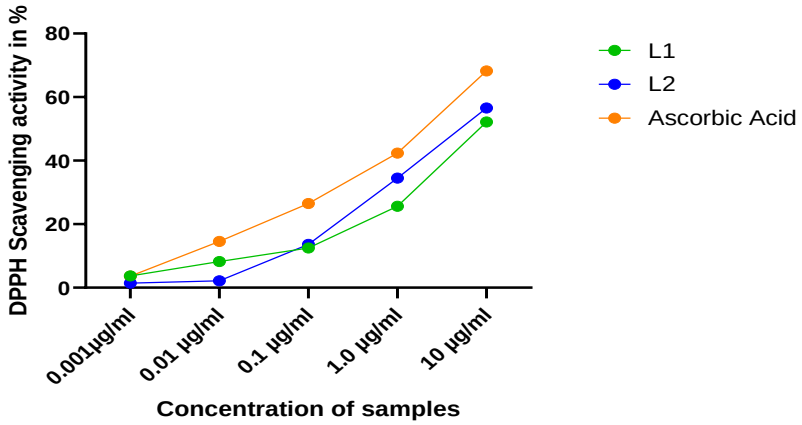

Figure 4: Graph showing % DPPH Radical Scavenging Activity of Hexane (L1) and Ethanolic (L2) Extracts of E. laevis Leaves Compared to Ascorbic Acid

SGOT/SGPT

Table 6: Effects of Hexane (L1) and Ethanolic (L2) Extracts of E. laevis Leaves on SGOT and SGPT Levels in Diabetic Rats

Group	Drug Concentration	SGOT (U/L)	SGPT (U/L)
Group-1	Normal control	109.8±6.7	114.48±3.6
Group-2	Diabetic control	154.49±6****	160.65±5.8****
Group-3	Metformin 200mg/kg	112.28±2.9****	117.53±4.65****
Group-4	L1 200 mg/kg	128.15±3.85****	138.73±4.34****
Group-5	L1 400 mg/kg	120.35±4.01****	130.68±2.7****
Group-6	L2 200 mg/kg	117.15±4.3****	124.8±3.74****
Group-7	L2 400 mg/kg	114.5±4.2****	119.18±3.83****

Values are given as mean ± SEM for groups of six animals each *P <0.0001 (Dunnet t-test). Diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.

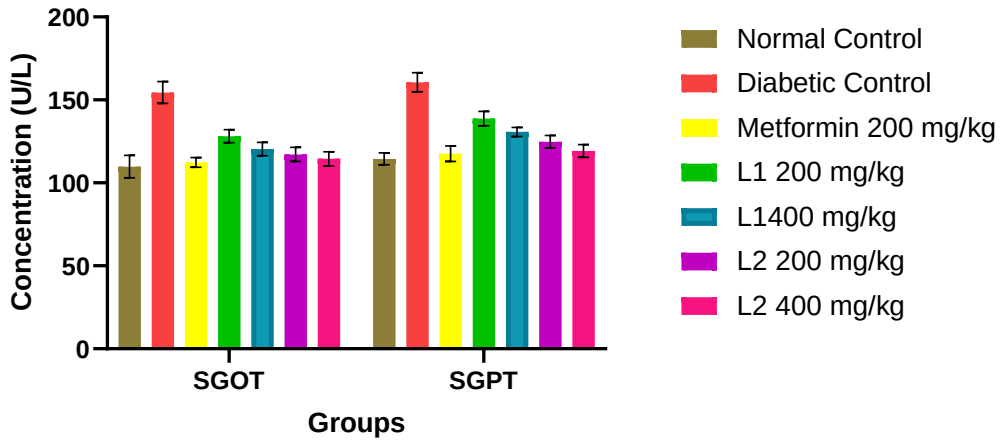

Figure 5: A Graph of Effects of Hexane (L1) and Ethanolic (L2) Extracts of E. laevis Leaves on SGOT and SGPT Levels in Diabetic Rats

Lipid Profile

Table 7: Effects of Hexane (L1) and Ethanolic (L2) Extracts of E. laevis Leaves on Lipid Profile in Diabetic Rats

Drug Concentration	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal control	75.68±1.42	78.48±3.75	30.08 ± 1.30	31.28±2.53	25.28±3.86
Diabetic control	105.3±4.50**	107.48±3.71****	20.07 ± 1.04****	57.78±1.79****	44.07±3.70****
Metformin 200mg/kg	82.33±4.10****	84±1.97****	30.97 ±1.47***	33.23±3.71****	26.6±2.79****
L1 200 mg/kg	95.43±4.59**	101.47±3.63	23.28± 2.86	49.78±5.59	36.25±1.81
L1 400 mg/kg	90.17±6.19****	95.28±2.68****	25.3 ± 2.26	40.55±3.08****	30.53±8.24****
L2 200 mg/kg	90.03±7.69****	91.38±1.73****	25.95 ±2.20	40.28±3.58****	29.42±2.40****
L2 400 mg/kg	83.95±5.40****	84.62±1.49****	29.55±1.31**	35.85±2.46****	27.43±3.68****

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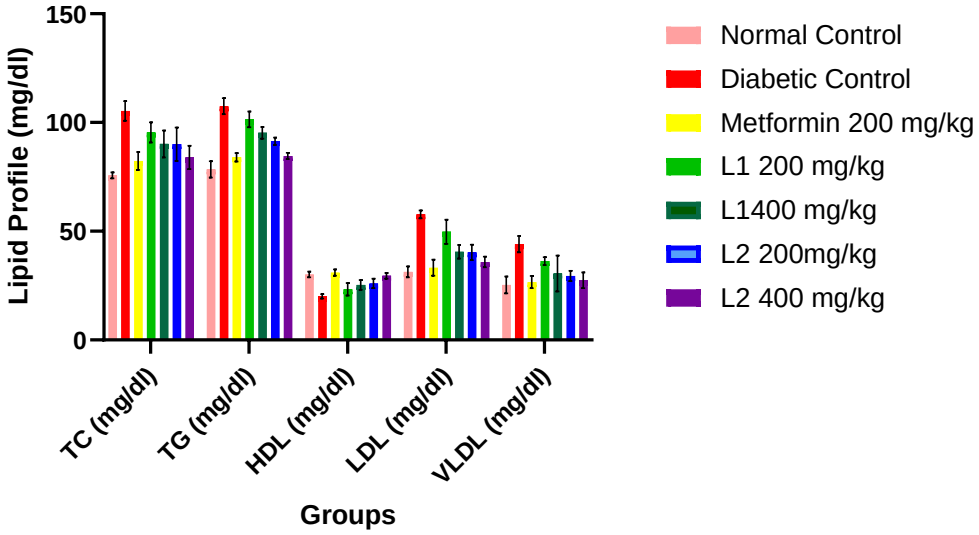

Figure 6: A Graph of Effects of Hexane (L1) and Ethanolic (L2) Extracts of E. laevis Leaves on Lipid Profile in Diabetic Rats

Blood Glucose

Table 8: Effects of Hexane (L1) and Ethanolic (L2) Extracts of Enantia laevis Leaves on Blood Glucose Levels in Diabetic Rats

Treatment(Dose)	0 day (mg/dl)	7th day (mg/dl)	14th day (mg/dl)	21st day (mg/dl)
Normal control	82.31 ± 3.66	82.35 ± 4.07	83.4 ± 2.56	81.62 ± 2.83
Diabetic control	322.8 ± 3.87****	322.7 ± 4.8****	323.8 ± 5.52****	324.5 ± 5.85****
Metformin 200mg/kg	322 ± 5.74****	165.9 ± 3.25****	141.5 ± 3.20****	81.33 ± 2.83****
L1 200 mg/kg	319.8 ± 3.87****	205.5 ± 6.09****	181.48 ± 6.99****	107.91 ±3.57****
L1 400 mg/kg	319.8 ± 1.96****	197.67 ± 3.82****	165 ± 6.45****	100.47 ± 4.4****
L2 200 mg/kg	320.3 ± 2.58****	203± 2.25****	158.38 ± 2.74****	95.08 ± 4.31****
L2 400 mg/kg	319.67 ± 1.86****	185.9 ± 4.74****	150.5 ± 5.09****	84.05 ± 2.79****

Values are given as mean ± SEM for groups of six animals each *P <0.001 (Dunnet t-test). Diabetic control was compared with the vehicle control and extract treated groups were compared with the diabetic control.

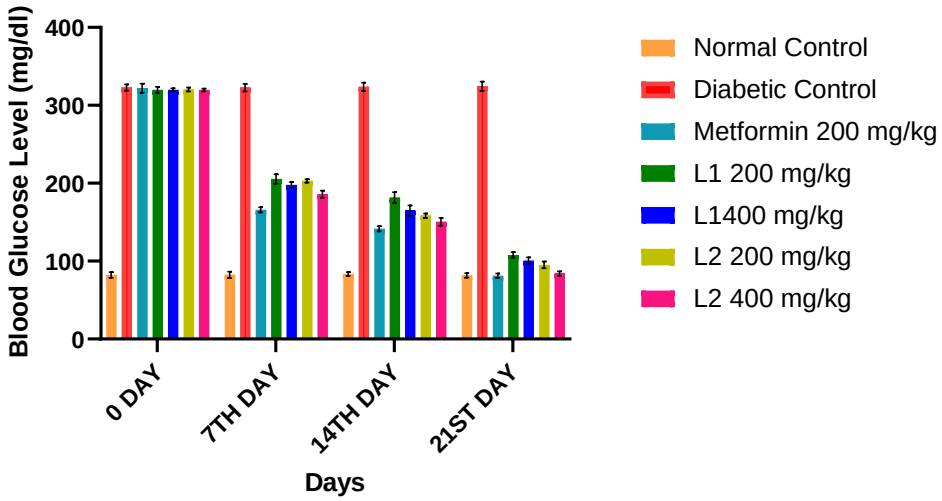

Figure 7: A Graph of Effects of Hexane (L1) and Ethanolic (L2) Extracts of E. laevis Leaves on Blood Glucose Levels in Diabetic Rats

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Discussion

In-vitro Enzyme inhibition Activity

The feasibility of leaf extracts from *Ehretia laevis* for postprandial hyperglycemia control was considered by their α-amylase and α-glucosidase inhibitory potential. Results revealed that at all tested concentrations, markedly inhibitory effects were observed and L2 was more effective in inhibition percentages compared to those of L1. This extract had IC50 values > 10 µg/ml for α -amylase and 7 g/ ml, while L2 favorably. These outcomes suggest that extracts may ban other carbohydrate metabolizing enzymes and abate glucose consumption comparatively swab the preserve blood sugar levels.

Antioxidant Activity

In the DPPH assay, antioxidant activity was moderate in both L1 and L2 extracts but more effective at all concentrations of scavenging inhibition by L2. The IC50 for DPPH scavenging was 10µg/mL (L1), and it is 5µg/mL (L2) while that of ascorbic acid, a positive control compound, was approximately equal to 2 µ g /m L. Thus, it possesses the free radical scavenging ability and effectively reduces oxidative stress associated with diabetes mellitus as well as its complications due to high antioxidant activity.

In-Vitro and In Vivo Anti-diabetic Studies

Anti-diabetic activity of E. laevis in-vivo The STZ induced diabetic rats, treated with the aqueous extracts from both leaves and stem bark of Ehretia laevis L were studied for 21 days to see their effects on blood glucose level(changes) against an untreated control group(table:3). This means that both L1 and L2 extracts lowered the blood glucose level, with a dose-dependent effect. Among all, L2 at 400 mg/kg demonstrated the most lowering effect on blood glucose level from 319. 67 ± 1. In Figure 1, on day-0 the mean serum Cholesterol was (86 mg/dl) whereas it raised to (236. 4 ± 2. At day 21 of the study: 70 mg/dl. They found that the reduction was comparable to what they observed in the metformin treated group.

Serum lipid levels and liver enzymes

Animals treated with extracts of E. laevis evidenced a significant improvement in lipid profiles compared to normal control animals The TC, TG and LDL decreased in patients treated with extracts of L1 or L2 (Figure 5), along with the HDL increased. The highest dose (400 mg/kg) of L2 made the most significant changes in lipid profile. The biochemical analysis of the extracts presented a hepatoprotective effect by lowering raised SGOT and SGPT levels in diabetic rats.

Phytochemical Analysis:

The HPTLC analysis of the extracts showed that the prescence of bioactive compounds including β-sitosterol (0.35%) in L1 and 1.72% gallic acid in L2. The compounds are associated with an array of pharmacological potentials like anti-diabetics, antioxidants which possibly contributed to the biological properties in terms of bioactivities by extracts.

Conclusion

Conclusively, from the studies above E. laevis leaf extracts showed promising antidiabetic effects via enzyme inhibitory activity and antioxidant properties These extorts brought down the blood glucose level, adjusting its profile and show hepatoprotective properties in STZ induce diabeties. The other phytochemicals beta-sitosterol and gallic acid also support for their medicaments properties. On the basis of these observations, E. laevis appears to be a good candidate for use as an ethnomedicine for diabetes treatment which warrants further investigation and possibly clinical trial screenings.

Abbreviations

p-N-Dglucopyranoside: p-nitrophenyl-alpha-D-glucopyranoside
DM: Diabetes Mellitus
STZ: Streptozotocin

Consent for publication

Not applicable.

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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