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

Enas H. Abdel Rahman, Azza R. Abdel Monem*, Amany A. Sleem

Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt. *Pharmacology Department, National Research Centre, Giza 12622, Egypt

**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, $^1$H-NMR and $^{13}$C-NMR spectral data as 3-O-[β-D-glucuronopyranosyl] oleanolic acid, 3-O-[α-D-glucuronopyranosyl]-[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 Caillaudi*\(^18\), 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. Oleane saponins were isolated from different organs of the plant\(^16,20\) 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 $^1$H-NMR and $^{13}$C-NMR, respectively. TLC was performed on precoated silica gel plates using chloroform: methanol [9:1 (S1) & 95:5 (S 2)] and chloroform: methanol: formic acid [75:20:5 (S 3)] as solvent systems. The chromatograms were visualized under UV light (at $\lambda _{max}$ 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.) 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 Trainder[21]. The percentage of change of blood glucose level was calculated [% of change = (G₀ – Gₜ) × 100/G₀], the data were statistically analyzed using student’s t-test[22], the obtained results were given in table 1.

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 (95:5) and then, purified on sephadex LH-20 using methanol: water (1:1) as eluent which afforded compounds 3 and 4.

Table 1: Antihyperglycemic activity of Meryta denhamii Seem. fruits

<table>
<thead>
<tr>
<th>Groups</th>
<th>Zero time M ± S.E.</th>
<th>After 4 weeks M ± S.E.</th>
<th>% of change</th>
<th>After 8 weeks M ± S.E.</th>
<th>% of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1 ml saline)</td>
<td>82.4 ± 1.9</td>
<td>83.6 ± 1.5</td>
<td>1.5</td>
<td>84.3 ± 1.2</td>
<td>2.3</td>
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<tr>
<td>Diabetic non treated</td>
<td>259.4 ± 9.20</td>
<td>262.2 ± 11.3</td>
<td>1.1</td>
<td>265.1 ± 10.4</td>
<td>2.2</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>
<td>142.7 ± 4.9*</td>
<td>42.8</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>
<td>157.8 ± 5.9*</td>
<td>38.4</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>
<td>86.9 ± 2.3*</td>
<td>67.1</td>
</tr>
</tbody>
</table>

*Statistically significant from control at P < 0.01.

Table 2: 13C NMR spectral data of compounds 1, 2, 3 and 4 (δ ppm)

<table>
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<tr>
<th>Aglycone</th>
<th>Carbon no. 1</th>
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<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar(s)</td>
<td>Glc</td>
<td>Glucur</td>
<td>Ara</td>
<td>Glucur</td>
</tr>
<tr>
<td>1`</td>
<td>104.45</td>
<td>105.47</td>
<td>108.62</td>
<td>105.68</td>
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<tr>
<td>2`</td>
<td>67.34</td>
<td>75.28</td>
<td>74.05</td>
<td>75.59</td>
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<tr>
<td>3`</td>
<td>71.09</td>
<td>79.73</td>
<td>81.02</td>
<td>77.74</td>
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<tr>
<td>4`</td>
<td>64.70</td>
<td>69.14</td>
<td>70.29</td>
<td>83.04</td>
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<tr>
<td>5`</td>
<td>72.64</td>
<td>76.93</td>
<td>67.93</td>
<td>77.54</td>
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<td>6`</td>
<td>62.82</td>
<td>174.93</td>
<td></td>
<td>177.30</td>
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<tr>
<td>Sugar(s)</td>
<td>Glc</td>
<td>Ara</td>
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<td></td>
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<tr>
<td>1`</td>
<td>102.34</td>
<td>109.26</td>
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<tr>
<td>2`</td>
<td>74.41</td>
<td>78.77</td>
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<tr>
<td>3`</td>
<td>77.27</td>
<td>76.85</td>
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<td>4`</td>
<td>72.22</td>
<td>87.69</td>
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<tr>
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<td>6`</td>
<td>60.21</td>
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- not detected

Compound 1: White powder, R, 0.693 (S), 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, 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-1-1), 3.54 - 4.46 (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-1-1), 5.14 (1H, broad s, H-1``) and 3.05 - 4.38 (sugars 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-1-1), 4.78 (1H, broad s, H-1``) and 3.03 - 4.11 (sugars 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 anti hyperglycemic 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 δH 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 (δH 5.08) which are characteristic for oleanane-type triterpene[9]. 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[18], it was identified as 3-O-[β-D-glucopyranosyl-(1-3)-α-L-arabinofuranosyl] oleanolic acid. This compound was previously isolated from the flowers of the same plant[18].

1H and 13C-NMR of compounds 2, 3 and 4 showed signals for the aglycone part resembling those of compound 1. Compound 2 have anomeric signals of a sugar molecule at δH 4.46 and δH 105.47. Signal at δH 174.93, corresponding to COOH[4,7,23] group of glucuronic acid, support the presence of glucuronic acid as the sugar molecule. Thus compound 2 was identified as 3-O-[α-D-glucuronopyranosyl] oleanolic acid[4,7,23].

1H and 13C NMR of compounds 3 and 4 revealed the presence of two sugar molecules in each compound. The 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-O-[β-D-glucopyranosyl-(1-3)-α-L-arabinofuranosyl].

Compound 4 displayed signals of two anomeric protons at δH 4.47 and 4.78 and two anomeric carbons at δH 105.68 and 109.26. Compound 4 was identified as 3-O-[α-L-arabinofuranosyl-(1-4)-β-D-glucuronopyranosyl] oleanolic acid by comparing its spectral data with the published data[9]. 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**


