Antihyperglycemic Effect of *Meryta denhamii* Seem. Fruits and Phytochemical study of its Saponin Content

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**ABSTRACT**

In this study *Meryta denhamii* Seem. fruits (Araliaceae) were tested for the antihyperglycemic effect against alloxan induced hyperglycemia in rats using metformin as standard drug. The alcoholic extract and n-butanol fraction (saponins rich fraction) of the fruits exhibited significant antihyperglycemic effect (42.8 and 38.4 % of change, respectively, comparing to 67.1% for metformin). The n-butanol fraction was subjected to chemical study which resulted in isolation of four monodesmosidic oleanane saponins. Their structures were established based on their MS, 1H-NMR and 13C-NMR spectral data as 3-O-[β-D-glucuronopyranosyl] oleanolic acid, 3-O-[α-D-glucuronopyranosyl] oleanolic acid, 3-O-[β-D-glucopyranosyl-(1-3)-α-L-arabinofuranosyl] oleanolic acid and 3-O-[α-Larabinofuranosyl-(1-4)-β-D-glucuronopyranosyl] oleanolic acid.

**Key words:** *Meryta denhamii* Seem. fruits, triterpenoid saponins, oleanane saponins, antihyperglycemic.

**INTRODUCTION**

Plants of family Araliaceae are rich in saponin content[1-5]. This class of constituents is characterized by a pronounced molluscicidal activity[6-10]. Antifungal[10-12], antidiabetic[13-15] and antiproliferative[16] activities were also recorded for saponins. *Meryta denhamii* Seem. is an evergreen tree cultivated in public gardens in Egypt, the plant is dioecious, giving globe-like fruits with 12-16 fused berries[17]. The alcoholic extracts of both the flowers and fruits exhibited molluscicidal activity against * Biomphalaria alexandrina* and *Lymnaea Caillaudii*[18], while the alcoholic extract of the stems exhibited anthelmintic activity against adult liver flukes, *Fasciola gigantica*[19]. These observed activities were attributed mainly to the saponin content of the plant. Oleanane saponins were isolated from different organs of the plant[16,18] except the fruits. Thus, this work was conducted on the fruits aiming for testing their antihyperglycemic activity and isolation of these bioactive compounds.

**MATERIAL AND METHODS**

**General experimental**

Mass spectra were performed on UPLC/MS/MS-Waters. NMR spectra were run using Jeol TMS Route instrument at 300 and 90 MHz for measuring 1H and 13C NMR, respectively. TLC was performed on precoated silica gel plates using chloroform: methanol [9:1 (S1) & 95:5 (S2)] and chloroform: methanol: formic acid [75:20:5 (S3)] as solvent systems. The chromatograms were visualized under UV light (at λ max 254 and 366 nm) before and after exposure to ammonia vapor, as well as spraying with p-anisaldehyde/ sulphuric acid spray reagent.

**Plant material**

The fruits of *M. denhamii* Seem. were collected from Faculty of Agriculture, Ein Shams University in July, 2011. The plant was kindly authenticated by Mrs T. Labib, taxonomist in El-Orman public garden, Giza, Egypt.

**Extraction**

About 2 kg of fresh fruits of *M. denhamii* seem. was extracted with cold methanol till exhaustion. After stripping of the solvent under reduced pressure, the residue (100 g) was suspended in water, and then fractionated by successive extraction with suitable volumes of petroleum ether (6 g), chloroform (0.5 g), ethyl acetate (1.2 g) and n-butanol (20 g).
**Experimental animals**

Sprague Dawley rats (100-150) were obtained from the animal house of National Research Center, Dokki, Giza, Egypt. They were maintained in standard environmental conditions of temperature (25 ± 2 °C), relative humidity (55 ± 10%) and they were kept in cages and maintained in well ventilated room under natural light and dark cycle.

**Drugs and Kits**

Alloxan: Sigma Co., Germany.


**Antihyperglycemic activity**

Rats were divided into five groups (6 animals each), the first group was kept as a control (received 1 ml saline), while for the other groups, diabetes mellitus was induced by intra-peritoneal injection of a single dose of alloxan (150 mg/kg b. wt.) followed by an overnight fasting[20]. A group of diabetic rats was kept non-treated served as negative control, another group received metformin (oral dose of 100 mg/kg b. wt.) as reference drug. The other two groups received the alcoholic extract and n-butanol fraction of *M. denhamii* Seem. fruits (oral dose of 100 mg/kg b. wt.). Blood samples were taken at zero time (G₀) and after 4 and 8 weeks (G₄ and G₈) from the retro-orbital venous plexus, the serum of the blood samples were isolated by centrifugation, then the blood glucose level was estimated using glucose kits according to the method described by Trinder[21]. The percentage of change of blood glucose level was calculated [% of change = (G₈ - G₀) × 100/G₀], the data were statistically analyzed using student’s t-test[22], the obtained results were given in table 1.

### Table 1: Antihyperglycemic activity of *Meryta dehamii* Seem. fruits

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose level (mg/dl)</th>
<th>% of change</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
<td>After 4 weeks</td>
<td>After 8 weeks</td>
</tr>
<tr>
<td></td>
<td>M ± S.E.</td>
<td>M ± S.E.</td>
<td>% of change</td>
</tr>
<tr>
<td>Control (1 ml saline)</td>
<td>82.4 ± 1.9</td>
<td>83.6 ± 1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Diabetic non treated</td>
<td>259.4 ± 9.20</td>
<td>262.2 ± 11.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Diabetic treated with alcohol extract</td>
<td>249.6 ± 10.3</td>
<td>188.1 ± 9.3*</td>
<td>24.6</td>
</tr>
<tr>
<td>Diabetic treated with n-butanol fraction</td>
<td>256.1 ± 11.2</td>
<td>209.4 ± 8.1*</td>
<td>18.2</td>
</tr>
<tr>
<td>Diabetic treated with metformin</td>
<td>264.2 ± 9.3</td>
<td>171.9 ± 6.4*</td>
<td>34.9</td>
</tr>
</tbody>
</table>

*Statistically significant from control at P < 0.01.

**Fractionation and isolation**

Ten g of n-butanol fraction was fractionated by VLC on silica gel G 60 column (10 × 7 cm). Gradient elution was carried out using chloroform: ethyl acetate mixtures, ethyl acetate and ethyl acetate: methanol mixtures as eluent. Fractions (200 ml each) were collected and monitored by TLC, similar fractions were collected together. Fractions eluted with 100% ethyl acetate, 1% methanol and 5% methanol were pooled and rechromatographed on silica gel column using solvent system chloroform: methanol (95:5) and then, purified on sephadex LH-20 using methanol as eluent to yield compounds 1 and 2. Fraction eluted with 10% methanol was rechromatographed on silica gel column using solvent system chloroform: methanol (9:1) and then, sephadex LH-20 using methanol: water (1:1) as eluent which afforded compounds 3 and 4.

**Compound 1:** White powder, R₂, 0.693 (S₂), MS m/z: 617 [M]+, ¹H-NMR (300 MHz, DMSO): δ: 0.58, 0.76, 0.84, 0.86, 0.88, 1.07 and 1.23 (each 3H, s, 7 CH₃), 5.08 (1H, broad s, H-12), 4.23 (1H, broad s, H-1’) and 3.07 - 3.62 (sugar protons). ¹³C-NMR (90 MHz, DMSO), see table 2.
Compound 2: White powder, Rf 0.671 (S), MS m/z 631 [M]+, 1H-NMR (300 MHz, CD3OD), δH 0.77, 0.80, 0.88, 0.90, 0.91, 0.95 and 0.97 (each 3H, s, 7CH3), 5.29 (1H, broad s, H-12), 4.46 (1H, broad s, H-1`), 5.14 (1H, broad s, H-1``) and 3.05 - 4.38 (sugar protons). 13C-NMR (90 MHz, CD3OD), see table 2.

Compound 3: White powder, Rf 0.437 (S), MS m/z 749 [M]+, 1H-NMR (300 MHz, DMSO): δH 0.53 (3H, s, CH3), 0.72 (3H, s, CH3), 0.87 (6H, s, 2CH3), 1.09 (3H, s, CH3), 1.23 (6H, s, 2CH3), 5.38 (1H, broad s, H-12), 4.54 (1H, broad s, H-1`), 5.14 (1H, broad s, H-1``) and 3.05 - 4.38 (sugar protons). 13C-NMR (90 MHz, DMSO), see table 2.

Compound 4: Needle crystals, Rf 0.166 (S), MS m/z 763 [M]+, 1H-NMR (300 MHz, DMSO): δH 0.74, 0.85, 0.86, 0.95, 1.07 (each 3H, s, 5CH3), 1.22 (6H, s, 2CH3), 5.10 (1H, broad s, H-12), 4.47 (1H, broad s, H-1`), 4.78 (1H, broad s, H-1``) and 3.03 - 4.11 (sugar protons). 13C-NMR (90 MHz, DMSO), see table 2.

RESULTS AND DISCUSSION

Both the alcoholic extract and n-butanol fraction of M. denhamii Seem. exhibited significant antihyperglycemic activity (42.8 and 38.4 % of change after 8 weeks, respectively) against alloxan induced hyperglycemia in rats compared to metformin (67.1 % of change after 8 weeks).

Four triterpenoidal saponins were isolated from the n-butanol fraction of Meryta denhamii, Seem. fruits by chromatographic fractionation on silica gel and sephadex columns.

1H-NMR of compound 1 displayed seven singlets at δ 0.58, 0.76, 0.84, 0.86, 0.88, 1.07 and 1.23 corresponding to seven tertiary methyls and a trisubstituted olefinic proton (δ 5.08) which are characteristic for oleanane-type triterpene[3]. Signals at δH 4.23 and δH 104.45 revealed the presence of a sugar molecule. By comparing the spectral data of compound 1 with the published data[1,16,24], it was identified as 3-O-[α-D-glucopyranosyl-(1-3)-α-L-arabinofuranosyl] oleanolic acid 3-

anomeric signals of compound 3 appeared at δH 4.54, δH 5.14, δH 102.34 and δH 108.62. By comparing the spectral data of compound 3 with the published data[1,16,24], it was identified as oleanolic acid 3-

Compound 4 displayed signals of two anomeric protons at δH 4.47 and 4.78 and two anomeric carbons at δC 105.68 and 109.26. Compound 4 was identified as 3-0-[α-L-arabinofuranosyl-(1-4)-β-D-glucuronopyranosyl] oleanolic acid by comparing its spectral data with the published data[6]. Compounds 2, 3 and 4 were for the first time isolated from this plant. The identity of the four compounds was further confirmed by acid hydrolysis[6] and comparison with reference materials.

CONCLUSION

Triterpenoidal saponins were reported to possess hypoglycemic activity[13-15], thus the observed antihyperglycemic activity of the n-butanol fraction could be attributed mainly to its saponin content. Other plant constituent viz. flavonoids also possess antihyperglycemic activity[25-27], this could explain the higher activity of the alcoholic extract compared to the n-butanol fraction.

REFERENCES