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-tetradecanoyloxygeraniol (1), 1-Benzoyloxy-8-(octadec-9′-enoyl) geraniol (2), and 1-Benzoyloxy-8-octadecanoyloxygeraniol (3), from the roots of *Premna herbacea*. Their structures were deciphered by 1H NMR, 13C 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-Benzyloxy-8-(octadec-9′-enoyl) geraniol from roots of *Premna herbacea* have significant antidiabetic and antioxidant activity, thus indicating a corrective effect on diabetes and its complications.

Keywords: *Premna herbacea*, 1-Benzoyloxy-8-tetradecanoyloxygeraniol, 1-Benzoyloxy-8-(octadec-9′-enoyl) geraniol, 1-Benzoyloxy-8-octadecanoyloxygeraniol, 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 peninsular including Kerala, Karnataka, and Tamilnadu (Reddy et al., 2010). It is known as *Bhoonjambu* in Sanskrit, *Bharangini* 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 apprised 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 *Premna herbacea*.

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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 IC50 value were determined (Madhusudhan et al., 2015; Nair et al., 2013). The results are reported as % IC50 ± 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.

In vivo antidiabetic activity

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. After 72 hours of injection, rats with fasting blood glucose levels greater than 260mg/dl were considered hyperglycemic and included in further study (Anandurai 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.

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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; Value (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ethanolic extract</strong></td>
<td></td>
</tr>
<tr>
<td>α-glucosidase inhibitory activity</td>
<td>107.32±0.451&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>α-amylase inhibitory activity</td>
<td>120.11±0.231&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Acarbose (standard)</strong></td>
<td></td>
</tr>
<tr>
<td>α-glucosidase inhibitory activity</td>
<td>72.30±0.883</td>
</tr>
<tr>
<td>α-amylase inhibitory activity</td>
<td>88.10±0.239</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n=3), <sup>a</sup> p ≤ 0.001 vs. standard.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (mg/dL)</th>
<th>Change in FBG on day 28 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 14</td>
</tr>
<tr>
<td>Normal</td>
<td>80.80±0.307</td>
<td>83.30±0.667</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>280.00±0.477&lt;sup&gt;a&lt;/sup&gt;</td>
<td>282.00±0.792&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+standard</td>
<td>264.39±0.477&lt;sup&gt;b&lt;/sup&gt;</td>
<td>131.01±0.872&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+PHE (200 mg/kg)</td>
<td>274.80±0.477&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>242.00±0.601&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+PHF (100 mg/kg)</td>
<td>265.00±0.307&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>225.00±0.477&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+PH1 (15 mg/kg)</td>
<td>279.02±0.307</td>
<td>279.00±0.803</td>
</tr>
<tr>
<td>Diabetic+PH2 (15 mg/kg)</td>
<td>262.00±0.872&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>189.00±0.715&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+PH3 (15 mg/kg)</td>
<td>278.80±0.792</td>
<td>279.00±0.654</td>
</tr>
</tbody>
</table>

Values are mean ±SEM (n=6), <sup>a</sup> p ≤ 0.001 vs. normal control, <sup>b</sup> p ≤ 0.001 vs. diabetic control, <sup>c</sup> p≤0.001 vs. standard treatment.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>HbA1C (%)</th>
<th>Creatinine (mg/dL)</th>
<th>BUN (mg/dL)</th>
<th>SGOT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 14</td>
<td>Day 28</td>
<td>Day 0</td>
<td>Day 14</td>
<td>Day 28</td>
</tr>
<tr>
<td>Normal</td>
<td>3.00±0.053</td>
<td>0.545±0.009</td>
<td>43.20±0.833</td>
<td>35.60±0.050</td>
<td>35.60±0.050</td>
<td>55.20±0.010</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>13.10±0.063&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.600±0.014&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.30±0.882</td>
<td>73.60±0.033&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.60±0.033&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.70±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+standard</td>
<td>4.83±0.106&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.862±0.005&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56.70±0.667&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.30±0.030&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47.30±0.030&lt;sup&gt;b&lt;/sup&gt;</td>
<td>61.20±0.017&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+PHE (200 mg/kg)</td>
<td>10.80±0.016&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.470±0.007&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>81.50±0.428&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>70.10±0.028&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>70.10±0.028&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>87.90±0.292&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+PHF (100 mg/kg)</td>
<td>9.05±0.020&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.410±0.005&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>74.70±0.494&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>63.30±0.030&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>63.30±0.030&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>77.31±0.273&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+PH1 (15 mg/kg)</td>
<td>13.00±0.021</td>
<td>1.620±0.009</td>
<td>89.80±0.601</td>
<td>73.60±0.019</td>
<td>73.60±0.019</td>
<td>87.50±0.342</td>
</tr>
<tr>
<td>Diabetic+PH2 (15 mg/kg)</td>
<td>6.58±0.029&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.230±0.006&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>61.50±0.563&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>51.70±0.171&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>51.70±0.171&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>70.70±0.143&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic+PH3 (15 mg/kg)</td>
<td>12.90±0.015</td>
<td>1.630±0.007</td>
<td>90.30±0.803</td>
<td>73.40±0.071</td>
<td>73.40±0.071</td>
<td>86.90±0.506</td>
</tr>
</tbody>
</table>

Values are mean ±SEM (n=6), <sup>a</sup> p ≤ 0.001 vs. normal control, <sup>b</sup> p<0.001 vs. diabetic control, <sup>c</sup> p≤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*.

**Compound 1-1-Benzoyloxy-8-tetradecanoxyloxy-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<sub>f</sub> value 0.67 (Chloroform: Ethylacetate (9.5:0.5)); Melting point: 82-84°C; IR <sub>λmax</sub> (KBr): 2954, 2855, 1729,1630, 1560, 1462, 1378, 1274, 1123, 1074, 979, 771cm<sup>-1</sup>; 1H NMR (CDCl<sub>3</sub>): 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'), 4.09 (2H, d, J=6.4 Hz, H-2'), 2.30 (2H, t, J=7.6 Hz, H-2''), 2.09 (1H, m, H-3), 2.05 (1H, m, H-7), 1.87 (1H, m, H-2n), 1.74 (1H, m, H-2β), 1.70 (1H, m, H-6α), 1.61 (1H, m, H-6β), 1.55 (2H, m, H-5), 1.44 (1H, m, H-5α), 1.39 (1H, m, H-5β), 1.32 (2H, m, H-3''), 1.28 (6H, brs, 3 x CH3), 1.25 (14H, brs, 7 x CH2), 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''); 13C NMR (CDCl<sub>3</sub>): δ
Novel compounds from Premna herbacea Roxb. with antidiabetic potential.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>CAT (nM H$_2$O$_2$ decomposed/min/g)</th>
<th>SOD (U/mg protein)</th>
<th>GSH (µmol/mg protein)</th>
<th>MDA (nmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>78.90±0.031</td>
<td>20.60±0.201</td>
<td>39.30±0.063</td>
<td>30.10±0.017</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>52.30±0.016$^a$</td>
<td>10.40±0.312$^a$</td>
<td>20.80±0.016$^a$</td>
<td>49.60±0.025$^a$</td>
</tr>
<tr>
<td>Diabetic+standard</td>
<td>72.70±0.016$^b$</td>
<td>18.90±0.293$^b$</td>
<td>34.00±0.021$^b$</td>
<td>34.00±0.013$^b$</td>
</tr>
<tr>
<td>Diabetic+PHE (200 mg/kg)</td>
<td>55.80±0.008$^a$</td>
<td>11.62±0.010$^a$</td>
<td>22.50±0.011$^a$</td>
<td>42.30±0.076$^a$</td>
</tr>
<tr>
<td>Diabetic+PHF (100 mg/kg)</td>
<td>59.00±0.016$^a$</td>
<td>12.89±0.006$^a$</td>
<td>26.80±0.012$^a$</td>
<td>39.50±0.042$^a$</td>
</tr>
<tr>
<td>Diabetic+PH1 (15 mg/kg)</td>
<td>52.60±0.191</td>
<td>10.12±0.009</td>
<td>21.00±0.022</td>
<td>49.30±0.009</td>
</tr>
<tr>
<td>Diabetic+PH2 (15 mg/kg)</td>
<td>67.80±0.006$^a$</td>
<td>15.12±0.010$^a$</td>
<td>39.30±0.063</td>
<td>30.10±0.017$^a$</td>
</tr>
<tr>
<td>Diabetic+PH3 (15 mg/kg)</td>
<td>52.70±0.225</td>
<td>10.23±0.009</td>
<td>20.80±0.016$^a$</td>
<td>49.60±0.025</td>
</tr>
</tbody>
</table>

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 (15 mg/kg) (H) Diabetic+PH3 (15 mg/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-1Þ), 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$_{13}$H$_{18}$O$_3$) (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 Rf 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$^{-1}$; $^1$H 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'), 7.56 (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), 4.09 (1H, d, J=6.8 Hz, H-2'), 2.52 (1H, m, H-3), 2.30 (2H, t, J=6.8 Hz, H-2''), 2.05 (2H, m, H-8''), 2.02 (2H, m, H-11''), 1.97 (1H, m, H-7''), 1.80 (1H, m, H-2a), 1.70 (1H, m, H-2b), 1.70 (1H, m, H-6a), 1.67 (1H, m, H-6a), 1.61 (1H, m, H-6b), 1.59 (2H, m, H-5), 1.44 (1H, m, H-4a), 1.39 (1H, m, H-4b), 1.33 (2H, m, CH$_3$), 1.29 (4H, brs, 2 x CH$_3$), 1.25 (8H, brs, 4 x CH$_3$), 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
CDCl₃): 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\''), 14.15 (C-18\''); LC-MS m/z (rel. int.): 542 [M]+ (C₃₅H₅₈O₄) (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 Rf 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 (CDCl₃): 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, H₂-1), 4.08 (1H, d, J=6.8 Hz, H₂-8), 2.51 (1H, m, H-3), 2.31 (2H, t, J=6.9 Hz, H₂-2\’), 2.05 (2H, m, CH₂), 2.02 (2H, m, H₂- CH₂), 1.86 (1H, m, H₂-2α), 1.76 (1H, m, H₂-3β), 1.74 (1H, m, H₂-6α), 1.61 (1H, m, H₂-6β), 1.55 (2H, m, H₂-5), 1.49 (1H, m, H₂-4α), 1.41 (1H, m, H₂-4β), 1.33 (2H, m, CH₂), 1.25 (2H, brs, 12 x CH₂), 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\’); ¹³C NMR (CDCl₃): 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\’), 132.36 (C-2\’), 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\’’), 29.46 (C-12\’’), 29.36 (C-13\’’), 29.36 (C-14\’’), 24.35 (C-15\’’), 22.69 (C-17\’’), 14.15 (C-18\’’); LC-MS m/z (rel. int.): 544 [M]+ (C₃₅H₆₀O₄) (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 IC₅₀ value of 120.11µg/ml and 107.32µg/ml, respectively, as shown in table 1. Under similar assay conditions, Acarbose (positive control) demonstrated an IC₅₀ value of 88.10µg/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 1.97-2.25 mg/dl.
and preserved islets with a significant decrease in fibrosis
administered with PH 2 showed normal lobules of acne
slight improvement. But a sample of the groups
noticed while extract and fraction treated groups showed
architecture since atrophy followed by extensively
PH3 exhibited no improvement in terms of pancreatic
decreased fibrosis. Groups administered with PH1 and
PH2 treated groups from 49.60nmol/mL to
the diabetic control group were significantly lowered in
damage, were also increased in this group. Table 4
reductase (GSH), a key component in preventing cellular
dismutase (SOD) level deficiency was restored to 67.80
IU/liter, 63.60 IU/liter and 70.70 IU/liter respectively
(p≤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$_2$O$_2$ 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 (Erennemmosghi et al., 1995; Choi et al., 1991).
This is the first study which was aimed to isolate and
characterize novel compounds from roots of
Premnährerbacea followed by its in vitro and in-vivo
antidiabetic evaluation. From the ethanolic extract of
roots of Premnährerbacea following important
phytoconstituents were isolated and structurally
elucidated.

**Compound 1- 1-Benzoyloxy-8-tetradecanoyloxy-
gerani lanilene**
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$_6$. The ion peaks generated at
m/z 348 [M- C$_6$H$_2$CO]$^+$, 332 [M- C$_6$H$_2$COO]$^+$, and 277
[M- CH$_3$ (CH$_2$)$_2$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 4.32 (J=6.0 Hz)
and a two proton doublet at δ 4.09 (J=6.4 Hz) were
assigned to oxygenated methylene H$_2$- 1 and H$_2$- 2 protons
respectively. The other methane 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 $^{1}$CNMR

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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 1H - 1H COSY spectra showed correlations of H-2' with H-3' and H-6'; H-2 with H-3 and H-3 with H-7 and H-6 and Me-9 and Me- 14 with H-13' and H-12'. The HMBC spectra of compound 1 showed interactions of H-2', H-3' and H-6' with C-1'; H-2, H-2' and H-6' with C-7' and H-2, H-2' and H-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.

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

The IR spectra suggest the presence of ester group (1732 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 542 established based on mass and 13CNMR spectra which corresponded to a molecular formula of a monoterpenic diester. The ion peaks arising at m/z 281 (C₇-O fission, CH₃(CH₂)₇CH=CH(CH₂)₇COO⁻), 265 (C₉-O fission, CH₃(CH₂)₅CH=CH(CH₂)₅COO⁻) and 121 (C₈H₁₀COO⁻) indicated that oleic and benzoic acids were esterified with a geranilidol. The 1H NMR 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 4.21 (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-8' protons respectively, other methine and methylene protons from δ 2.51 to δ 1.25 and methyl protons from δ 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 13CNMR 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 1H COSY spectra showed correlations of H-1 with H-2, and H-3; H-2 with H-7, H-6 and Me-9 and H-2' with H-3', H-4', and H-6'. The HMBC spectra of compound 3 showed that H-1, H-2', and H-6' interacted with C-7'; H-2 and H-2' with C-1' and H-2, H-8' and H-2'' with C-1'' and H-2', H-9', and H-2' and H-3' with C-10'. 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 ≤ 0.001) which indicates the relapse of the pancreas to normal. Increased HbA1C 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
Novel compounds from *Premna herbacea* Roxb. with antidiabetic potential.

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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