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Characterization and bioassay guided hypoglycemic activity of *Premna herbacea* Roxb. against streptozotocin-induced hyperglycaemic rats.

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ABSTRACT

Diabetes mellitus is a global epidemic, and India being the major epicenter of it. However, many synthetic drugs are available in the market. But produce specific side effects or residual effects. The concept of plant-based inhibitors has come up to overcome the adverse effects of synthetic drugs. Plant-derived inhibitors are less toxic, have minimal side effects, and are easily adaptable by the human body. Sequential solvent extraction of *Premna herbacea* leaves was carried out to evaluate the *in-vitro* and *in-vivo* anti-diabetic properties against streptozotocin (STZ) induced Wister albino diabetic rats. Among all the extracts, methanol showed the highest inhibitory potential of α-amylase (IC_{50} 68.62 ± 2.11) and α-glucosidase (IC_{50} 75.01 ± 2.40) significantly in comparison to standard acarbose. The toxicity, carbohydrate tolerance, and biochemical parameters among the experimental animal groups were performed using the methanol extract. The methanol extract showed no toxicity and side effects. It had significant tolerance against glucose, maltose, and starch compared to the diabetic control and standard groups. The methanol extract has shown effective results in controlling body weight loss, decreased blood glucose level, and other biochemical parameters like cholesterol, triglycerides, and glycosylated hemoglobin. The *in-vitro* and *in-vivo* results suggested that the methanolic *Premna herbacea* leaves extract is a dose-dependent inhibitor of α-amylase and α-glucosidase in managing type 2 diabetes mellitus. Furthermore, the GC-MS analysis validates the hypoglycemic property of *Premna herbacea* by reporting the presence of major active anti-diabetic compounds like Beta-Sitosterol, Octadecenoic Acid, Triacontanoic Acid, Phytol, Methyl Eicosatetraenoate, Stigmasterol, Thymol, and D-Mannitol.

Keywords: Diabetes, α-amylase, α-glucosidase, Acarbose, Streptozotocin.

1. Introduction

Diabetes mellitus is an acute metabolic syndrome characterized by an increase in blood glucose levels. It may be either due to inherited or acquired deficiency in insulin secretion leading to a gradual decrease in responsiveness of the secreted insulin by the vital organs, which damages the organs, blood vessels, and nerves (Matsui *et. al., 2007*). The most promising therapeutic approach to retard glucose absorption is inhibiting the carbohydrate hydrolyzing enzymes, such as α-amylase and α-glucosidase. It will eventually decrease the postprandial hyperglycemia (Bhandari *et. al.,* 2008). Many commercial drugs are available today in controlling diabetes mellitus, which inhibits α-amylase and α-glucosidase. The most commonly used synthetic drugs are acarbose, miglitol, and voglibose (Saito *et. al.,* 1998). But these drugs have adverse side effects such as abdominal distension, meteorism, flatulence, bloating, and diarrhea (Fujisawa *et. al.,* 2005). To overcome dependency on synthetic drugs, focus on active, effective, and safe inhibitors of α-amylase and α-glucosidase from natural sources is targeted to treat diabetes (Kao *et. al.,* 2006; Zhishen *et. al., 1999;* Kazeem *et. al.,* 2013; Kim *et. al.,* 2009; Oboh *et. al.,* 2013). Many plants reported being beneficial for the treatment of diabetes in various traditional literature of the world. But very few of the claimed hypoglycemic properties have received authentic scientific validation, with the majority yet to assess their efficacy.

Premna herbacea Roxb. is a small herb *c.a.* 8-20 cm, which dies off during winter and regenerates from the underground woody rootstock in spring. Leaves are sessile, rosettes, opposite, obovate, toothed, and membranous. The inflorescence is in terminal corymbose panicles, with white or greenish-yellow flowers. Fruit are drupe, globose and glossy black (Patiri and Borah, 2007). *Premna herbacea* Roxb. leaves, stem, or root decoction helps treat asthma, neuralgia, rheumatism, diarrhea, obesity, and hyperglycemia, as reported in Ayurveda (Khare, 2004; Khare, 2004; Thirumalai *et. al.,* 2013). The present investigation will assist in analyzing the anti-diabetic activity of *Premna herbacea* Roxb. leaves extracts.

2. Materials and methods

2.1 Identification of plant material

Leaves of *Premna herbacea* Roxb. (Verbenaceae) was collected from Holtugaon Reserve Forest under Manas National Park in Kokrajhar, Assam (India). It falls under the Indo-Burma biodiversity hotspot region. Local indigenous people of Bodo tribes collect it from the forest under the Forest Act provision of Non-Timber Forest Product. The plant is locally known as Matiphesua or Matiajam in Assamese and Keradaphini or Matiagaldab in Bodo. The herbarium was prepared (***Accession no: 18205***) for identification and submitted to Gauhati University Botanical Herbarium, Department of Botany, Gauhati University, Assam, India.

2.2 Preparation of crude extract

The leaves of *Premna herbacea* were washed and cleaned with distilled water. Then it was allowed to shade dry under a controlled environment to prevent the sample from dust and

decay. After drying, it was ground to a fine powder and stored in an air-tight container. The powder was subjected to sequential solvent extraction in Soxhlet Apparatus at 20° C for 15 hours using petroleum ether, ethyl acetate, methanol, and water, based on the polarity index. Solvents used in the study for extract preparation were Excess solvents were separated from the crude extracts using a Rotary Vacuum Evaporator (Buchi Rotavapor® R-100) at 40 °C. The maceration process carried out the aqueous extraction, and the Freeze Dryer (Delvac LYODEL-55) removed excess water from the extract. The extracts were stored in Borosil screw cap glass vials in a 4 °C refrigerator for further analysis.

2.3 In-vitro anti-diabetic studies

2.3.1 α-amylase inhibition activity

The α-amylase inhibitory assay of the extracts was determined according to a standardized method with slight modification (Yonemoto *et. al.*, 2014). In brief, 0.2 ml of extract solutions at different concentrations (20-100 mg/mL) and 0.2ml of starch solution (1% w/v, in 20 mM phosphate-buffered saline, pH 6.9, with 6 mM sodium chloride) was mixed. Incubated for 12 min at 30° C and 0.2 ml α-amylase solution (0.5 mg/mL) was added. Incubated for 12 min at 30° C. The reaction terminated by adding 0.4 ml dinitrosalicylic acid (DNS) color reagent. Then, reactions were heated in the water bath for 10 min. After cooling the reaction solutions to room temperature, samples were diluted by adding distilled water of 0.5 ml, and absorbance reading was measured at O.D. 540 nm using Thermo Scientific Multiskan GO Multiplate Reader (N10588). Acarbose was used as the positive control. The α-amylase inhibition was calculated using the following formula:

$$\text{Inhibition (\%)} = (1 - As/Ac) \times 100.$$

Where, As = absorbance of the sample.

Ac = absorbance of the control.

2.3.2 α-glucosidase inhibition activity

The α-glucosidase inhibitory assay of the extracts was determined according to a standardized method with slight modification (Kim, 2013). In brief, 0.2 ml of extract solutions at different concentrations (20-100 mg/mL) was added with 0.4 ml α-glucosidase solution (1.0 U/mL) dissolved in 0.1 M phosphate buffer, pH 6.9. Reactions were incubated for 12 min at 30° C. Then, 0.2ml 5mM PNPG (p-nitrophenyl-α-D-glucopyranoside) was dissolved in 0.1 M phosphate buffer, pH 6.9, and added. Incubated for 18 min at 37° C and the reaction was terminated by adding 0.8ml sodium carbonate solution (Na2CO3). All samples were diluted with distilled water 0.4ml. Absorbance was recorded at O.D. 405 nm using Thermo Scientific Multiskan GO Multiplate Reader (N10588). Acarbose was used as the positive control. The α-glucosidase inhibition was calculated using the following formula:

$$\text{Inhibition (\%)} = (1 - As/Ac) \times 100.$$

Where, As denotes, the absorbance of sample; Ac denotes absorbance of control.

2.4 In-vivo anti-diabetic studies

2.4.1 Animal used in experiments

Prior approval (***IAEC/Per/2019/HoD/2019-029***) was taken from the Institutional Animal Ethics Committee (Gauhati University) and carried out following the National Institutes of Health guideline (NIH Publications No. 8023, revised 1978) for the use and maintenance of laboratory animals. Wistar albino rats of both sexes, male and female,

weighing 160g ± 20g, were taken into consideration for the study. Animals are routinely provided with a pellet feed diet enriched with maize bran, rice bran, protein, vitamins, and salt. Water administered ad libitum, maintained in a controlled environment at 27 ± 2^0 C temperatures and a 12-hour day/night cycle. The Wistar albino rats were procured from the College of Veterinary Science (Assam Agriculture University), Khanapara, Assam (India). Feed procured from Bajaj Instrument Works Ghaziabad, Uttar Pradesh (India).

2.4.2 Acute toxicity study

The toxicity level of the plant extract was determined considering Organization for Economic Cooperation & Development (OECD) guidelines (Khandewal, 2001).

2.4.3 Induction of diabetes in the studied animal model

Wister albino rats fasted overnight. Intraperitoneally (i.p.) diabetes was induced in rats with 110 mg/kg nicotinamide in normal saline, and 60 mg/kg streptozotocin (STZ) prepared in 0.1 M citrate buffer, pH 4.5 (Badole, 2013). The blood glucose level of rats was measured after 72 hours of streptozotocin (STZ) injection. Fasting blood glucose levels above 250 mg/dL among the rats were considered diabetic and further selected to investigate (Atsuo, 2008).

2.4.4 Oral carbohydrate tolerance test

The carbohydrate tolerance test was performed by oral administration of glucose, maltose, and starch after a gap period of 7 days sequentially to all the study groups of rats (Subramanian and Asmawi, 2008).

Rats fasted overnight were treated with concentrations of plant extract (200 and 400 mg/kg/body weight), standard drug acarbose (100 mg/kg/body weight), and distilled water orally. After 15 min, rats were administered orally with glucose (4 g/kg/body weight) on Day 1, maltose (4 g/kg/body weight) on Day 7, and starch (4 g/kg/body weight) on Day 14. After glucose/maltose/starch treatment, blood sample analyzed by tail puncture method for blood glucose estimation at 0, 30, 60, and 120 min using an Accu-Chek Glucometer (Make: Germany) biochemical kit.

2.4.5 Experimental design for biochemical study

The rats were grouped into 5 cages (n = 6 rats per cage).

Group 1: Normal control, ad libitum distilled water.

Group 2: Diabetic control, ad libitum distilled water.

Group 3: Streptozotocin (STZ) induced diabetic rats, treated with standard drug Glibenclamide (5 mg/kg body weight).

Group 4: Streptozotocin (STZ) induced diabetic rats treated with plant extract (200 mg/kg body weight).

Group 5: Streptozotocin (STZ) induced diabetic rats treated with plant extract (400 mg/kg body weight).

The treatments were carried out daily for 21 days. In addition, the fasting blood glucose level and body weight were monitored at regular intervals on days 0, 7, 14, and 21. The fasting glucose level of blood was determined from the tail tip cut using the glucose oxidase method, using an Accu-Chek Glucometer (Make: Germany) biochemical kit. On the 21st day, the experimental rats of all the groups were sacrificed. The blood sample was

collected from the retro-orbital bleeding under anesthesia to carry out the biochemical parameters using standard procedures (Annadurai *et. al.*, 2012).

2.5 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The Gas Chromatography-Mass Spectrometry (GC-MS) was analyzed using Clarus 680 GC & Clarus 600C MS Liquid Autosampler (PerkinElmer, USA) in the Central Analytical Instrumentation Facility of Guwahati Biotech Park. The carrier gas, helium, is used at a 1 ml per min flow rate in split mode (10:1) v/v. The extract was injected into the column (60.0 m × 250 µm) at 280 ℃ injector temperature. The initial temperature of the oven started at 60 ℃ and was held for 1 min. Then, it was gradually raised at a rate of 10 ℃ per min to 300 ℃. Holding was allowed for 5 min. The ion sources temperature was maintained at 180 ℃. The source and detector temperature were maintained at 150 ℃. The detector operates in scan mode ranging from 50–600 atomic mass units. The compounds identification was made by direct spectrum comparison of the extract's retention times with Turbo-mass NIST 2008 (National Institute of Standard and Technology) library software.

2.6 Fourier transformed infrared (FTIR) spectroscopic analysis

Fourier transform infrared spectroscopic (FTIR) was analyzed using Thermo Nicolet iS10 FT-IR Spectromer (Thermo Scientific, USA) in the Central Analytical Instrumentation Facility of Guwahati Biotech Park. The extract was dried to get powders for FTIR analysis. The dried extracts powder (8 mg) was encapsulated in 80 mg of KBr pellet to prepare a translucent sample disc. The sample was scanned from the 400–4000 cm^{-1} wave-number frequency range with a 4 cm^{-1} resolution. The peak values of FTIR were recorded.

2.7 Statistical analysis

The data expressed as Mean ± SEM (standard error of the mean). Groups were compared performing ANOVA (one-way analysis of variance) using SPSS for Windows version 12.0 (SPSS Inc., Chicago, IL, USA). Dunnette's multiple comparison tests were used to determine the significance level between means ($P < 0.05$).

3. Results

3.1 In-vitro α-amylase and α-glucosidase inhibitory effects of the leaves extracts of *Premna herbacea*

The inhibitory effect of *Premna herbacea* leaves extracts on the activity of α-amylase showed a dose-dependent pattern (20 - 100 mg/ml) in Table 1; Figure 1. Compared to the standard drug acarbose (IC50 = 68.56 ± 2.13 mg/ml), the methanol extract (IC50 = 68.62 ± 2.11 mg/ml) demonstrated highest inhibitory effect on α-amylase. While among all the extracts, petroleum ether (IC50 = 91.23 ± 1.91 mg/ml) showed the least inhibition. On the other hand, the potentiality of the extracts in inhibiting α-glucosidase showed in Table 2; Figure 2. The methanol extract (IC50 = 75.01 ± 2.40 mg/ml) showed highest α-glucosidase inhibitory activity and aqueous extract (IC50 = 91.97 ± 2.11 mg/ml) showed lowest activity among all the extracts, in comparison to the standard drug acarbose (IC50 = 71.76 ± 2.41 mg/ml).

Table 1: Inhibition of α - amylase by *Premna herbacea* leaves extracts (% inhibition).

Concentration (mg/ml)	20	40	60	80	100	IC_{50}
Petroleum Ether	20.91 ± 0.18	24.57 ± 0.37	36.33 ± 0.39	42.32 ±	56.46 ± 0.37	91.23 ± 1.91

				0.24		
Ethyl Acetate	23.51 ± 0.45	33.78 ± 0.19	39.25 ± 0.26	49.18 ± 0.14	58.62 ± 0.17	81.33 ±1.81
Methanol	23.97 ± 0.08[a]	38.58 ± 0.40[ab]	44.76 ± 0.21[a]	56.58 ± 0.27[a]	64.68 ± 0.24[ab]	68.62 ± 2.11
Aqueous	21.67 ± 0.26	32.18 ± 0.13	37.58 ± 0.34	45.66 ± 0.21	59.23 ± 0.09	84.23 ± 1.88
Acarbose	24.27 ± 0.16	36.43 ± 0.21	45.55 ± 0.28	59.62 ± 0.17	62.69 ± 0.15	68.56 ± 2.13

Values expressed as Mean ± SD; n = 3.
[a] Significantly ($p < 0.05$) higher compared to other extracts at the same concentration.
[b] Significantly ($p < 0.05$) higher compared to the standard drug acarbose at the same concentration.

Table 2: Inhibition of α - glucosidase by *Premna herbacea* leaves extracts (% inhibition).

Concentration (mg/ml)	20	40	60	80	100	IC_{50}
Petroleum Ether	20.19 ± 0.15	23.95 ± 0.17	33.48 ± 0.21	47.81 ± 0.13	62.24 ± 0.17	83.09 ± 2.21
Ethyl Acetate	19.45 ± 0.27	24.40 ± 0.25	36.87 ± 0.19[a]	49.37 ± 0.27	57.49 ± 0.29	84.70 ± 2.12
Methanol	21.58 ± 0.22[ab]	25.62 ± 0.27[a]	34.38 ± 0.24	55.59 ± 0.38[a]	67.28 ± 0.21[a]	75.01 ± 2.40
Aqueous	17.49 ± 0.31	23.49 ± 0.17	29.73 ± 0.16	41.37 ± 0.27	58.33 ± 0.29	91.97 ± 2.11
Acarbose	20.53 ± 0.13	27.39 ± 0.23	39.81 ± 0.14	54.55 ± 0.19	70.39 ± 0.24	71.76 ± 2.41

Values expressed as Mean ± SD; n = 3.
[a] Significantly ($p < 0.05$) higher compared to other extracts at the same concentration.
[b] Significantly ($p < 0.05$) higher compared to the standard drug acarbose at the same concentration.

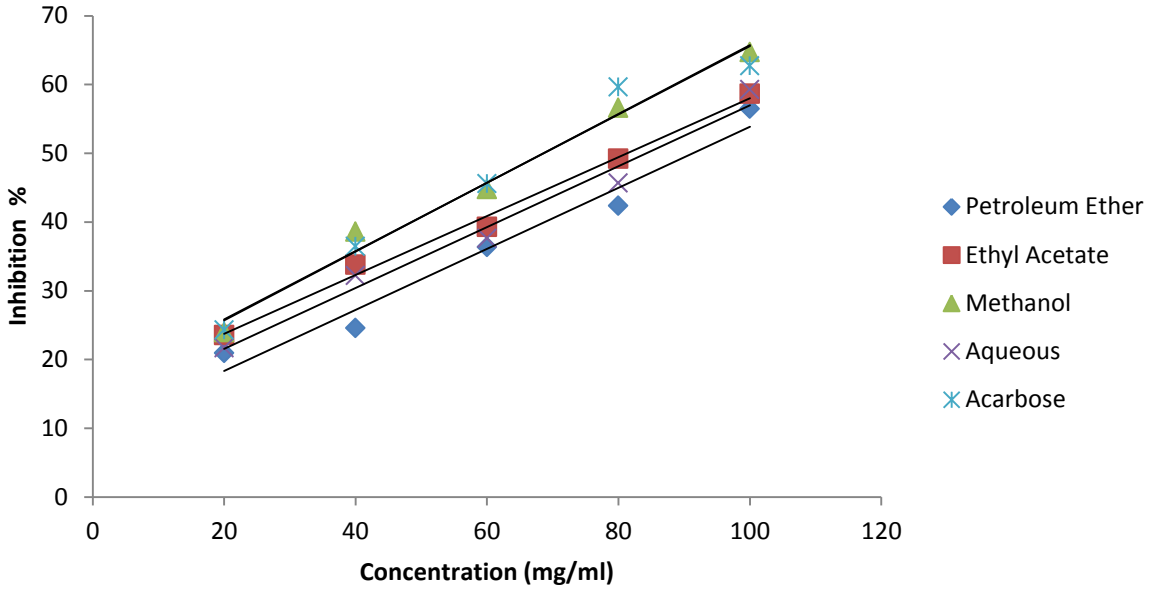

Figure 1: Concentration vs. inhibition % of α - amylase exhibited in *Premna herbacea* leaves extracts compared to standard acarbose.

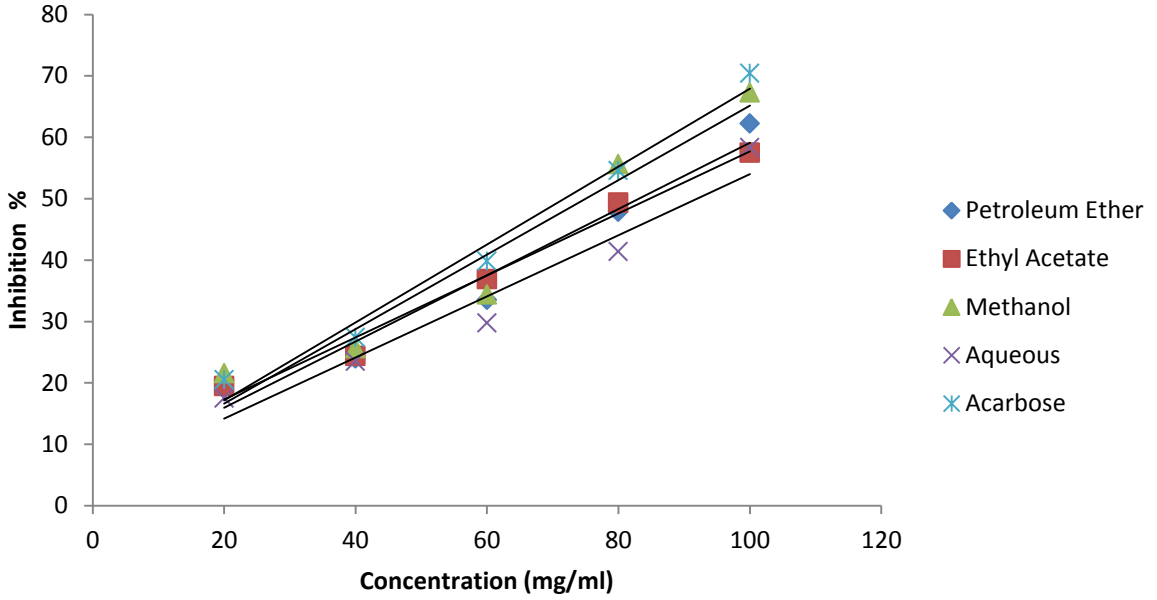

Figure 2: Concentration vs. inhibition % of α - glucosidase exhibited in *Premna herbacea* leaves extracts compared to standard acarbose.

3.2 *In vivo* study of the selected extract of *Premna herbacea*

Based on the *in-vitro* studies, the methanol extract was most potent among all the extracts in inhibiting α-amylase and α-glucosidase when compared to the standard acarbose. So, a further *in-vivo* study will be carried out on the methanolic extract of *Premna herbacea* (PHME) leaves.

3.2.1 Acute toxicity study

The crude methanolic extract of *Premna herbacea* leaves was subjected to an acute toxicity study. No behavioral changes in the studied animals were seen during the 14 days observation period (Table 3). Thus, the acute toxicity result concludes that the methanolic extract of *Premna herbacea* leaves is safe to administer orally up to 2000 mg/kg/body weight without any toxic symptoms as it did not produce any mortality or restlessness.

Table 3: Acute toxicity study of methanolic extract of *Premna herbacea* leaves on Wister albino rats.

PARAMETERS	OBSERVATIONS
Toxic	No
Pre-terminal deaths	No
Bodyweight	No significant change
Motor activity	Normal
Tremors	No
Convulsions	No
Lacrimation and salivation	Normal
Sedation	No
Body temperature	Normal
Analgesia	No
Diarrhea	No
Skin color	Normal
Yellowing of fur	Absent
Scratching	No
Respiration	Normal
Aggressiveness and restlessness	No

3.2.2 Oral carbohydrate tolerance test

All the three carbohydrate tolerance tests, *i.e.*, glucose (Table 4), maltose (Table 5), and starch (Table 6), showed a sudden elevation in blood glucose level in normal and diabetic rats at 30 min when compared with the initial blood glucose level at 0 min. The rats were either administered glucose, maltose, or starch in their respective tests after 0 min. A sudden rise in blood glucose level was monitored in all the experimental rats. During the 60 min and 120 min observations, blood glucose level gradually decreases and comes near the initial baseline (0 min). But in the diabetic control untreated group, the blood glucose level tends to increase even at 60 min, and a very marginal decrease was observed at 120 min. The *Premna herbacea* methanolic leaves extract (400 mg/kg/ body weight) showed the most significant dose-dependent ($P < 0.05$) reduction in the blood glucose level of diabetic rats when compared with the acarbose and methanolic extract at low concentration (200 mg/kg/ body weight). It showed that the methanolic extract of *Premna herbacea* at a higher concentration dose is significantly more effective than the reference drug acarbose. While at low concentration, the methanolic extract of *Premna herbacea* decreases the blood glucose level closer to the initial at 0 min.

Table 4: Effect of *Premna herbacea* methanolic leaves extract (PHME) on oral glucose tolerance test (OGTT)				
Minutes	0	30	60	120
Normal Control	92.85±1.14	129.17±2.72	124.83±1.35	108.83±1.35
Diabetic Control	259.59±1.11	312.50±0.95	322.50 ±0.95	297.83±0.83
Acarbose (100 mg/kg/b.w.)	258.58±0.95	295.17±1.01	282.17±1.01	269.00±1.15
PHME (200 mg/kg/b.w.)	261.59±1.08	307.33±1.30	298.33±1.30	281.67±1.17
PHME (400 mg/kg/ b.w.)	257.39±2.13	296.17±1.13	285.37±1.13	266.17±1.13
Values expressed in mean ± SEM (n=6), compared with diabetic control using one-way ANOVA followed by Dunnette's multiple comparison test ($p<0.05$).				

Table 5: Effect of *Premna herbacea* methanolic leaves extract (PHME) on oral maltose tolerance test (OMTT)				
Minutes	0	30	60	120
Normal Control	98.12±0.33	138.16±0.72	131.83±1.25	111.83±0.85
Diabetic Control	272.17±0.16	329.50±0.95	344.50 ±0.95	317.83±0.33
Acarbose (100 mg/kg/ b.w.)	228.17±0.08	292.17±1.14	277.17±0.38	256.40±0.15
PHME (200 mg/kg/ b.w.)	252.67±1.02	311.33±1.30	294.33±1.30	274.67±1.17
PHME (400 mg/kg/ b.w.)	232.50±2.04	302.17±1.13	284.37±1.13	251.17±1.13
Values expressed in mean ± SEM (n=6), compared with diabetic control using one-way ANOVA followed by Dunnette's multiple comparison test ($p<0.05$).				

Table 6: Effect of *Premna herbacea* methanolic leaves extract (PHME) on oral starch tolerance test (OSTT)				
Minutes	0	30	60	120
Normal Control	96.12±0.33	144.16±0.72	139.83±1.25	124.83±0.85
Diabetic Control	278.17±0.16	349.50±0.95	336.50 ±0.95	327.83±0.33
Acarbose (100 mg/kg/b.w.)	181.17±0.08	226.17±1.14	219.17±0.38	199.40±0.15
PHME (200 mg/kg/b.w.)	215.67±1.02	241.33±1.30	233.33±1.30	219.67±1.17
PHME (400 mg/kg/b.w.)	193.50±2.04	229.17±1.13	217.37±1.13	196.17±1.13
Values expressed in mean ± SEM (n=6), compared with diabetic control using one-way ANOVA followed by Dunnette's multiple comparison test ($p<0.05$).				

3.2.3 Effect of *Premna herbacea* leaves methanolic extract on body weight

The mean bodyweight of the groups of rats during the study period of all groups is shown in Table 7. Except in Normal Control, the mean body weight gradually decreased in Group 2-5 up to Day 21 compared to Day 1. A significant reduction in body weight $p<0.05$ is seen in Diabetic Control when compared within the groups.

Table 7: Effect of *Premna herbacea* leaves methanolic extract (PHME) on body weight				
Treatment	Bodyweight (gm)			
Groups	Day 0	Day 7	Day 14	Day 21
Normal Control	157.9±2.4	159.63±2.7	158.5±2.7	161.85±2.9
Diabetic Control	154.65±1.3	149.21±2.3	143.05±2.1	135.3±2.0
Glibenclamide (5 mg/kg/b.w.)	162.06±2.7	157.9±1.8	154.14±1.2	148.05±1.07
PHME (200 mg/kg/b.w.)	151.7±1.4	147.5±1.3	142.75±1.9	136.5±3.15
PHME (400 mg/kg/b.w.)	156.42±2.1	152.95±1.9	149.33±1.6	143.8±2.9
Values expressed in mean ± SEM (n=6), compared with diabetic control using one-way ANOVA followed by Dunnette's multiple comparison test ($p<0.05$).				

3.2.4 Effect of *Premna herbacea* leaves methanolic extract on blood glucose level

In Table 8, the *Premna herbacea* methanolic extract (400 mg/kg/b.w.) exhibited significant hypoglycemic potentiality against streptozotocin (STZ) induced diabetic rats. A significant ($p<0.05$) decrease of 43.30%, 36.48% and 44.81% in blood glucose levels has been observed among the glibenclamide, PHME (200 mg/kg/b.w.) and PHME (400 mg/kg/b.w.) treated groups respectively from its initial glucose level (Day 0). The blood glucose level showed a significant ($p<0.05$) reduction within the group of glibenclamide (43.3%), PHME 200 mg/kg/b.w. (36.48%) and PHME 400 mg/kg/b.w. (44.81%) on comparing Day 21 with Day 0.

Table 8: *Premna herbacea* leaves methanolic extract (PHME) on blood glucose level in streptozotocin (STZ) induced diabetic rats.				
Treatment	Blood Glucose Level (mg/dl)			
Groups	Day 0	Day 7	Day 14	Day 21
Normal Control	84.50±2.07	89.33±2.10	86.33±1.89	91.16±2.02
Diabetic Control	269.08±1.18	306.67±1.22	342.67±4.58	387.67±2.83
Glibenclamide (5 mg/kg/b.w.)	253.67±1.33	272.67±1.33	224.67±2.84	143.83±1.53
PHME (200 mg/kg/b.w.)	265.83±0.79	279.83±0.79	235.83±0.79	168.83±0.79
PHME (400 mg/kg/b.w.)	267.33±2.65	278.67±1.02	228.67±2.01	147.67±2.01
Values expressed in mean ± SEM (n=6), compared with diabetic control using one-way ANOVA followed by Dunnette's multiple comparison test ($p<0.05$).				

3.2.5 Estimation of lipid profiling, glycosylated hemoglobin, and liver glycogen

The lipid, glycosylated hemoglobin, and liver glycogen estimation were done and

tabulated below (Table 9). A significant ($p< 0.05$) decrease in the cholesterol, triglycerides and glycosylated hemoglobin level was observed among the studied groups compared to the diabetic control. While substantial ($p<0.05$) increase in the High-Density Lipoprotein Cholesterol and Liver glycogen was seen in contrast to the diabetic control.

Table 9: Estimation of lipid, glycosylated hemoglobin, and liver glycogen

Group	Cholesterol (mg/dl)	High Density Lipoprotein Cholesterol (HDL) (mg/dl)	Triglycerides (mg/dl)	Glycosylated Hemoglobin HbA1c (mmol/mol)	Liver glycogen (mg/g) of wet tissue
Normal Control	39.16±0.83	64.00±0.36	96.50±0.76	4.22±0.07	14.66±0.33
Diabetic Control	82.33±0.71	28.83±0.30	184.33±1.47	8.58±0.09	7.16±0.60
Glibenclamide standard (0.25 mg/kg/day)	53.66±0.66	43.83±0.47	126.50±0.76	4.95±0.07	13.00±0.25
PHME (200 mg/kg/day)	59.16±0.64	37.83±0.54	143.50±0.99	5.27±0.06	11.41±0.37
PHME (400 mg/kg/day)	50.83±0.76	47.83±0.33	120.54±0.84	4.83±0.04	12.58±0.23

Values expressed in mean ± SEM (n=6), compared with diabetic control using one-way ANOVA followed by Dunnette's multiple comparison test ($p<0.05$).

3.3 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Since the *Premna herbacea* leaves methanolic extract elicited the most potent inhibitory effect, it was subjected to GC-MS profiling as depicted in Table 10 and Figures 3. Some of the primary compounds found to be identified in the methanol extract are; Beta-Sitosterol (25.06%), Octadecenoic Acid (15.08%), Hexadecadienoic Acid (9.66%), Tridecanoic Acid (4.60%), Triacontanoic Acid (4.49%), Heptadecanoic Acid (2.74%), Phytol (1.56%), Methyl Eicosatetraenoate (1.19%), Stigmasterol (0.37%), Thymol (0.37%) and D-Mannitol (0.35%).

Table 10: GC-MS Identified compounds of the methanol extract of the *Premna herbacea* leaves.

SI NO	Retention Time	Peak Area (%)	Compound name	Molecular Formula	Molecular Weight
1	24.705	0.58	Trans-2,4-Dimethylthiane, S,S-Dioxide	$C_{10}H_{20}O_2S$	162
2	28.822	4.60	Tridecanoic Acid, 12-Methyl-, Methyl Ester	$C_{15}H_{30}O_2$	242
3	30.182	0.29	Propanoic Acid, 3-Mercapto-, Dodecyl Ester	$C_{15}H_{30}O_2S$	274

4	31.148	9.66	9,12-Hexadecadienoic Acid, Methyl Ester	$C_{17}H_{30}O_2$	266
5	31.223	15.08	13-Octadecenoic Acid, Methyl Ester	$C_{19}H_{36}O_2$	296
6	31.273	0.29	13-Methyltetradec-9-Enoic Acid Methyl Ester	$C_{16}H_{30}O_2$	254
7	31.358	1.56	Phytol	$C_{20}H_{40}O$	296
8	31.498	2.74	Heptadecanoic Acid, 16-Methyl-, Methyl Ester	$C_{19}H_{38}O_2$	298
9	31.709	0.56	Methyl 9,10-Octadecadienoate	$C_{19}H_{34}O_2$	294
10	32.208	0.64	Linoelaidic Acid	$C_{18}H_{32}O_2$	280
11	32.658	4.49	Triacontanoic Acid, Methyl Ester	$C_{31}H_{62}O_2$	466
12	32.819	1.19	Methyl 8,11,14,17-Eicosatetraenoate	$C_{21}H_{34}O_2$	318
13	33.669	0.27	Heptacosanoic Acid, 25-Methyl-, Methyl Ester	$C_{29}H_{58}O_2$	438
14	33.714	0.28	1-Nonylcycloheptane	$C_{16}H_{32}$	224
15	35.43	0.35	D-Mannitol, 1-Decylsulfonyl-	$C_{16}H_{34}O_7S$	370
16	37.085	25.06	Beta-Sitosterol	$C_{29}H_{50}O$	414
17	37.711	0.37	Thymol, Tbdms Derivative	$C_{16}H_{28}OSi$	264
18	37.776	0.25	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236
19	37.826	0.37	Stigmasterol, Tms Derivative	$C_{32}H_{56}OSi$	484
20	37.976	0.28	2,4,6-Cycloheptatrien-1-One, 3,5-Bis-Trimethylsilyl-	$C_{13}H_{22}OSi_2$	250
21	38.676	0.36	2-Trimethylsiloxy-6-Hexadecenoic Acid, Methyl Ester	$C_{20}H_{40}O_3Si$	356
22	38.721	0.45	Oxiraneoctanoic Acid, 3-Octyl-, Methyl Ester	$C_{19}H_{36}O_3$	312

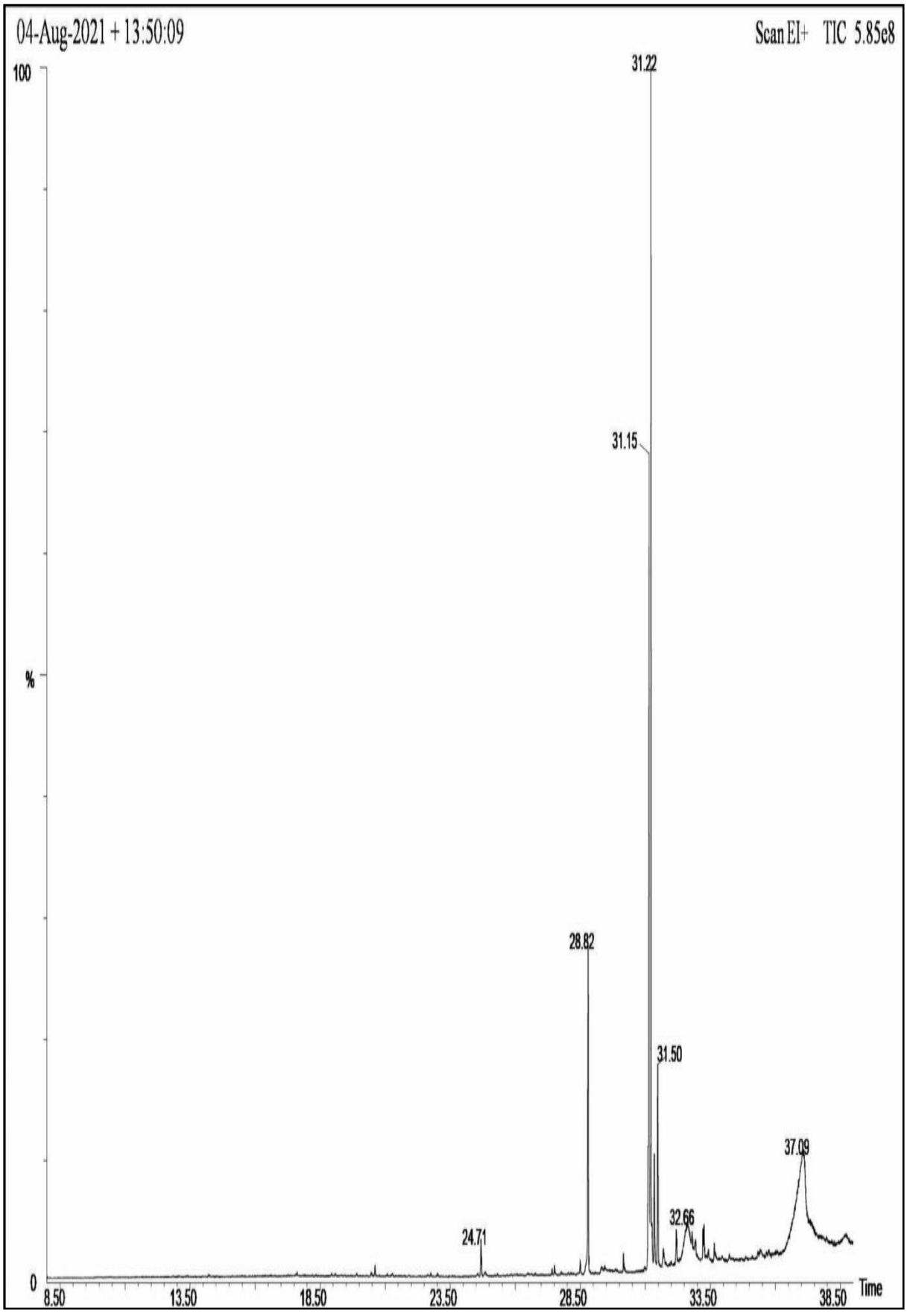

Figure 3. GC-MS Chromatogram of methanol extract of *Premna herbacea* leaves.

3.4 Fourier transformed infrared (FTIR) spectroscopic analysis

The result of FTIR interpretation is presented in Table 11, and the FTIR spectrum is illustrated in Figure 4. The major functional groups found in the methanol extract of *Premna herbacea* leaves are –C=C, -P-O-C, -P-O, -C-O, -O-H, -CH, and –NO.

Table 11: FTIR interpretation of the methanol extract of the *Premna herbacea* leaves.

SI NO	Absorption (cm^{-1})	Reference standard (cm^{-1})	Functional group	Identified compound
1	717.91	665-730	C=C bend	Alkene
2	894.52	850-995	P-O-C	Aromatic phosphates
3	1039.12	1030-1130	P-O stretch	Orthophosphate
4	1156.5	1150-1210	C-O stretch	Ester
5	1250.7	1220-1275	C-O stretch	Alkyl aryl ether
6	1377.79	1310–1390	O-H bend	Phenol
7	1444.65	1350-1480	C-H bend	Alkane
8	1558.03	1500-1550	N-O stretch	Nitro compound
9	1603.92	1566-1650	C=C stretch	Cyclic alkene
10	2849.87	2840-3000	C-H stretch	Alkane
11	2919.08	2840-3000	C-H stretch	Alkane
12	3370.43	3200-3550	O-H stretch	Alcohol

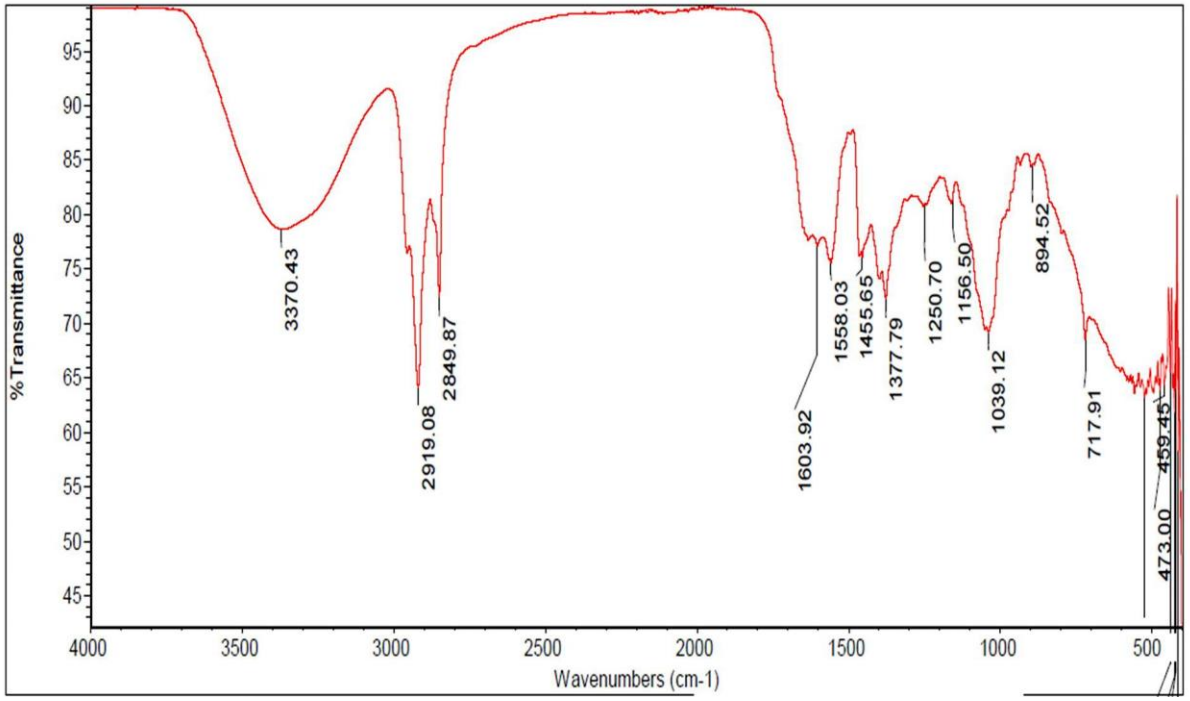

Figure 4. FTIR spectrum of methanol extract of *Premna herbacea* leaves.

4. Discussion

Although synthetic anti-diabetic drugs like acarbose and glibenclamide exist, herbal medicines and their preparations are flourishing with greater interest among the ethnobotanical community. They are less toxic and have minimal adverse effects than synthetic agents (Pari and Umamaheswari, 2000; Atmakuri and Dathi, 2010). Streptozotocin is an antibiotic isolated from *Streptomyces achromogenes*. In the Wister albino rats, it causes irreversible rupture destroying the beta cells of the pancreas. It is mainly used in the induction of diabetes mellitus in experimental animals (Matteucci and Giampietro, 2008).

The present study validates the traditional claim of *Premna herbacea* exhibiting hypoglycaemic activity as reported in Ayurveda. The methanolic extract showed potency in inhibiting α-amylase and α-glucosidase. It also reduces the blood glucose levels in streptozotocin-induced diabetic rats. The hypoglycaemic activity may be due to active phytoconstituents present in the methanolic extract, which triggers and acts as an inhibitor of type 2 diabetes mellitus. An increase in total cholesterol and triglycerides levels in serum causes significant risk factors in cardiovascular diseases. The *Premna herbacea* methanol leaves extract-treated groups showed reduced total cholesterol and triglycerides than the untreated diabetic rats. The crude methanolic extract (400 mg/kg/b.w.) treated group showed more reduction in blood glucose level compared to a low dose of 200 mg/kg/b.w. Severe bodyweight loss occurred in the streptozotocin-induced diabetic rats. But the methanolic extract (200 and 400 mg/kg/b.w.) treated group showed improvement in their body weight. This improvement in the bodyweight of rats could be due to the hypoglycaemic ability of *Premna herbacea* extracts.

Characterization of methanol extract using Gas Chromatography-Mass Spectrometry (GC-MS) revealed that Beta-Sitosterol, Octadecenoic Acid, Triacontanoic Acid, Phytol, Methyl Eicosatetraenoate, Stigmasterol, Thymol, and D-Mannitol are the major anti-diabetic bioactive compounds present in the methanol extract of *Premna herbacea* leaves. Previous reports suggest these compounds are of remarkable anti-diabetic potentials to regenerate pancreatic β-cell and inhibit α-amylase or α-glucosidase enzymes (Elmazar *et. al.,* 2013; Moloney *et. al.,* 2007; Ponnulakshmi *et. al.,* 2019; Chukwuma *et. al.,* 2019; Saravanan and Pari, 2015; Wang *et. al.,* 2017). The FTIR spectroscopic peak values of methanol extract have confirmed the presence of functional groups in the sample. It indicates the plant is rich in flavonoids and saponins along with other phytoconstituents. The –OH group in saponins and flavonoids links up to antioxidants to inhibit the anti-diabetic processes (Maobe and Nyarango, 2013). Thus, the work on the anti-diabetic potential of *Premna herbacea* validates previously reported work from the same Genus in *Premna latifolia* Roxb. (Gowtham *et. al.,* 2018).

5. Conclusion

Premna herbacea Roxb. is consumed by the local tribal Bodo community of Assam. Moreover, the plant is found to be non-toxic and without any side effects. Furthermore, both the *in-vitro* and *in-vivo* results suggested that the plant is a dose-dependent inhibitor of α-amylase and α-glucosidase. Thus, the plant extract of *Premna herbacea* can be used to manage type 2 diabetes mellitus. Thus, further isolation of pure active compounds from the

plant will help develop a medicinal formulation, nutraceuticals products, and functional foods for diabetes-related metabolic symptoms.

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