Antiulcerogenic Activity of *Alhagi maurorum*

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Abstract

Six main flavonoid glycosides were isolated, for the first time, from the ethanol extract of *Alhagi maurorum* Boiss (Leguminosae). They were identified as kaempferol, chrysoeriol, isorhamnetin, chrysoeriol-7-O-xyloside, kaempferol-3-galactoorhamnoside, and isorhamnetin 3-O-β-L-apiofuranosyl (1→2) β-D-galactopyranoside. Their identities were established by m.p., UV, EI-mass, Fab-mass, 600 MHz 1H and 13C NMR. The total extract (300 and 400 mg/kg) and two of the isolated compounds (chrysoeriol 7-O-xyloside and kaempferol-3-galactorhamnoside, 100 mg/kg each) showed a very promising antiulcerogenic activity with curative ratios 66.31%, 69.57%, 75.49%, and 77.93%, respectively.

Keywords: *Alhagi maurorum*, antiulcerogenic activity, chrysoeriol-7-O-xylosidoflavonoids, isorhamnetin, kaempferol-3-galactorhamnoside.

Introduction

The family Leguminosae is comprised of about 550 genera and more than 13,000 species (Bolus, 2000), including several members that are used in folklore medicine (Lewis & Lewis 1977). The family provides us with many edible plants as well as a variety of medicinal plants that constitute an important source of raw materials used in the pharmaceutical industries. Chemical investigation of the *Alhagi* species revealed the presence of several contents such as fatty acids and sterols (Ghosal et al., 1974; Kudlik et al., 1991; Kalhoro et al., 1997), flavonoids (Al-Yahya et al., 1987; El-Saayed et al., 1993; Singh et al., 1999), coumarins (Behari & Gupt, 1980), alkaloids (Behari & Gupt, 1980), and vitamins. Twelve flavonoids (El-Saayed et al. 1993; Singh et al., 1999) were isolated from *Alhagi graeorum* Boiss. These flavonoids were identified as tamarixtin 3-O-dirhamnoside, isorhamnetin 3-O-glucosylneo-hesperidoside, isorhametine 3-O-robinoside, isorhamnetin 3-O-rotinoside, quercetin 3-O-rhamnoside, kaempferol 3-O-galactoside, quercetin 3,7-diglycoside, isorhamnetin 3-rotinoside, daidzein 7,4-di-hydroxyisoflavone, calycisin 3'-hydroxyformononetin, and isorhamnetin and tamarixin aglycones.

*Alhagi maurorum* Boiss is customarily used in folk medicine as a remedy for rheumatic pains, bilharziasis, liver disorders, and for various types of gastrointestinal discomfort (Bolus, 1983), but there is no scientific background that supports this use. The aim of the current work was to study the antiulcerogenic effect of *Alhagi maurorum* Boiss to reveal its possible use for the treatment of gastric ulcer and to discover the compounds responsible for this activity.

Materials and Methods

Plant Material

The aerial parts of *Alhagi maurorum* Boiss were collected from the Sewa Oasis during summer 2002. Identification of the plant was verified by the late Prof. N. El-Hadidi, Professor of Taxonomy, Botany Department, Faculty of Science, Cairo University, and by comparison with plant description in *Flora of Egypt* (Tackholm, 1974; Bolus 2000). A voucher specimen was kept in the herbarium of the Desert Research Center. Plant sample was air-dried in shade, reduced to fine powder, packed in tightly closed containers, and stored for phytochemical and pharmacological studies.

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Solvent Systems
The solvent systems (a) chloroform:methanol (95:5), (b) ethyl acetate:methanol:water (30:5:4), (c) butanol:acetic acid:water (4:1:5), (d) ethyl acetate:methanol:acetic acid:water (65:15:10:10), (e) acetic acid:water (15:85) were used for developing the chromatoplates. Visualization of chromatograms was achieved under UV before and after exposure to ammonia vapor or by spraying with aluminum chloride (Stahl, 1969).

Apparatus
Melting points were determined on a Kofler hot-stage apparatus (UK) and are uncorrected; mass spectra (electrospray negative ion) sample was dissolved in acetonitrile on a Micromass Quattro spectrometer (Germany). 1H and 13C NMR spectra, using external electronic referencing through the deuterium resonance frequency of the solvent, were determined at 600.17 or 150.91 MHz, respectively, with a JEOL ECA 600 spectrometer fitted with an auto 5 mm X/H probe, Mannheim Hitachi 717 automatic analyzer (Japan). Carbon atom types were established in the 13C NMR spectrum by employing a combination of broad- and proton-decoupled and distortionless enhancement by polarization transfer (DEPT) experiments with 64 K data points over a spectrum width of 17,605.6 Hz. 1H-13C correlations were established using heteronuclear multiple quantum correlation (HMQCC) and heteronuclear multiple bond correlation (HMBC) pulse sequences, respectively. 1H–1H correlations were by double quantum filtered correlation spectroscopy (COSY).

Phytochemical study

Extraction and isolation
Defatted powder (1 kg) of the aerial parts of the plant was extracted in a Soxhlet apparatus with 95% ethanol. The ethanol extract was concentrated under reduced pressure (150 g) and diluted with water (200 ml), filtered over a piece of cotton, then successively extracted with ether, chloroform, ethyl acetate, and n-butanol. Each extract was dried over anhydrous sodium sulfate and concentrated to yield 4, 10, 18.5, and 30 g dry extracts, respectively.

TLC examination (systems a and b) revealed the presence of the same spots in ether and chloroform extracts. Accordingly, both extracts were combined together (14 g) and subjected to preparative TLC (system b) with repeated PPC (systems c and f). Bands corresponding to flavonoids were separated extracted with methanol, concentrated, and resubmitted to a column Silica gel G and eluted with the same system (b) where compounds 4–7 were isolated (Figure 1).

Compound 1: (20 mg) yellow crystals, Rf = 0.91 (system b), m.p. 277°C, UV, λ max in MeOH: nm 367, 268; (AlCl3): 265, 350, 420; (AlCl3/HCl): 265, 350, 420; (NaOA): 275, 300 (sh), 380; (NaOAc/H3BO3): 267, 319 (sh), 380; (NaOMe): 285, 322, 430. 1H-NMR (MSO-d6): δ 8.0 (2H, d, J = 8 Hz, H2 and H6), δ 6.9 (2H, d, J = 8 Hz, H3 and H5), δ 6.4 (1H, d, J = 2.5 Hz, H8) and δ 6.2 (1H, d, J = 2.5 Hz, H6). EI-MS m/z (% re. int): 285 (M+/100), 258 (15), 229 (16), 184 (8), 121 (22) and 93 (10). From the previous data and by comparison with authentic and published data (Mabry et al., 1970), this compound is identified as kaempferol.

Figure 1. Compounds isolated from Alhagi maurorum.
(sh), 266, 254; (AlCl\textsubscript{3}): 422, 363, 305 (sh), 262; (AlCl\textsubscript{3}/HCl): 419, 355, 303 (sh), 264; (NaOAc): 395, 317, 274, 264; (NaOAc/H\textsubscript{2}BO\textsubscript{3}): 366, 305, 269, 257; (NaOMe): 417, 238, 272, 242. \textsuperscript{1}H-NMR (DMSO-d\textsubscript{6}): \( \delta \) 7.96 (d, \( J = 2.1 \) Hz, H\textsubscript{2}), \( \delta \) 7.50 (d, \( J = 2.1,8.5 \) Hz, H\textsubscript{6}), \( \delta \) 6.92 (d, \( J = 8.5 \) Hz, H\textsubscript{5}) \( \delta \) 6.37 (d, \( J = 2 \) Hz, H\textsubscript{8}) and 6.17(d, \( J = 2 \) Hz, H\textsubscript{6}). EI-MS m/z (% re. int.): 316 (M\textsuperscript{+}) (100), 301 (50), 285 (16), 164 (30), 152 (22) and 124 (10). From the previous data and by comparison with published data (Markham, 1982), this compound is identified asisorhamnetin.

**Compound 4:** (500 mg) yellow crystals, \( R_f = 0.432 \) (system b), m.p. 350°C, UV, \( \lambda \) max in MeOH: nm 250, 270 (sh), 350: (AlCl\textsubscript{3}): 276, 297 (sh), 356, 392; (AlCl\textsubscript{3}/HCl): 276, 297 (sh), 392; (NaOAc): 253, 355, 410; (NaOAc/H\textsubscript{2}BO\textsubscript{3}): 253, 265 (sh), 350; (NaOMe): 260, 390. \textsuperscript{1}H-NMR (DMSO-d\textsubscript{6}): \( \delta \) 7.66 (d, \( J = 2.8 \) Hz, H\textsubscript{6}), \( \delta \) 7.59 (d, \( J = 2 \) Hz, H\textsubscript{2}), \( \delta \) 6.98 (d, \( J = 8 \) Hz, H\textsubscript{5}), \( \delta \) 6.96 (s, H\textsubscript{3}), \( \delta \) 6.90 (d, \( J = 2.1 \) Hz, H\textsubscript{8}), \( \delta \) 6.20 (d, \( J = 2.1 \) Hz, H\textsubscript{6}), \( \delta \) 5.06 (d, \( J = 6.9 \) Hz H\textsubscript{1}), \( \delta \) 3.87 (s, OCH\textsubscript{3}) and \( \delta \) 3.7–3.2 (m, rest of sugar proton).\textsuperscript{13}C NMR (DMSO) 154.8 (C-2), 103.3 (C-3), 176.9 (C-4), 162.1 (C-5), 98.8 (C-6), 168.9 (C-7), 94.3 (C-8), 156.5 (C-9), 103.9 (C-10), 131.1 (C-1’), 111.8 (C-2’), 146.8 (C-3’), 149.8 (C-4’), 116.5 (C-5’), 121.9 (C-6’), 106.2 (C-1’’), 73.8 (C-2’’), 76.8 (C-3’’), 67.8 (C-4’’), 66.7 (C-5’’), 55.5 (OCH\textsubscript{3}). EI-MS m/z (% re. int): 432 (M\textsuperscript{+}) (100), 301 (50), 273 (36), 269 (8), 152 (22), 152 (12), 148 (20).

From the previous data and by comparison with published data (Mabry et al., 1970) this compound is identified as chrysoeriol-7-O-β-D-apio-furanosyl (1-2) β-D-galactopyranoside.

**Pharmacological study**

**Preparation of the plant extract**

Dried aerial parts of *Alhagi maurorum* were extracted in a Soxhlet apparatus with ethanol 95%. The ethanol extract was completely dried under vacuum, weighed and the residue was used in testing. The dried plant extract was freshly suspended in distilled water just before administration.

**Determination of median lethal dose (LD\textsubscript{50})**

LD\textsubscript{50} of the ethanol extract was determined as described before (Finny, 1964). For this purpose, five groups of five mature albino mice (20–25 g body weight) each were used. The tested extract was administered orally in doses of 2000–4000 mg/kg body weight in addition to a group used as a control (given the solvent). Rats were kept under observation for 24 h during which time the number of dead animals in each group was recorded.

**Antilcerogenic effect**

Forty male albino rats of 180–200 g body weight were obtained from the Laboratory Animal Colonies (Helwan, Egypt). Animals were kept under good hygienic conditions and fed on standard diet and watered ad libitum. They were divided into eight equal groups and starved for 48 h before use to ensure an empty stomach (Galvin & Mikhail 1976). To avoid dehydration during the period of fasting, rats were supplied with sucrose (BDH) 8% (w/v) solution in NaCl (BDH) 0.2% (w/v), which was removed 1 h before experimentation. The first group was kept as a normal control, and the second one was kept as a positive control. Three treatment groups received the ethanol extract of *Alhagi maurorum* Boiss in doses of 200, 300, and 400 mg/Kg b.wt. and another two treatment groups were administered compounds 4 and 5, respectively, in a dose
of 100 mg/Kg.b.wt. In addition, a group of rats was given ranitidine as a reference drug in a dose of 100 mg/kg/Kg.b.wt. The ethanol extract, compounds 4, 5, and ranitidine were given orally via a stomach tube. Two doses were given in the first day at 0800 and 1600 h; a third dose was given on the second day 1.5 h before induction of gastric ulceration. All rats except the normal control were given ethanol (Merck) 50% (v/v) (in distilled water) in a dose of 10 ml/kg orally to induce gastric ulceration. Normal control rats received equivolumes of distilled water only at the same time intervals. One hour after ethanol administration, all rats were sacrificed by an overdose of chloroform, and the stomachs were rapidly removed, opened along their greater curvature, and gently rinsed under running tap water. Lesions in the glandular part of the stomach were measured under an illuminated magnifying microscope (10×). Long lesions were counted and their lengths were measured. Petchial lesions were counted, and then each five petchial lesions were taken as 1 mm of ulcer (Cho & Ogle, 1979). To calculate the ulcer index (mm), the sum of the total length of long ulcers and petchial lesions in each group of rats was divided by its number. The curative ratio was determined according to the formula:

The results obtained were statistically analyzed using \( t \)-test (Snedecor & Cochran, 1976).

Results and Discussion

Compounds 1–3 were identified as kaempferol, chrysoeriol, and isorhamnetin by comparing their EI-MS, \( ^1\)H NMR, \( ^{13}\)C NMR, UV spectrum in methanol and different shift reagents with published data.

Acid hydrolysis of compound 4 revealed the sugar xylose that was identified by HPLC, PC, and TLC (systems e and f), and an aglycone that was found to be identical with compound 2 when compared with its TLC, UV shift reagents. It is substituted at position 3 as indicated by their UV spectra upon addition of diagnostic shift reagent. From the obtained data and by their comparison with other published data (Mabry et al., 1970; Markham, 1982), these compounds were identified as chrysoeriol 7-0-xylosid. Compound 5 revealed the sugars galactose and rhamnose after acid hydrolysis, which were identified by PC and TLC (systems c and d), and an aglycone that was found to be identical with compound 8 when compared with its TLC, UV shift reagents. It is substituted at position 3 as indicated by its UV spectra upon addition of diagnostic shift reagent, so from data given and by comparison with published data, these compounds were identified as kaempferol-3-galactorhamnoside. Acid hydrolysis for compound 6 gave galactose and isorhamnetin, a milder hydrolysis with 0.1 N \( \text{H}_2\text{SO}_4 \) afforded galactose, apiose, and isorhamnetin. The two sugars and the aglycone were identified by TLC comparison with authentic samples. The presence of two doublets at 6 5.62 and 5.32 with \( J = 7.5 \text{Hz} \) demonstrated the \( \beta \)-configuration of both galactosyl and apiosyl residues. \( ^{13}\)C NMR, C-2" was shifted downfield (3.00 ppm), which indicated the position of attachment of apiosyl moiety at the 2" hydroxyl. From all the above data, this compound was identified as isorhamnetin-3-\( O-\beta-D\)-apiofurano(1-2) \( \beta-D\)-galactopyranoside (Mabry et al., 1970).

Gastric damage induced by ethanol was characterized by both long ulcers and petechial lesions. The number of ulcers and the ulcer index in control rats (received ethanol) were highly significant \( (p < 0.001) \) when compared with normal untreated animals (receiving distilled water). Repeated oral administration of the ethanol extract of \textit{Alhagi maurorum} in doses of 300 and 400 mg/kg reduced the severity of gastric damage, as the ulcer index was significantly decreased to 2.90 and 2.62 mm, respectively, compared with 8.61 mm in the positive control group. The curative ratio was 66.31% and 69.57% after administration of both doses (Table 1). Compounds 4 and 5 in a dose of 100 mg/kg showed the best results. They significantly decreased the ulcer index into 2.11 and 1.90 mm, respectively, compared with 8.61 mm in the positive control group and elevated the curative ratio

\[ \text{Table 1. Antiulcerogenic effect of the ethanol extract of } \textit{Alhagi maurorum} \text{ Boiss and its isolated compounds in rats (n = 5).} \]

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mg/kg b.wt)</th>
<th>Number of ulcers (M ± SE)</th>
<th>Ulcer index (mm)</th>
<th>Curative ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Positive control</td>
<td>0</td>
<td>6.8 ± 0.48</td>
<td>8.61 ± 0.46</td>
<td>—</td>
</tr>
<tr>
<td>Total extract</td>
<td>200</td>
<td>6.1 ± 0.24</td>
<td>7.54 ± 0.32</td>
<td>12.42</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>4.7 ± 0.35</td>
<td>2.90 ± 0.25</td>
<td>66.31</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>3.9 ± 0.20</td>
<td>2.62 ± 0.21</td>
<td>69.57</td>
</tr>
<tr>
<td>Compound 4</td>
<td>100</td>
<td>2.8 ± 0.17</td>
<td>2.11 ± 0.12</td>
<td>75.49</td>
</tr>
<tr>
<td>Compound 5</td>
<td>100</td>
<td>2.2 ± 0.10</td>
<td>1.90 ± 0.10</td>
<td>77.93</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>100</td>
<td>6.2 ± 0.30</td>
<td>8.40 ± 0.44</td>
<td>2.43</td>
</tr>
</tbody>
</table>

\(^a\)Compared with normal control \( (p < 0.001) \).

\(^*\) \( p < 0.01 \) compared with positive control.

\(^***\) \( p < 0.001 \) compared with positive control.
to 75.49% and 77.93%, respectively. The curative ratio after ranitidine administration was only 2.43%. Failure of ranitidine to decrease gastric damage induced by ethanol could be attributed to its mechanism of action, as it blocks the histaminergic receptors, so prevents the stomach from producing excess acid. This mechanism cannot protect the gastric mucosa against the irritating and damaging effects of ethanol.

It could be concluded that the ethanol extract of *Alhagi maurorum* and the isolated compounds are highly safe for human use. Due to their antiulcerogenic activity, they could be used orally either for prophylaxis or for treatment of gastric ulcer.

### References


