Introduction

Pathogenesis of liver fibrosis is so far not clear. However, reactive oxygen species (ROS) were found to play a pivotal role in pathological changes in the liver, especially in alcoholic and toxic liver disease (Poli and Parola, 1997). Several protective mechanisms have been suggested and applied to limit the damage caused by ROS (Sies, 1993). Nevertheless, drugs targeting ROS inhibition did not provide complete protection which prompts the search for new antioxidants. Many natural antioxidants have been proposed to prevent and treat hepatopathies induced by oxidative stress (Cervinkova and Drahota, 1998; Lieber, 1997). With increasing evidence for the hepatoprotective role of hydroxylated and polyhydroxylated organic compounds from vegetables, fruits, and herbs (Bass, 1999), these could provide excellent sources for the isolation of effective antioxidants.

Onopordum (Onopordon) L. is a genus of about 40 species of thistles belonging to the family Asteraceae, native to Europe (mainly the Mediterranean region), Northern Africa, the Canary Islands, the Caucasus, and Southwest and Central Asia. It grows on disturbed land, roadsides, arable land, and pastures (Briese et al., 1990). From the leaves and stems of O. alexandrinum, eleven flavonoid compounds have been isolated and identified as apigenin, luteolin, chrysoeriol, and their 7-galactosides and 7-glucosides together with the 7-diglucosides of apigenin and chrysoeriol (Kawashty et al., 1996). Taraxasterol, lupeol, β-sitosterol, stigmasterol, scutellarein 4’-methyl ether, and takakin were isolated from the aerial parts of O. alexandrinum (Saif-El Din et al., 1994). The lignan glucoside arctiin was isolated from the seeds of O. alexandrinum (Abdallah, 1978). Three lactones were isolated from the aerial parts of O. alexandrinum, and one of them was identified as onopordopicrin, in addition to two flavone rhamnosides (Khafagy et al., 1977). The study of the nitrogenous bases of O. alexandrinum resulted in the isolation of stachydrine and choline (Wassel, 1975).

Reviewing the current literature, nothing was traced regarding the flavonoid content of the flowers and their hepatoprotective activity. Thus this study was carried out with the aim of isolating natural hepatoprotective and antioxidant compounds.
Material and Methods

Chemicals

Authentic flavonoids were obtained from Merck (Darmstadt, Germany). Silica gel H (Merck) for vacuum liquid chromatography (VLC), silica gel 60 (70–230 mesh ASTM; Fluka, Steinheim, Germany), silica gel 100 C18 (Fluka) and Sephadex LH-20 (Pharmacia, Stockholm, Sweden) were used for column chromatography (CC). Thin-layer chromatography (TLC) was performed on silica gel GF254 precoated plates (Fluka) using the following solvent systems: S1, chloroform/methanol (90:10); S2, chloroform/methanol (80:20); S3, ethyl acetate/methanol/water (100:16.5:13.5). The chromatograms were visualized under UV light (at 254 and 366 nm) before and after exposure to ammonia vapour and spraying with AlCl3, as well as after spraying with anisaldehyde/sulfuric acid spray reagent. Ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA), silymarin from Sedico Pharmaceutical (6 October City, Egypt), and carbon tetrachloride from El-Gomhoreya (Cairo, Egypt). Kits for assessment of the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were from Bio-Mérieux (Craponne, France).

Melting points (uncorrected) were determined on an electrothermal 9100 instrument (Labequip, Ontario, Canada). UV spectra were recorded in a Shimadzu UV 240 (P/N 204–58000) spectrophotometer (Kyoto, Japan). 1H NMR (300 MHz) and 13C NMR (75 MHz) spectra were recorded in a Varian VX-300 instrument (Palo Alto, CA, USA) in CDCl3 and DMSO-d6, and chemical shifts are given in δ (ppm) relative to TMS as internal standard.

Plant material

The flowers of O. alexandrinum L. were obtained from thistles growing on the north coast of Alexandria, Egypt, during spring 2008. The plant was kindly identified at the Botany Department, Faculty of Science, Cairo University, Giza, Egypt. A voucher specimen (No. O-3) is deposited at the museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Animals

Adult male albino rats of Sprague Dawley strain, weighing 100–150 g, and albino mice (20–25 g) were purchased from the animal breeding unit of National Research Center, Dokki, Giza, Egypt. Rats were fed on standard laboratory diet and water ad libitum. All rats were allowed to acclimatize for 10 d prior to experimentation.

Fractionation of extracts

The air-dried powdered flowers of O. alexandrinum L. (750 g) were extracted by cold percolation with 95% ethanol (5 x 3 l) till exhaustion. The combined ethanol extract was concentrated under reduced pressure to give 75 g of a brown residue. Twenty grams of the ethanol extract were kept for the biological study, the residue left was suspended in distilled water and partitioned successively with n-hexane (3 x 400 ml), chloroform (4 x 400 ml), ethyl acetate (6 x 400 ml), and n-butanol saturated with water (5 x 400 ml). Each fraction was concentrated to dryness under reduced pressure to obtain 15, 14, 22, and 12 g, respectively.

Assessment of LD50 values

The LD50 values of the ethanolic extract of the flowers was calculated according to Karber (1931).

Assessment of hepatoprotective activity

The ethanolic extract of the flowers as well as its four fractions, n-hexane, chloroform, ethyl acetate, and n-butanol, were tested for their hepatoprotective activity at two doses (50 and 100 mg/kg body weight), while the new compound 9 was tested at 10 and 25 mg/kg body weight. The test samples were administered daily (for each tested dose) for 15 d before induction of liver damage by intraperitoneal injection of 5 ml/kg body weight of 25% carbon tetrachloride (CCl4) in liquid paraffin according to the method described by Klassan and Plaa (1969) using silymarin (25 mg/kg body weight) as a reference drug. The test samples as well as the reference drug were further administered to the rats for another 15 d after liver damage. Blood samples of each group were collected at zero time, 15 d after receiving the test sample, 72 h after induction of liver damage, and 15 d after treatment with the test samples, and allowed
to clot, centrifuged at 1000 x g for 40 min and the separated sera were used for estimation of the levels of AST (Thewfweil, 1974), ALT (Thewfweil, 1974), and ALP (Kind and King, 1954) (Table I).

Assessment of free radical scavenging activity using the DPPH assay

Qualitative assay: Test compounds or extracts were applied to a TLC plate and sprayed with DPPH solution (0.2% in methanol) using an atomizer. The plate was allowed to develop for 30 min in the dark, and the colour changes (yellow on purple background) were noted.

Quantitative assay: The method used by Takao et al. (1994) and modified by Delazar et al. (2004) was adopted. DPPH (4 mg) was dissolved in methanol (50 ml) to obtain a concentration of 80 μg/ml. A serial dilution of test compounds, fractions, and extracts was prepared in methanol (compounds, 5–100 μg/ml; extracts and fractions, 20–400 μg/ml). Diluted solutions (1.0 ml each) were mixed with equal volumes of DPPH and allowed to stand for 30 min at room temperature. The control sample was prepared by mixing 1.0 ml of DPPH with 1.0 ml of methanol. The absorbance was recorded at 517 nm. The experiment was performed in triplicate, and the average absorbance for each concentration was recorded. The same procedure was followed for ascorbic acid, silymarin, and quercetin used as positive controls. The percent quenching of the UV absorption of DPPH was followed for ascorbic acid, silymarin, and quercetin used as positive controls. The percent quenching of the UV absorption of DPPH was calculated using the following equation:

\[ \text{Inhibition} \% = \left( \frac{A_{\text{B}} - A_{\text{A}}}{A_{\text{B}}} \right) \times 100, \]

where \( A_{\text{B}} \) is the absorbance of the control sample (\( t = 0 \)), and \( A_{\text{A}} \) is the absorbance of the test sample after 30 min. The \( IC_{50} \) value was calculated as the concentration (in μg/ml) of test sample that causes 50% quenching of the UV absorption of DPPH.

Statistical analysis

All data are expressed as the mean ± S.E.M (n = 10). Means were compared by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test. The values were considered to be significantly different when \( p \) values were less than 0.01.

Isolation of the components of the active ethyl acetate fraction

The ethyl acetate fraction (15 g) was fractionated on a Sephadex LH-20 column using methanol as eluent with increasing percentage of methanol from 10% up to 100%. The fractions were monitored by TLC, similar fractions were pooled together to yield five major fractions.

Fraction V (1 g, 3 major spots) was purified on a silica gel column using a methanol/chloroform mixture (95:5) as eluent, to yield compounds 1 (123 mg), 2 (117 mg), and 3 (122 mg).

Fraction IV (2.9 g, 5 spots, 3 major spots) was purified on a Sephadex LH-20 column using methanol as eluent followed by a silica gel column using a methanol/chloroform mixture (90:10) as eluent. Three pure compounds were isolated and designated as compounds 4 (140 mg), 5 (128 mg), and 6 (127 mg).

Fraction III (1 g, 6 spots with two major spots) was repeatedly purified on a Sephadex LH-20 column using methanol/water mixtures followed by purification on a silica gel 100 C18 reversed phase column using a methanol/water mixture (60:40) as eluent to afford compounds 7 (125 mg) and 8 (130 mg).

Fraction II was purified on several Sephadex LH-20 columns using methanol and methanol/water mixtures, followed by purification on silica gel 100 C18 reversed phase columns using a methanol/water mixture (1:1) as eluent to yield compound 9 (213 mg).

Finally, fraction I was purified like fraction II to yield compound 10 (119 mg).

6-Methoxy-apigenin (1): Yellow microcrystalline powder. – M.p. 228–230 °C. – Rf 0.88 [chloroform/methanol (90:10, v/v)]. – UV/Vis: \( \lambda_{\text{max}} \) (MeOH) = 289, 326; (NaOMe) 254, 323; (AlCl3) 312, 375; (AlCl3/HCl) 311, 371; (NaOAc) 284 sh, 323; (NaOAc/H,BO3) 290, 332 nm. – 1H NMR (300 MHz, DMSO): \( \delta_s = 3.76 \) (3H, s, OCH3), 6.43 (1H, d, J = 8.4 Hz, H-3`), 6.70 (1H, s, H-8), 6.97 (1H, s, H-3), 6.87 (1H, d, J = 8.4 Hz, H-3`,5`), 7.89 (2H, d, J = 8 Hz, H-2`,6`). – 13C NMR (75 MHz, DMSO): \( \delta_c = 59.83 \) (OCH3), 93.74 (C-8), 102.79 (C-3), 103.63 (C-10), 115.87 (C-3`), 115.87 (C-5`), 121.48 (C-1'), 123.81 (C-2'), 128.31 (C-6`), 131.29 (C-6), 157.20 (C-4'), 161.04 (C-9), 161.38 (C-5), 163.86 (C-2), 164.01 (C-7), 181.65 (C-4).

Acacetin (2): Yellow microcrystalline powder. – M.p. 228–230 °C. – Rf 0.85 [chloroform/methanol (90:10, v/v)]. – UV/Vis: \( \lambda_{\text{max}} \) (MeOH) = 267, 302 sh, 328; (NaOMe) 276, 295 sh, 364; (AlCl3) 260 sh, 277, 292 sh, 344, 383; (AlCl3/HCl) 260 sh, 277, 292 sh, 344, 383; (NaOAc) 276, 297 sh, 356;
**Apigenin (3):** Yellow microcrystalline powder.
- M.p. 345–347 °C. – Rf 0.67 [chloroform/methanol (90:10, v/v)]. – UV/Vis: λ\text{max} (MeOH) = 267, 294 sh, 331 nm. – 1H NMR (300 MHz, DMSO): δ\text{H} = 3.89 (3H, s, OCH3), 6.18 (1H, d, J = 2.1 Hz, H-6), 3.47 (1H, d, J = 2.1 Hz, H-8), 6.76 (1H, s, H-3), 7.64 (1H, d, J = 8.7 Hz, H-3'), 7.90 (2H, d, J = 8.7 Hz, H-2',6').
- 13C NMR (75 MHz, DMSO): δ\text{C} = 94.02 (C-6), 98.75 (C-8), 102.33 (C-3), 104.00 (C-10), 116.21 (C-3'), 121.50 (C-1'), 131.30 (C-2'), 131.30 (C-6'), 156.46 (C-2), 157.26 (C-9), 161.00 (C-4), 161.18 (C-5), 167.80 (C-7), 177.77 (C-4).

**Eriodictyol (6):** Orange microcrystalline powder.
- M.p. 257–258 °C. – Rf 0.79 [chloroform/methanol (95:5, v/v)]. – UV/Vis: λ\text{max} (MeOH) = 289, 324 sh; (NaOMe) 242 sh, 323; (AlCl3) 272, 306; (AlCl3/HCl) 307, 375; (NaOAc) 289 sh, 324; (NaOAc/H3BO3) 290, 333 sh nm. – 1H NMR (300 MHz, DMSO): δ\text{H} = 2.64 (1H, dd, J = 3.3 Hz eq* ax, J = 1.7 Hz gem coupling, H-3 eq), 3.12 (1H, dd, J = 12.4 Hz ax* ax, J = 17.1 Hz gem coupling, H-3 ax), 5.35 (1H, dd, J = 3 ax eq, J = 12.6 Hz ax* ax, H-2 ax), 5.87 (2H, br, s, H-6,8), 6.74 (2H, br, s, H-5',6'), 6.87 (1H, br, s, H-2'). – 13C NMR (75 MHz, DMSO): δ\text{C} = 42.06 (C-3), 78.47 (C-2), 95.01 (C-8), 95.81 (C-6), 101.83 (C-10), 114.30 (C-2'), 115.38 (C-5'), 118.01 (C-6'), 129.49 (C-1'), 145.19 (C-4'), 145.70 (C-3'), 162.89 (C-5), 163.47 (C-9), 166.64 (C-7), 196.29 (C-4).

**Apigenin-7-O-glucoside (7):** Yellow microcrystalline powder.
- M.p. 178–179 °C. – Rf 0.61 [chloroform/methanol (80:20, v/v)]. – UV/Vis: λ\text{max} (MeOH) = 268, 332; (NaOMe) 248 sh, 269, 385; (AlCl3) 275, 300, 348, 386; (AlCl3/HCl) 273, 300, 350, 385; (NaOAc) 256 sh, 267, 355, 388; (NaOAc/H3BO3) 267, 340 nm. – 1H NMR (300 MHz, DMSO): δ\text{H} = 5.11 (1H, d, J = 7.5 Hz, H-1'''), 6.43 (1H, br, s, H-6), 6.81 (1H, s, H-8), 6.89 (1H, s, H-3), 7.02 (2H, d, J = 8.1 Hz, H-3',5'), 7.90 (2H, d, J = 8.1 Hz, H-2',6'). – 13C NMR (75 MHz, DMSO): δ\text{C} = 61.04 (C-6'''), 71.77 (C-4'''), 72.88 (C-2''), 76.23 (C-3'''), 76.24 (C-5'''), 94.65 (C-8), 99.52 (C-6), 100.5 (C-1''), 102.93 (C-3), 105.27 (C-10), 115.98 (C-3',5'), 120.91 (C-1'), 128.46 (C-2',6'), 156.88 (C-9), 161.01 (C-4'), 161.51 (C-5'), 162.85 (C-2''), 164.24 (C-7), 181.89 (C-4).

**Luteolin (4):** Yellow microcrystalline powder.
- M.p. 226–229 °C. – Rf 0.54 [chloroform/methanol (90:10, v/v)]. – UV/Vis: λ\text{max} (MeOH) = 254, 266 sh, 353; (NaOMe) 270, 399; (AlCl3) 276, 301 sh, 420; (AlCl3/HCl) 274, 378, (NaOAc) 270, 386; (NaOAc/H3BO3) 260, 370 nm. – 1H NMR (300 MHz, DMSO): δ\text{H} = 6.17 (1H, br, s, H-6), 6.43 (1H, br, s, H-8), 6.63 (1H, d, J = 8.4 Hz, H-5'), 7.30 (2H, d, J = 8.4 Hz, H2'-6'). – 13C NMR (75 MHz, DMSO): δ\text{C} = 94.04 (C-8), 98.99 (C-6), 102.77 (C-3), 103.58 (C-10), 113.23 (C-2''), 116.09 (C-5'), 119.04 (C-6'), 121.42 (C-1'), 145.77 (C-3'), 149.87 (C-4'), 157.36 (C-9), 161.38 (C-5), 163.95 (C-2'), 164.46 (C-7), 181.54 (C-4').

**Kaempferol (5):** Yellow microcrystalline powder. – M.p. 279–280 °C. – Rf 0.51 [chloroform/methanol (90:10, v/v)]. – UV/Vis: λ\text{max} (MeOH) = 253, 266, 294 sh, 367; (NaOMe) 278, 316, 416; (AlCl3) 260 sh, 268, 303 sh, 424; (AlCl3/HCl) 256 sh, 269, 303 sh, 424; (NaOAc) 274, 303, 387; (NaOAc/H3BO3) 267, 297 sh, 372 nm. – 1H NMR (300 MHz, DMSO): δ\text{H} = 6.18 (1H, d, J = 1.8 Hz, H-6), 6.47 (1H, d, J = 1.8 Hz, H-8), 6.91 (2H, d, J = 8.4 Hz, H-3',5'), 7.90 (2H, d, J = 8.4 Hz, H-2',6').

**Luteolin-7-O-glucoside (8):** Yellow microcrystalline powder. – M.p. 237–239 °C. – Rf 0.59 [chloroform/methanol (80:20, v/v)]. – UV/Vis: λ\text{max} (MeOH) = 255, 266 sh, 348; (NaOMe) 264, 300 sh, 394; (AlCl3) 274, 298 sh, 329, 432; (AlCl3/HCl) 371, 294 sh, 357, 387; (NaOAc) 259, 266 sh, 364 sh, 405; (NaOAc/H3BO3) 259, 372 nm. – 1H NMR (300 MHz, DMSO): δ\text{H} = 5.05 (1H, d, J = 7.5 Hz, H-1'''), 6.43 (1H, d, J = 1.5 Hz, H-6), 6.73 (1H, s, H-3), 6.77 (1H, d, J = 1.5 Hz, H-8), 6.8 (1H, d, J = 8.1 Hz, H-5'), 7.45 (2H, d, J = 8 Hz, H-2',6'). – 13C
Results and Discussion

The ethanolic extract of the flowers of *O. alexandrinum* L. was found to be non-toxic to rats up to 6.3 mg/kg body weight and this study was thus undertaken to assess its hepatoprotective activity. Oral administration of CCl₄ to male rats showed significant elevations of serum activities of ALT, AST, and ALP when compared with negative control rats (Table I). In comparison with the CCl₄-treated group, significant improvements were noticed in the serum activity of ALT, AST, and ALP in rats that previously had been treated with silymarin and *O. alexandrinum* ethanolic extract. This improvement was observed at the two doses used (50 and 100 mg/kg body weight), but it was more significant at 100 mg/kg body weight of the ethanolic extract. Fractionation of the ethanolic extract was performed, and the resultant four fractions (*n*-hexane, chloroform, ethyl acetate, and *n*-butanol) were tested for their hepatoprotective activity at the same two dose levels. The ethyl acetate fraction proved to be the most active fraction in improving the serum activity of ALT, AST, and ALP in rats, whose activity exceeded that of the ethanolic extract especially at the dose of 100 mg/kg body weight.

The free radical scavenging activity of the ethanolic extract was assessed in the DPPH system. The results showed that it has an IC₅₀ value of 200 μg/ml; consequently, its four fractions were also tested. The ethyl acetate fraction showed the highest free radical scavenging activity of all tested fractions (IC₅₀ = 65 μg/ml), followed by the *n*-butanol fraction (IC₅₀ = 150 μg/ml), whereas the *n*-hexane and chloroform fractions showed negligible activities.

The biochemical mechanism of CCl₄ toxicity is based on mitochondrial damage that leads to an accumulation of fat within 60 min, damage of endoplasmic reticulum within 30 min (Christie and Judah, 1954), and damage of lysosomes (Judah, 1969), which eventually leads to the death of the hepatocytes. Liver microsomal oxidizing systems connected with cytochrome P-450 produce reactive metabolites of CCl₄; for example, the trichloromethyl radical (CCl₃·) or the trichloroperoxy radical (CCl₃O₃·). These radical species induce lipid peroxidation, disturbance in Ca²⁺ homeostasis, and finally death (Recknagel, 1967). In this respect, these improvements could be attributed to the free radical scavenging activity of both the ethanolic and ethyl acetate fraction (Al-Qarawi et al., 2004; Gupta et al., 2005).

The most active hepatoprotective and free radical scavenging fraction was then used for the chemical study. Investigation of the active ethyl
acetate fraction yielded a new compound, designated as acacetin-7-O-galacturonide (9), and nine known compounds viz; four flavones, 6-methoxy-apigenin (hispidulin) (1), acacetin (2), apigenin (3), luteolin (4); a flavonol, kaempferol (5); and a flavanone, eriodictyol (6); as well as three glycosides, apigenin-7-O-glucoside (7), luteolin-7-O-glucoside (8), and kaempferol-3-O-rutinoside (10), identified by comparing their UV, 1H NMR and 13C NMR spectra with published data (Fig. 1).

The new compound 9, showed the characteristic 15 signals of an acacetin aglycone in the 1H NMR and 13C NMR spectra, in addition to 6 extra signals of β-D-galacturonic acid (Agrawal, 1989) which is characterized by the presence of C-6 at δC 175.00 ppm and C-3 and C-5 at δC 72.85 and 74.64 ppm (cf. β-D-glucuronic acid in which C-3 and C-5 appear at about δC 76.00 and 77.00 ppm; Agrawal, 1989). Substitution at position 7 was indicated by the UV spectrum upon addition of the diagnostic shift reagents and from the downfield shift of H-6 and H-8 to δH 6.44 and 6.88 ppm, respectively (cf. compound 2) and confirmed by HMBC correlated NMR spectra, which exhibited cross-peaks between the anomeric proton at C-1 and C-7. Thus, compound 9 was identified as acacetin-7-O-galacturonide.

Table I. Effect of Onopordum alexandrinum L. on serum AST, ALT, and ALP levels of adult male albino rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST [U/L]</th>
<th>ALT [U/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero time</td>
<td>15 d</td>
</tr>
<tr>
<td>Control</td>
<td>29.2 ± 1.1</td>
<td>28.6 ± 0.4</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>Silymarin</td>
<td>32.4 ± 1.1</td>
</tr>
<tr>
<td>Ethanollic extract</td>
<td>33.4 ± 1.2</td>
<td>33.5 ± 1.3</td>
</tr>
<tr>
<td>n-Hexane fraction</td>
<td>28.7 ± 0.1</td>
<td>28.9 ± 0.8</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>29.8 ± 0.7</td>
<td>29.4 ± 0.7</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>31.9 ± 1.1</td>
<td>31.6 ± 0.9</td>
</tr>
<tr>
<td>n-Butanol fraction</td>
<td>32.6 ± 1.3</td>
<td>31.5 ± 0.8</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>Compound 9</td>
<td>33.1 ± 1.2</td>
</tr>
<tr>
<td>Ethanollic extract</td>
<td>31.6 ± 1.1</td>
<td>31.2 ± 1.3</td>
</tr>
<tr>
<td>n-Hexane fraction</td>
<td>38.4 ± 1.4</td>
<td>39.9 ± 1.1</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>39.4 ± 1.5</td>
<td>41.9 ± 1.1</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>32.8 ± 1.4</td>
<td>29.7 ± 0.5</td>
</tr>
<tr>
<td>n-Butanol fraction</td>
<td>33.4 ± 1.2</td>
<td>31.9 ± 1.1</td>
</tr>
<tr>
<td>25 mg/kg</td>
<td>Compound 9</td>
<td>30.4 ± 1.1</td>
</tr>
</tbody>
</table>

a Statistically significant from zero time at p < 0.01.
b Statistically significant from 72 h after CCl₄ at p < 0.01.
Compounds 1–6 were identified as 6-methoxy-apigenin (hispidulin) (1), acacetin (2), apigenin (3), luteolin (4), kaempferol (5), and eriodictyol (6) and their spectral data were in agreement with those published for these compounds (Abdel-Sattar et al., 2000; Agrawal, 1989; Islam and Sleem, 2006; Lazari et al., 1998; Mabry, 1970; Markham, 1982; Youssef, 2003). The 1H NMR spectra of 7 and 8 agreed with those of 3 and 4, respectively, in addition to an anomeric proton with a large coupling constant of 7.5 Hz in each case indicating a β-linked sugar which was identified as β-glucose from 13C NMR spectra of the two compounds. The UV spectra and the downfield shift of H-6 and H-8 of the two compounds indicated that the glycosylation is at C-7. Thus, compounds 7 and 8 were identified as apigenin-7-O-glucoside and luteolin-7-O-glucoside, respectively. The 1H NMR spectrum of 10 was identical to that of 5, in addition to two anomeric protons assigned for α-rhamnose and β-glucose, and this was confirmed by their characteristic signals in the 13C NMR spectra (Agrawal, 1989). The glycosylation at C-3 was deduced from the UV spectrum of the compound and from the downfield shift of the anomeric proton of glucose (δH 5.60 ppm) and presence of the anomeric carbon atom C-1 at δC 101.4 ppm (Agrawal, 1989; Harborne et al., 1975;
Compound 10 was identified as kaempferol-3-O-rutinoside.

The new compound acacetin-7-O-galacturonide (9) was also tested for its hepatoprotective activity, and it proved to have significant activity as it caused an improvement in the serum levels of ALT, AST, and ALP in rats (Table I). In addition, 9 showed potent free radical scavenging activity (IC$_{50}$ = 25 $\mu$g/ml) relative to silymarin (IC$_{50}$ = 45 $\mu$g/ml), but less than shown by ascorbic acid and quercetin (7.5 and 0.09 $\mu$g/ml, respectively).

In conclusion, applying a bioactivity-guided assay served as tool for the isolation of a new hepatoprotective and antioxidant compound of natural origin that might have a role in the treatment of hepatic failure. More studies are needed to confirm its efficacy in clinical trials.


