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Novel compounds from *Premna herbacea* Roxb. with antidiabetic potential

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Abstract: Diabetes mellitus is an epidemic that is growing worldwide at an alarming rate. The need for plant-based drugs to combat this epidemic led to the isolation of three new compounds, namely 1-Benzoyloxy-8-tetradecanoyloxy-geranilane (1), 1-Benzoyloxy-8-(octadec-9"-enoyl) geranilane (2), and 1-Benzoyloxy-8- octadecanoyloxygeranilane (3), from the roots of *Premnaherbacea*. Their structures were deciphered by ^{1}H NMR, ^{13}C NMR and 2D-NMR. *In vitro* (α-amylase and α-glucosidase inhibitory activity) and *in vivo* antidiabetic activity (streptozotocin- nicotinamide model) were conducted using various groups for which various biochemical parameters and histopathology studies were done. This study confirmed that 1-Benzoyloxy-8-(octadec-9"-enoyl) geranilane from roots of *Premna herbacea* have significant antidiabetic and antioxidant activity, thus indicating a corrective effect on diabetes and its complications.

Keywords: *Premnaherbacea*, 1-Benzoyloxy-8-tetradecanoyloxy-geranilane, 1-Benzoyloxy-8-(octadec-9"-enoyl) geranilane, 1-Benzoyloxy-8- octadecanoyloxygeranilane, antidiabetic, antioxidant.

INTRODUCTION

Diabetes mellitus, a disease that was recognized in antiquity, is characterized by impaired glucose utilization. Leading researchers and clinical investigators have used their best cutting-edge techniques for understanding and controlling diabetes but not fully succeeded in it. Medicinal plants with interesting pharmacological activities have been successfully employed in the treatment of various diseases like diabetes.

Premna herbacea (synonym-*Pygmaeopremna herbacea*) is a herbaceous perennial plant or a dwarf undershrub distributed through the subtropical Himalayas, Assam, West Bengal, Bihar, Orissa and Deccan peninsula including Kerala, Karnataka, and Tamilnadu (Reddy *et al*., 2010). It is known as *Bhoomjambu*in Sanskrit, *Bharangi*in Hindi, *Sirutekku* in Tamil, and *Cherrutekku* in Malayalam (Moldenke 1980). Most of the stem (15 to 30 cm tall) is underground, the plant has a creeping woody rhizome, the above-ground part is slender and simple or with single dichotomous branching. Leaves in rosette are closely appraised to the ground while greenish-yellow flowers are seen in small corymbs (Narayananan *et al*., 2000; Nayar *et al*., 1976). *Premna herbacea* is extensively used in Ayurveda and Siddha. Crushed leaves are used for relieving headaches (Sankaram *et al*., 1988). The decoction of fresh leaves and roots are used in rheumatic treatment and for relieving cough, cold and fever (Sandhya *et al*., 1988). Root paste is used in ulcer and gout treatment while juice extracted from roots is used for dropsy and cholera (Srihari *et al*., 2011). This study was designed and executed to explore the *in vitro* and *in vivo* antidiabetic potential of isolated compounds from *Premnaherbacea.*

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MATERIALS AND METHODS

Procurement and authentication of plant material

Roots of *Premna herbacea* were purchased from local vendors in Kerala, India and authenticated by NISCAIR, New Delhi, India[authentication number–NISCAIR/ RHMD/2011-12/1922/222/02].

Chemicals and reagents

Streptozotocin, nicotinamide, and glibenclamide were products of Sigma Chemical Co. (St. Louis USA). Toluene, chloroform, ethanol, sodium chloride, and sodium citrate were purchased from Global Chemicals, India. All other chemicals used were of analytical grade.

Extraction and isolation from plant material

Soxhlet extraction was done with absolute ethanol (50 liters) for 24 hrs on powdered dried roots of *Premna herbacea* (5kg). The concentrated extract was subjected to gradient elution by column chromatography using solvents like toluene, chloroform, ethyl acetate, and methanol in varying proportions on silica gel G (60-120). The fractions of the plant were collected and based on the TLC pattern they were combined and stored. Rechromatography was done followed for purification of individual compounds in the presence of Chloroform: Ethylacetate (9.5:0.5) as the mobile phase.

In vitro antidiabetic activity

α-amylase and α-glucosidase inhibitory activity of the extract were performed according to procedures reported by Ramasamy *et al*. (2016) and Anindita *et al*. (2017) respectively with minor modifications and IC_{50}value were determined (Madhusudhan *et al*., 2015; Nair *et al*., 2013). The results are reported as % IC_{50} ± SD.

In vivo antidiabetic activity

Research protocol

The *in vivo* hyperglycemic activity was approved by IAEC (approval reference no-RGIP/03/2012) and based on the Committee for Control and Supervision of Experiment on Animal guidelines.

Experimental animals

Experiments were performed using adult Wister rats of either sex weighing 150-180 g. Animals were maintained in clean stainless steel polypropylene cages at the temperature of 22°C (±1°C), under controlled relative humidity (40% -50%), and 12 h light-dark cycle (Badole *et al*., 2013). The rats were fed with a standard rat pellet diet (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*.

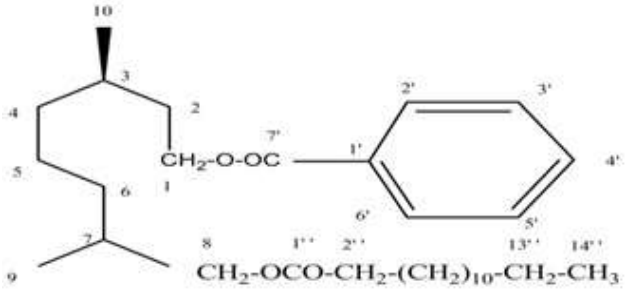

Fig. 1: Structure of compound 1

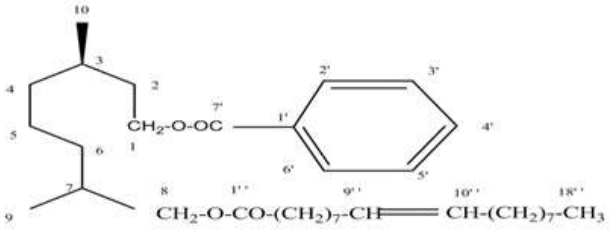

Fig. 2: Structure of compound 2

Treatment protocol

Diabetic animals were grouped randomly to the following groups of six animals each.

Group I- Normal control- 0.5% sodium CMC (1mg/kg)
Group II- Diabetic control- 0.5% sodium CMC (1mg/kg)
Group III- Standard group- Glibenclamide (5mg/kg)
Group IV- Diabetic rats treated with ethanolic extract of *Premna herbacea* (200mg/kg)

Group V- Diabetic rats treated with chloroform fraction of *Premna herbacea* (100mg/kg)
Group VI- Diabetic rats receive compound 1 (15mg/kg)
Group VII- Diabetic rats receive compound 2 (15mg/kg)
Group VIII- Diabetic rats receive compound 3 (15mg/kg)

Induction of diabetes

Nicotinamide (110mg/kg, i.p.) was injected to overnight fasted rats in normal physiological saline. Streptozotocin (60mg/kg, i.p.) to be injected was prepared extemporaneously in 0.1M citrate buffer (pH- 4.5) and injected later after 15 minutes to all groups except normal which received only the vehicle (Badole *et al*., 2011; Ananda *et al*., 2012). Animals were fed with a 15% glucose solution to avoid streptozotocin-induced sudden hyperglycemia. After72 hours of injection, rats with fasting blood glucose levels greater than 260mg/dl were considered hyperglycemic and included in further study (Annadurai *et al*., 2012). FBS levels were measured from blood samples collected using tail vein puncture technique at 0, 14, and 28 days. All other major biochemical parameters were analyzed.

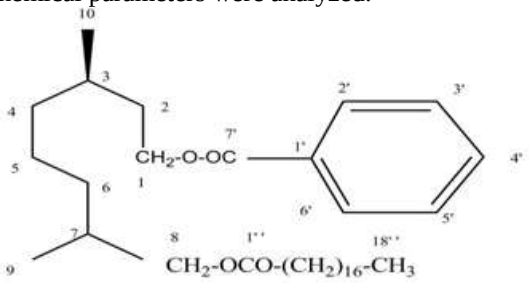

Fig. 3: Structure of compound 3

Histopathological examination

On the 28th day, animals were sacrificed, and isolated pancreatic and liver samples were rinsed, stained with hematoxylin and eosin dye (H&E), and examined under an optical microscope and necessary photomicrographs were taken.

Ethical approval

All the experimental procedures were performed according to the committee for control and supervision of experiments on animals (CPCSEA), ministry of social justice and empowerment Government of India, norms and approved by the Institutional Animal Ethics Committee (IAEC).

STATISTICAL ANALYSIS

All the results were expressed as mean ± SEM. One way ANOVA followed by Tukey's Multiple Comparison Test was applied for the statistical analysis using Graph Pad Prism 5 statistical package (Graph Pad Software, USA) and data were considered to be significantly different at $p<0.001$.

RESULTS

The semi-solid ethanolic extract of *Premna herbacea* was dark brown colored of 580g (11.60% yield). When the column was eluted with Toluene: Chloroform (9:1), it yielded three compounds from *Premna herbacea* (compound 1, 2 and 3) followed by purification using preparative TLC. The following compounds were isolated and characterized:-

Table 1: Inhibitory potency of *Premna herbacea* root extract against α-glucosidase and α-amylase.

Sample	IC_{50} Value (µg/mL)
Ethanolic extract	
α-glucosidase inhibitory activity	107.32±0.451[a]
α-amylase inhibitory activity	120.11±0.231[a]
Acarbose (standard)	
α-glucosidase inhibitory activity	72.30±0.883
α-amylase inhibitory activity	88.10±0.239

Values are mean ± SEM (n=3),[a] $p \leq 0.001$ vs. standard.

Table 2: Effects of *Premna herbacea* on fasting blood glucose

Group	Mean (mg/dL)			Change in FBG on day 28 (%)
	Day 0	Day 14	Day 28	
Normal	80.80±0.307	83.30±0.667	82.30±0.667	+1.860
Diabetic control	280.00±0.477[a]	282.00±0.792 [a]	301.00±0.725 [a]	+7.500
Diabetic+standard	264.39±0.477[b]	131.01±0.872[b]	95.70±0.577 [b]	-63.803
Diabetic+PHE (200 mg/kg)	274.80±0.477[bc]	242.00±0.601[bc]	238.00±0.667[bc]	-13.390
Diabetic+PHF (100 mg/kg)	265.00±0.307[bc]	225.00±0.477[bc]	214.00±0.494[bc]	-19.488
Diabetic+PH1 (15 mg/kg)	279.02±0.307	279.00±0.803	299.00±0.730	+7.091
Diabetic+PH2 (15 mg/kg)	262.00±0.872[bc]	189.00±0.715[bc]	150.33±0.882[bc]	-42.665
Diabetic+PH3 (15 mg/kg)	278.80±0.792	279.00±0.654	298.00±0.422	+6.886

Values are mean ±SEM (n=6), [a] $p \leq 0.001$ vs. normal control, [b] $p \leq 0.001$ vs. diabetic control, [c] $p \leq 0.001$ vs. standard treatment.
FBG- fasting blood glucose; PHE- ethanolic extract of *P. herbacea*; PHF- chloroform fraction of *P. herbacea*; PH1– PH3-compounds 1-3 from *P. herbacea*

Table 3: Effect of *Premna herbacea* on blood biochemical parameters and liver enzymes on day 28

Group	HbA_1C (%)	Creatinine (mg/dL)	BUN (mg/dL)	SGOT (IU/L)	SGOT (IU/L)	ALP (IU/L)
Normal	3.00±0.053	0.545±0.009	43.20±0.833	35.60±0.050	35.60±0.050	55.20±0.010
Diabetic control	13.10±0.063[a]	1.600±0.014[a]	90.30±0.882	73.60±0.033[a]	73.60±0.033[a]	87.70±0.008[a]
Diabetic+standard	4.83±0.106[b]	0.862±0.005[b]	56.70±0.667[b]	43.70±0.030[b]	43.70±0.030[b]	61.20±0.017[b]
Diabetic+PHE (200 mg/kg)	10.80±0.016[bc]	1.470±0.007[bc]	81.50±0.428[bc]	70.10±0.028[bc]	70.10±0.028[bc]	82.90±0.292[bc]
Diabetic+PHF (100 mg/kg)	9.05±0.020[bc]	1.410±0.005[bc]	74.70±0.494[bc]	66.30±0.030[bc]	66.30±0.030[bc]	77.31±0.273[bc]
Diabetic+PH1 (15 mg/kg)	13.00±0.021	1.620±0.009	89.80±0.601	73.60±0.019	73.60±0.019	87.50±0.342
Diabetic+PH2 (15 mg/kg)	6.58±0.029[bc]	1.230±0.006[bc]	61.50±0.563[bc]	51.70±0.171[bc]	51.70±0.171[bc]	70.70±0.143[bc]
Diabetic+PH3 (15 mg/kg)	12.90±0.015	1.630±0.007	90.30±0.803	73.40±0.071	73.40±0.071	86.90±0.506

Values are mean ±SEM (n=6), [a] $p \leq 0.001$ vs. normal control, [b] $p \leq 0.001$ vs. diabetic control, [c] $p \leq 0.001$ vs. standard treatment.
ALP- alkaline phosphatase; BUN- blood urea nitrogen; PHE- ethanolic extract of *P. herbacea*; PHF- chloroform fraction of *P. herbacea*; PH1 – PH3-compounds 1–3 from *P. herbacea*; SGOT- serum glutamic oxaloacetic transaminase; SGPT- serum glutamic pyruvic transaminase.

Compound 1- 1-Benzoyloxy-8-tetradecanoyloxy-geranilane

When the column was eluted with Toluene: Chloroform (9:1) furnished a light brown solid mass of compound 1 of yield 1.384 mg (0.223% yield) with R_f value 0.67 (Chloroform: Ethylacetate (9.5:0.5)); Melting point: 82-84°C; IR λ_{max} (KBr): 2954, 2855, 1729,1630, 1560, 1462, 1378, 1274, 1123, 1074, 979, 771cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.72 (1H, m, H-2'), 7.70 (1H, m, H-6'), 7.53 (1H, m, H-3'), 7.51 (1H, m, H-5'), 7.35 (1H, m, H-4'), 4.21 (2H, t, J=6.0 Hz, H_2-1), 4.09 (2H, d, J=6.4 Hz, H_2-1), 2.30 (2H, t, J=7.6 Hz, H_2-2''), 2.09 (1H, m, H-3), 2.05 (1H, m, H-7), 1.87 (1H, m, H_2-2α), 1.74 (1H, m, H_2-2β), 1.70 (1H, m, H_2-6α), 1.61 (1H, m, H_2-6β), 1.55 (2H, m, H_2-5), 1.44 (1H, m, H_2-5α), 1.39 (1H, m, H_2-5β), 1.32 (2H, m, H_2-3''), 1.28 (6H, brs, 3 x CH_2), 1.25 (14H, brs, 7 x CH_2), 0.98 (3H, d, J=6.8 Hz, Me-10), 0.94 (3H, d, J=7.6 Hz, Me-9), 0.88 (3H, t, d, J=6.4 Hz, Me-14''); ^{13}C NMR ($CDCl_3$): δ

Table 4: Effect of *Premna herbacea* on levels of CAT, SOD, GSH and MDA on day 28

Group	CAT (nM H_2O_2 decomposed/min/g)	SOD (U/mg protein)	GSH (µmol/mg protein)	MDA (nmol/mL)
Normal	78.90±0.031	20.60±0.201	39.30±0.063	30.10±0.017
Diabetic control	52.30±0.0165[a]	10.40±0.312[a]	20.80±0.016[a]	49.60±0.025[a]
Diabetic+standard	72.70±0.016[b]	18.90±0.293[b]	34.00±0.021[b]	34.00±0.013[b]
Diabetic+PHE (200 mg/kg)	55.80±0.008[bc]	11.62±0.010[bc]	22.50±0.011[bc]	42.30±0.760[bc]
Diabetic+PHF (100 mg/kg)	59.00±0.016[bc]	12.89±0.006[bc]	26.80±0.012[bc]	39.50±0.428[bc]
Diabetic+PH1 (15 mg/kg)	52.60±0.191	10.12±0.009	21.00±0.022	49.30±0.009
Diabetic+PH2 (15 mg/kg)	67.80±0.006[bc]	15.12±0.010[bc]	39.30±0.063	30.10±0.017[bc]
Diabetic+PH3 (15 mg/kg)	52.70±0.225	10.23±0.009	20.80±0.016[a]	49.60±0.025

Values are mean ±SEM (n=6).

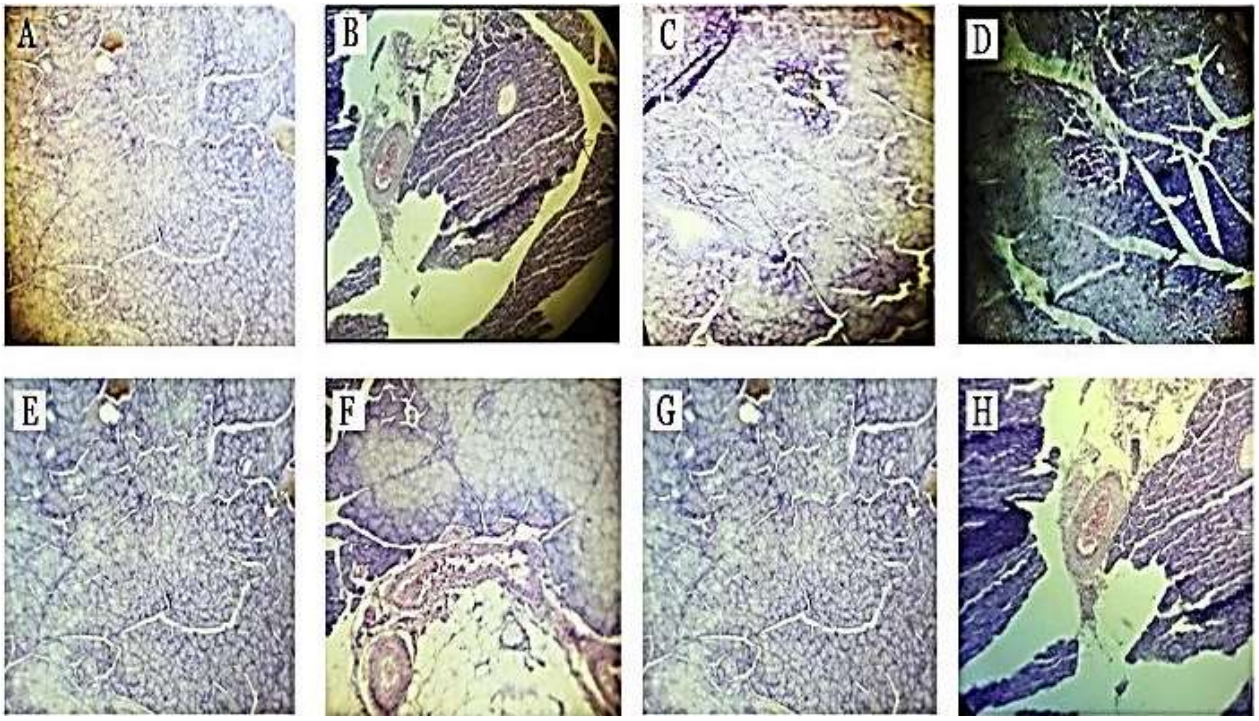

Fig. 4: Histopathological examination of pancreatic tissues of different experimental groups (A) Normal (B) Diabetic control (C) Diabetic+standard (D) Diabetic+PHE (200mg/kg) (E) Diabetic+PHF (100 mg/kg) (F) Diabetic+PH1 (15 mg/kg) (G) Diabetic+PH2 (15mg/kg) (H) Diabetic+PH3 (15mg/kg).

66.17 (C-1), 34.06 (C-2), 38.75 (C-3), 31.43 (C-4), 23.77 (C-5), 22.97 (C-6), 34.50 (C-7), 71.79 (C-8), 10.95 (C-9), 19.15 (C-10), 138.23 (C-1'), 132.40 (C-2'), 130.89 (C-3'), 128.84 (C-4'), 130.86 (C-5'), 128.80 (C-6'), 167.87 (C-7'), 172.26 (C-1"), 51.41 (C-2"), 30.20 (C-3"), 29.68 (C-4"), 29.94 (C-5"), 29.15 (C-6"), 28.93 (C-7"), 31.92 (C-8"), 29.05 (C-9"), 31.89 (C-10"), 27.72 (C-11"), 24.36 (C-12"), 22.67 (C-13"), 14.09 (C-14"); LC-MS m/z (rel. int.): 488 $[M]^+$ ($C_{31}H_{52}O_4$) (3.1), 348 (34.5), 332 (50.8), 277 (1.8).

Compounds2- 1-Benzoyloxy-8-(octadec-9''-enoyl) geranilane

On further elution with Toluene: Chloroform (9:1) it resulted in a golden yellow solid mass of compound 2 of yield 1.431mg (0.230% yield) with R_f value 0.59 (Chloroform: Ethylacetate (9.5:0.5)); Melting point: 80-82°C; IR λ max (KBr): 2926, 2854, 1731,1635, 1562, 1450, 1379, 1274, 1172, 1073, 1042, 979, 757 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.73 (1H, m, H-2'), 7.71 (1H, m, H-6'), 7.54 (1H, m, H-3'), 7.52 (1H, m, H-5'), 7.36 (1H, m, H-4"), 5.37(1H, m, H-9"), 5.34 (1H, m, H-8"), 4.25 (2H, t, J=6.0 Hz, H_2-1), 4.09 (1H, d, J=6.8 Hz, H_2-8), 2.52 (1H, m, H-3), 2.30 (2H, t, J=6.8 Hz, H_2-2"), 2.05 (2H, m, H_2-8"), 2.02 (2H, m, H_2-11"), 1.97 (1H, m, H-7), 1.80 (1H, m, H_2-2α), 1.70 (1H, m, H_2-2β), 1.70 (1H, m, H_2-6α), 1.67 (1H, m, H_2-6α), 1.61 (1H, m, H_2-6β), 1.59 (2H, m, H_2-5), 1.44 (1H, m, H_2-4α), 1.39 (1H, m, H_2-4β), 1.33 (2H, m, CH_2), 1.29 (4H, brs, 2 x CH_2), 1.25 (8H, brs, 4 x CH_2), 0.99 (3H, d, J=6.8 Hz, Me-10), 0.92 (3H, d, J=6.7 Hz, Me-9), 0.86 (3H, t, J=6.5 Hz, Me-18"); ^{13}C NMR

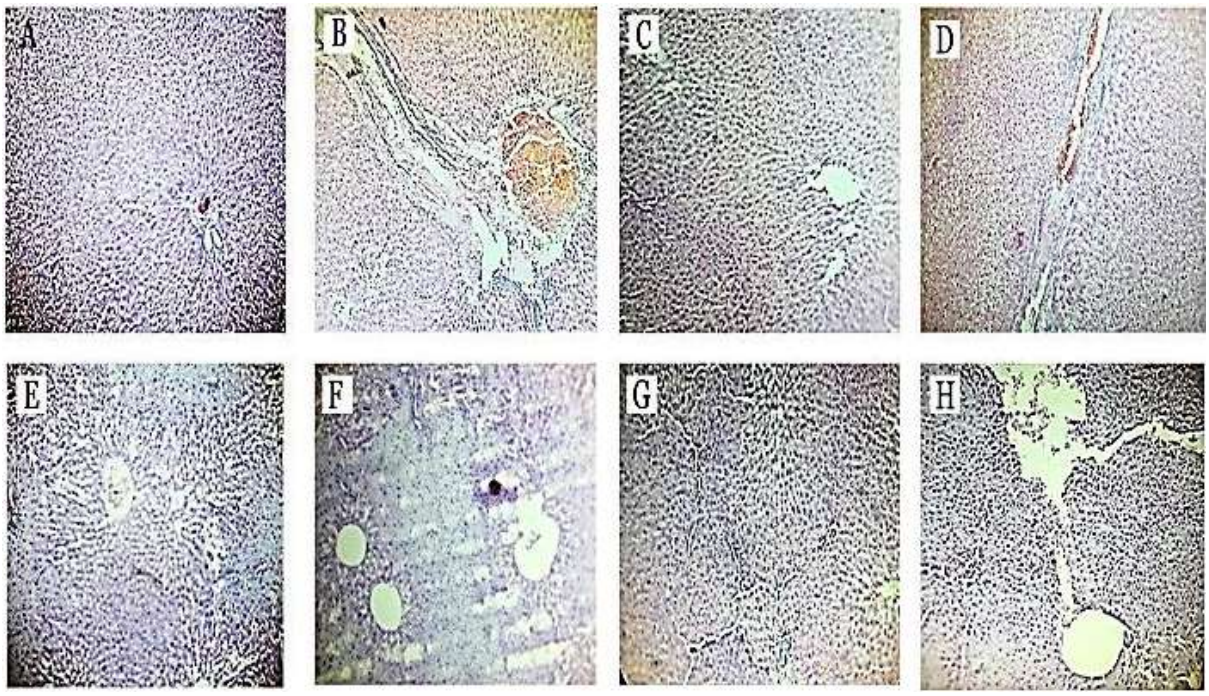

Fig. 5: Histopathological examination of hepatic tissues of different experimental groups (A) Normal (B) Diabetic control (C) Diabetic+standard (D) Diabetic+PHE (200 mg/kg) (E) Diabetic+PHF (100 mg/kg) (F) Diabetic+PH1 (15 mg/kg) (G) Diabetic+PH2 (15 mg/kg) (H) Diabetic+PH3 (15 mg/kg).

($CDCl_3$): δ 68.16 (C-1), 34.06 (C-2), 38.72 (C-3), 31.44 (C-4), 23.74 (C-5), 22.99 (C-6), 34.01 (C-7), 71.81 (C-8), 10.87 (C-9), 19.16 (C-10), 138.19 (C-1'), 132.36 (C-2'), 130.93 (C-3'), 129.06 (C-4'), 130.49 (C-5'), 128.85 (C-6'), 167.87 (C-7'), 172.83 (C-1``), 51.48 (C-2"), 33.87 (C-3"), 29.16 (C-4"), 28.92 (C-5"), 27.72 (C-6"), 26.83 (C-7"), 31.44 (C-8"), 132.40 (C-9"), 127.33 (C-10"), 30.35 (C-11"), 30.19 (C-12"), 29.70 (C-13"), 29.37 (C-14"), 24.89 (C-15"), 24.59 (C-16"), 22.70 (C-17"), 14.13 (C-18"); LC-MS m/z (rel. int.): 542 $[M]^+$ ($C_{35}H_{58}O_4$) (2.3), 348 (5.1), 332 (4.9), 265 (28.30), 127 (3.60).

Compounds3- 1-Benzoyloxy-8-octadecanoyloxygeranilane.

Later a light brown solid mass of compound 3 of yield 1.397 mg (0.225% yield) with R_f value 0.50 (Chloroform: Ethylacetate (9.5:0.5)) was obtained; Melting point: 120-122°C; IR λ max (KBr): 2954, 2851, 1728, 1635, 1561, 1458, 1378, 1275, 1123, 1072, 978, 771 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.74 (1H, m, H-2'), 7.72 (1H, m, H-6'), 7.53 (1H, m, H-3'), 7.51(1H, m, H-5'), 7.35 (1H, m, H-4'), 4.21 (2H, t, J=6.0 Hz, H_2-1), 4.08 (1H, d, J=6.8 Hz, H_2-8), 2.51 (1H, m, H-3), 2.31 (2H, t, J=6.9 Hz, H_2-2``), 2.05 (2H, m, CH_2), 2.02 (2H, m, H_2- CH_2), 1.86 (1H, m, H_2-2α), 1.76 (1H, m, H_2-2β), 1.74 (1H, m, H_2-6α), 1.61 (1H, m, H_2-6β), 1.55 (2H, m, H_2-5), 1.49 (1H, m, H_2-4α), 1.41 (1H, m, H_2-4β), 1.33 (2H, m, CH_2), 1.25 (24H, brs, 12 x CH_2), 0.98 (3H, d, J=6.8 Hz, Me-10), 0.92 (3H, d, J=6.7 Hz, Me-9), 0.86 (3H, t, J=6.5 Hz, Me-18"); ^{13}C NMR ($CDCl_3$): δ 68.19 (C-1), 34.87 (C-2), 36.74 (C-3), 31.42 (C-4), 23.75 (C-5), 22.99 (C-6), 33.87 (C-7), 71.80 (C-8), 10.86 (C-9), 19-+
.16 (C-10), 138.19 (C-1'), 132.38 (C-2'), 130.92 (C-3'), 128.85 (C-4'), 130.92 (C-5'), 128.85 (C-6'), 167.69 (C-7'), 173.46 (C-1"), 51.48 (C-2"), 33.85 (C-3"), 30.19 (C-4"), 28.93 (C-5"), 27.72 (C-6"), 29.12 (C-7"), 29.35 (C-8"), 29.49 (C-9"), 29.49 (C-10"), 29.49 (C-11"), 29.49 (C-12"), 29.46 (C-13"), 29.36 (C-14"), 24.35 (C-15"), 29.49 (C-16"), 22.69 (C-17"), 14.15 (C-18"); LC-MS m/z (rel. int.): 544 $[M]^+$ ($C_{35}H_{60}O_4$) (2.6), 348 (4.5), 267 (11.30).

In vitro hyperglycemic activity

The *Premna herbacea* extract exhibited substantial inhibition of α -amylase, and α -glucosidase with an IC_{50} value of 120.11µg/ml and 107.32µg/ml, respectively, as shown in table 1. Under similar assay conditions, Acarbose (positive control) demonstrated an IC_{50} value of 88.10µ/ml for α -amylase and 72.30µg/ml for α-glucosidase.

In vivo antidiabetic activity

Effect on fasting rat blood glucose levels

Fasting rat blood glucose was measured for all groups on 0, 14, and 28 days of drug treatment as shown in table 2 where a significant increase was seen in the case of diabetic control rats when compared to normal. Elevated glucose levels of diabetic rats were decreased 63.80% by glibenclamide. The treatment with extract and fractions of *Premna herbacea*, exhibited a slight diminution on increased fasting blood glucose levels in the range of

13.39% to 19.48%. As shown in table 2, after the administration of PH2 (15mg/kg), blood glucose levels dropped to 42.67%. PH1 and PH3 showed no remarkable action.

Effects of Premna herbacea on HbA_1C, creatinine, blood urea nitrogen, and liver enzymes

In diabetic rats, the effect of PH2 was found as potent as that of the reference drug because the levels of HbA_1C were reduced from elevated levels of 13.10% to 6.58% ($p \leq 0.001$). In the diabetic control group, blood urea nitrogen levels (BUN) and creatinine were raised to 90.30 mg/dl and 1.60 mg/dl and respectively. The extract and fraction reduced the increased creatinine and blood urea nitrogen levels to a lower level. In experimental diabetic animals, PH2 treatment decreased levels of creatinine and blood nitrogen urea to 1.23mg/dl and 61.50mg/dl ($p \leq 0.001$) respectively as shown in table 3 thus decreasing the chances of the end-stage renal disorder. Standard glibenclamide treated groups showed reduced levels of serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and alkaline phosphatase (ALP). PH2 group showed a significant fall in the increased levels of SGOT, SGPT, and ALP to 51.70 IU/liter, 63.60 IU/liter and 70.70 IU/liter respectively ($p \leq 0.001$) as shown in table 3, thus indicating an improvement in liver function.

Effect of Premna herbacea on antioxidant enzyme levels

Diabetes induced Catalase (CAT) and superoxide dismutase (SOD) level deficiency was restored to 67.80 nM H_2O_2 decomposed/ min/g and 15.12 U/mg proteins in PH2 administered groups. Reduced levels of glutathione reductase (GSH), a key component in preventing cellular damage, were also increased in this group. table 4 indicates that raised levels of malondialdehyde (MDA) in the diabetic control group were significantly lowered in PH2 treated groups from 49.60nmol/mL to 30.10nmol/mL. But groups administered with PH1 and PH3 showed no effect.

Effects of Premna herbacea on pancreatic and liver histopathology

In the histopathology studies of the pancreas (fig. 4) normal group exhibited no much change in pancreatic surface and architecture. But in diabetic control, they showed extensive and comprehensive pancreatic damage along with fibrosis and necrotic damage. The glibenclamide treated groups liver architecture showed decreased fibrosis. Groups administered with PH1 and PH3 exhibited no improvement in terms of pancreatic architecture since atrophy followed by extensively congested blood vessels and degenerated islets were noticed while extract and fraction treated groups showed slight improvement. But a sample of the groups administered with PH2 showed normal lobules of acne and preserved islets with a significant decrease in fibrosis and necrotic damage followed by dilation of islets. Histopathological studies of normal rat liver samples showed normal liver anatomy including normal portal triads and central vein. But in the case of diabetic control, they showed severe fatty depositions in hepatocytes. Focal necrotic areas were seen with inflammatory hepatocyte collections. Groups treated with PHE and PHF showed slight decrease degeneration, while PH2 samples showed minimal hepatocyte damage when compared to other samples which showed a change in liver architecture (fig. 5).

DISCUSSION

The prevalence of diabetes is increasing worldwide. Diabetes not only causes disturbances in blood glucose levels but also causes renal dysfunction and oxidative degeneration. So a multi-approach is necessary in the effective management of diabetes. Continuous use of synthetic drugs is not advisable as it causes toxic effects (Chattopadhyay, 1993; Holmann, 1991), which in turn increases the need for effective and efficacious use of herbal medicines with less side effects and relatively cheap (Erenmemsoghi *et al*., 1995; Choi *et al*., 1991). This is the first study which was aimed to isolate and characterize novel compounds from roots of *Premnaherbacea* followed by its *in vitro* and *in-vivo* antidiabetic evaluation. From the ethanolic extract of roots of *Premnaherbacea* following important phytoconstituents were isolated and structurally elucidated.

Compound 1- 1-Benzoyloxy-8-tetradecanoyloxy-geranilane

Compound 1, a geranilanyldiester, showed characteristic IR spectra which suggests the presence of ester groups (1729 cm^{-1}), aromatic ring (1630 cm^{-1}, 1560 cm^{-1}, 1074 cm^{-1}), an aliphatic chain (771 cm^{-1}). Based on mass spectroscopy the molecular ion peak was determined at m/z 488 consistent with the molecular formula of a monoterpenicdiester $C_{31}H_{52}O_4$. The ion peaks generated at m/z 348 [M- C_6H_5CO] $^+$, 332 [M- C_6H_5COO] $^{+,}$ and 277 [M- CH_3 $(CH_2)_{12}CO$] $^+$ suggested that benzoyl and myristyl groups were attached to a geranilanyl group unit. The ^{1}H NMR spectra of compound 1 exhibited five one proton multiplets from δ 7.72 to δ 7.35 assigned to aromatic protons. A two proton triplet at δ 4.21 (J=6.0 Hz) and a two proton doublet at δ 4.09 (J=6.4 Hz) were ascribed to oxygenated methylene H_2- 1 and H_2-8 protons respectively. The other methylene protons appeared as a two proton triplet at δ 2.30 (J=7.6 Hz) due to H_2- 2" adjacent to the ester group, as multiplets between δ 2.09-1.32 and as broad singlets at δ 1.28 (6H) and 1.25 (14H). Two-three proton doublets, at δ 0.94 (J= 6.8 Hz) and 0.94 (J= 7.6 Hz) and a three proton triplet at δ 0.88 (J= 6.4 Hz) were accounted correspondingly to secondary C-10 and C-9 and primary C-14" methyl protons. The ^{13}CNMR

spectra of compound 1 displayed signals for ester carbons at δ 167.87 (C-7') and δ 172.26 (C-1"), aromatic carbons between δ 138.23-128.80, methyl carbons at δ 10.95 (C-9), δ 19.15 (C-10), and δ 14.09 (C-14"), oxygenated methylene carbons at δ 66.17 (C-1) and δ 71.79 (C-8) and the remaining methine and methylene carbons from δ 51.41- δ 22.67. The ^{1}H - ^{1}H COSY spectra showed correlations of H-2' with H-3' and H-6'; H_2-1 with H_2-2 and H-3; H_2-8 with H-7 and H_2-6 and Me-9 and Me- 14" with H_2-13" and H_2- 12". The HMBC spectra of compound 1 exhibited interactions of H-2', H-3' and H-6' with C-1'; H_2-2, H_2-1, H_2-2' and H-6' with C-7' and H_2-8, H_2-2" and H_2-3" with C-1". Based on the above data it can be concluded that compound 1 is unsaturated and structure has been established as- 1-Benzoyloxy-8-tetradecanoyloxy-geranilane (fig. 1). This is a new monoterpenic ester.

Compounds2- 1-Benzoyloxy-8-(octadec-9''-enoyl) geranilane

The IR spectra suggest the presence of ester group (1731 cm^{-1}), unsaturation (1635 cm^{-1}), aromatic ring (1562 cm^{-1}, 1073 cm^{-1}) and aliphatic chain (757cm^{-1}). It had a molecular ion peak at m/z 542 established based on mass and ^{13}CNMR spectra which corresponded to a molecular formula of a monoterpenicdiester. The ion peaks arising at m/z 281 (C_8-O fission, $CH_3(CH_2)_7CH{=}CH(CH_2)_7COO]^+$), 265 [$C_{1"}$-O fission, $CH_3(CH_2)_7CH{=}CHCH_2CO]^+$) and 121 $[C_6H_5COO]^+$ indicated that oleic and benzoic acids were esterified with a geranilandiol. The ^{1}HNMR exhibited seven one proton multiplets from δ 7.73- δ 5.34 assigned to five aromatic and two vinylic protons. A two proton triplet at δ 4.25 (J=6.0 Hz) and a two proton doublet at δ 4.09(J= 6.8 Hz) were ascribed to oxygenated methylene H_2-1and H_2-8 protons respectively. Two-three proton doublets at δ 0.99 (J= 6.8 Hz) and 0.92 (J=6.7 Hz) and a three proton triplet at δ 0.86 (J= 6.5 Hz) were associated correspondingly with the secondary C-10, C-9, and primary C-18" methyl protons. The remaining methylene and methine protons appeared between δ 2.52-1.25. The ^{13}CNMR spectra of compound 2 displayed signals for ester carbon at δ 167.71 (C-7') and 172.83 (C-1"), aromatic and vinylic carbons in the range of δ 138.19-128.85, oxygenated methylene carbons at δ 68.16 (C-1) and δ 71.81 (C-8) and methyl carbons at δ 10.87 (C-9), δ 19.16 (C-10) and δ 14.13 (C-18"). The ^{1}H-^{1}H COSY spectra showed correlations of H_2-1 with H_2—2 and H-3; H_2-8 with H-7, H_2-6, and H_3-9; H-2' with H-3', H-4' and H-6' and H-9" with H-8", H-10", and H_2-11". The HMBC spectra of compound 2 showed that H-2', H-3', and H-6' interacted with C-1'; H_2-1, H-2', and H-6' interacted with C-7'; H_2-8 and H-2" with C-1"; and H_2-8", H-9", and H_2-11" interacted with C-10". Based on the above discussion compound 2 has been established as- 1-Benzoyloxy-8-(octadec-9"-enoyl) geranilane (fig. 2). This is a new monoterpenicdiester.

Compounds3- 1-Benzoyloxy-8-octadecanoyloxygeranilane

The IR spectra suggest the presence of ester group (1728 cm^{-1}), aromatic ring (1635 cm^{-1}, 1561 cm^{-1}, 1072 cm^{-1}) and aliphatic chain (771 cm^{-1}). It had a molecular ion peak at m/z 544 established based on mass and ^{13}CNMR spectra which corresponded to a molecular formula of a monoterpenicdiester. The ion peaks arising at m/z 267 [$C_{1"}$-O fission, CH_3 $(CH_2)16CO]^+$) indicated that stearyl group was attached to the benzoyloxygeranilane unit. The ^{1}HNMR exhibited five one proton multiplets from δ 7.74-δ 7.35 assigned to aromatic protons. A two proton triplet at δ 4.21 (J=6.1 Hz) and a two proton doublet at δ 4.08 (J= 6.8 Hz) were ascribed to oxygenated methylene H_2-1and H_2-8 protons respectively, other methine and methylene protons from δ 2.51 to δ 1.25 and methyl proton as three proton doublets at δ 0.98 (J=6.8 Hz) and 0.92 (J=6.7 Hz) and a triplet at δ 0.86 (J=6.5 Hz) accounted to secondary C-10 and C-9 and primary C-18" methyl protons respectively. The ^{13}CNMR spectra of compound 3 displayed signals for ester carbon at δ 167.69 (C-7') and 173.46 (C-1"), aromatic carbons in the range of δ 138.19- δ 128.85, oxygenated methylene carbons at δ 68.19 (C-1) and δ 71.80 (C-8) and methyl carbons at δ 10.86 (C-9), δ 19.16 (C-10) and δ 14.15 (C-18"). The ^{1}H-^{1}H COSY spectra showed correlations of H-1 with H_2- and H-3; H_2-8 with H-7, H_2-6 and Me-9 and H-2' with H-3', H-4', and H-6'. The HMBC spectra of compound 3 showed that H_2-1, H-2', and H-6' interacted with C-7'; H_2-8 and H-2" with C-1" and H_2-16", H-17" with C-18". Based on the above discussion compound 3 is established as- 1-Benzoyloxy-8- octadecanoyloxygeranilane (fig. 3).

In the preliminary assessment, the ethanolic extract revealed significant α -amylase and α -glucosidase inhibitory activity. The major indication of diabetes mellitus is elevated fasting glucose levels (Postic *et al*., 2004). The administration of compound 2 showed a significant dose-dependent decrease in fasting sugar levels which ($p \leq 0.001$) which indicates the relapse of the pancreas to normal. Increased HbA_1C levels are used as an objective for indicating the onset of diabetes mellitus (Florkowski 2013) and long-standing diabetes is an adverse factor in the progression of renal dysfunction indicated by high levels of creatinine and blood urea nitrogen leading to diabetic nephropathy (Nasri and Kopaei 2015). These levels were reduced in compound 2 treated groups which indicate a checkpoint in the complications associated with diabetes.

The liver is a vital organ involved in glucose homeostasis. So long term diabetes mellitus is closely associated with hepatic damage indicated by increased levels of liver enzymes (Haris 2005). Here in this study treatment with PH2 reduced the levels of liver enzymes significantly when compared with the standard group. Based on this it can be observed that compound **2** exerts a beneficial

effect on the liver. In diabetic patients when the major focus is given in controlling hyperglycemia, little or no attention is given to other vascular complications (Ullah *et al*., 2016). This may pave way for the development of oxidative stress due to the free radical generation which impairs the endogenous antioxidant defense system (Giacco and Brownlee 2010; Moron *et al*., 2016). This is accompanied by the decreased levels of CAT, SOD, and GSH as well as increased levels of MDA which was further supported by the histopathology studies of the liver and pancreas. It was also noticed that compound **2**, 1-Benzoyloxy-8-(octadec-9"-enoyl) geranilane, from *Premna herbacea* root extract not only showed a hyperglycemic effect but also exhibited antioxidant effect by regulating the levels of antioxidant enzymes.

CONCLUSION

This study yielded a new promising compound namely 1-Benzoyloxy-8-(octadec-9"-enoyl) geranilane from roots *Premna herbacea* with antidiabetic and antioxidant activity. This duo activity in a single compound may reduce oxidative stress in diabetic patients which prevents diabetic complications to a greater extend.

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