# A New Hepatoprotective Flavone Glycoside from the Flowers of *Onopordum alexandrinum* Growing in Egypt

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A bioactivity-guided fractionation of the ethyl acetate fraction of the flowers of *Onopordum alexandrinum* L. (Asteraceae) yielded a new flavonoidal glycoside designated as acacetin-7-*O*-galacturonide (**9**), alongside with nine known flavonoids; 6-methoxy-apigenin (hispidulin) (**1**), acacetin (**2**), apigenin (**3**), luteolin (**4**), kaempferol (**5**), eriodictyol (**6**), apigenin-7-*O*-glucoside (**7**), luteolin-7-*O*-glucoside (**8**), and kaempferol-3-*O*-rutinoside (**10**). The compounds were assayed for their hepatoprotective activity against $CCl_4$-induced hepatic cell damage in rats and free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Compounds **4**, **6**, **9**, and **10** have not been previously reported from flowers of *O. alexandrinum* L., and this is the first report of acacetin-7-*O*-galacturonide (**9**) in nature which has also shown significant hepatoprotective and free radical scavenging effects. The isolated compounds were identified using different spectroscopic methods (UV, $^1$H NMR, $^{13}$C NMR, HMQC, HMBC, and COSY).

*Key words: Onopordum alexandrinum*, Acacetin-7-*O*-galacturonide, Hepatoprotective

## 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 $C_{18}$ (Fluka) and Sephadex LH-20 (Pharmacia, Stockholm, Sweden) were used for column chromatography (CC). Thin-layer chromatography (TLC) was performed on silica gel $GF_{254}$ precoated plates (Fluka) using the following solvent systems: $S_1$, chloroform/methanol (90:10); $S_2$, chloroform/methanol (80:20); $S_3$, 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 $AlCl_3$, 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). $^1$H NMR (300 MHz) and $^{13}$C NMR (75 MHz) spectra were recorded in a Varian VX-300 instrument (Palo Alto, CA, USA) in $CDCl_3$ and DMSO-$d_6$, and chemical shifts are given in $\delta$ (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 $LD_{50}$ values*

The $LD_{50}$ 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 acitivity 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 ($CCl_4$) 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 (Thewfweld, 1974), ALT (Thewfweld, 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 $\mu$g/ml. A serial dilution of test compounds, fractions, and extracts was prepared in methanol (compounds, 5–100 $\mu$g/ml; extracts and fractions, 20–400 $\mu$g/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 percentage of DPPH radicals scavenging effect of test samples was calculated using the following equation: inhibition (%) = $[(A_B - A_A)/A_B] \cdot 100$, where $A_B$ is the absorbance of the control sample ($t$ = 0), and $A_A$ is the absorbance of the test sample after 30 min. The $IC_{50}$ value was calculated as the concentration (in $\mu$g/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 in $H_2O$ 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 $C_{18}$ 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 $C_{18}$ 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. – $R_f$ 0.88 [chloroform/methanol (90:10, v/v)]. – UV/Vis: $\lambda_{max}$ (MeOH) = 289, 326 sh; (NaOMe) 254, 323; ($AlCl_3$) 312, 375; ($AlCl_3$/HCl) 311, 371; (NaOAc) 284 sh, 323; (NaOAc/$H_3BO_3$) 290, 332 nm. – $^1$H NMR (300 MHz, DMSO): $\delta_H$ = 3.76 (3H, *s*, $OCH_3$), 6.43 (1H, *s*, H-8), 6.70 (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`). – $^{13}$C NMR (75 MHz, DMSO): $\delta_C$ = 59.83 ($OCH_3$), 93.74 (C-8), 102.79 (C-3), 103.63 (C-10), 115.87 (C-3`), 115.87 (C-5`), 121.48 (C-1`), 128.31 (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. – $R_f$ 0.85 [chloroform/methanol (90:10, v/v)]. – UV/Vis: $\lambda_{max}$ (MeOH) = 267, 302 sh, 328; (NaOMe) 276, 295 sh, 364; ($AlCl_3$) 260 sh, 277, 292 sh, 344, 383; ($AlCl_3$/HCl) 260 sh, 277, 292 sh, 344, 383; (NaOAc) 276, 297 sh, 356;

($NaOAc/H_3BO_3$) 267, 309 sh, 331 nm. – $^1H$ NMR (300 MHz, DMSO): $\delta_H$ = 3.89 (3H, *s*, $OCH_3$), 6.18 (1H, *d, J* = 2.1 Hz, H-6), 6.47 (1H, *d*, *J* = 2.1 Hz, H-8), 6.76 (1H, *s*, H-3), 6.91 (1H, *d*, *J* = 8.7 Hz, H-3`,5`), 7.90 (2H, *d*, *J* = 8.7 Hz, H-2`,6`). – $^{13}C$ NMR (75 MHz, DMSO): $\delta_C$ = 55.94 ($OCH_3$) 93.91 (C-8), 98.79 (C-6), 102.80 (C-3), 103.64 (C-10), 115.91 (C-3`), 115.91 (C-5`), 121.14 (C-1`), 128.39 (C-2`), 128.39 (C-6`), 157.25 (C-4`), 161.09 (C-5), 161.39 (C-9), 163.68 (C-2), 164.09 (C-7), 181.66 (C-4).

*Apigenin* (**3**): Yellow microcrystalline powder. – M.p. 345–347 °C. – $R_f$ 0.67 [chloroform/methanol (90:10, v/v)]. – UV/Vis: $\lambda_{max}$ (MeOH) = 267, 294 sh, 331; (NaOMe) 274, 323, 390; ($AlCl_3$) 274, 300, 346, 381; ($AlCl_3$/HCl) 274, 300, 346, 381; (NaOAc) 274, 301, 376; ($NaOAc/H_3BO_3$) 267, 302 sh, 338 nm. – $^1H$ NMR (300 MHz, DMSO): $\delta_H$ = 6.18 (1H, *d*, *J* = 2.1 Hz, H-6), 6.46 (1H, *d*, *J* = 2.1 Hz, H-8), 6.73 (1H, *s*, H-3), 6.89 (2H, *d*, *J* = 8.4 Hz, H-3`,5`), 7.89 (2H, *d*, *J* = 8.4 Hz, H-2`,6`). – $^{13}C$ NMR (75 MHz, DMSO): $\delta_C$ = 94.02 (C-6), 98.75 (C-8), 102.33 (C-3), 104.00 (C-10), 115.29 (C-3`), 115.29 (C-5`), 121.14 (C-1`), 128.30 (C-2`), 128.30 (C-6`), 157.10 (C-4`), 161.36 (C-9), 161.38 (C-5), 163.66 (C-2), 163.80 (C-7), 181.59 (C-4).

*Luteolin* (**4**): Yellow microcrystalline powder. – M.p. 226–229 °C. – $R_f$ 0.54 [chloroform/methanol (90:10, v/v)]. – UV/Vis: $\lambda_{max:}$ (MeOH) = 254, 266 sh, 353; (NaOMe) 270, 399; ($AlCl_3$) 276, 301 sh, 420; ($AlCl_3$/HCl) 274, 378; (NaOAc) 270, 386; ($NaOAc/H_3BO_3$) 260, 370 nm. – $^1H$ NMR (300 MHz, DMSO): $\delta_H$ = 6.17 (1H, *br. s*, H-6), 6.43 (1H, *br. s*, H-8), 6.63 (1H, *br. s*, H-3), 6.87 (1H, *d*, *J* = 8.4 Hz, H-5`), 7.30 (2H, *d*, *J* = 8.4 Hz, H2`,6`). – $^{13}C$ NMR (75 MHz, DMSO) $\delta_C$ = 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), 181.65 (C-4).

*Kaempferol* (**5**): Yellow microcrystalline powder. – M.p. 279–280 °C. – $R_f$ 0.51 [chloroform/methanol (90:10, v/v)]. – UV/Vis: $\lambda_{max}$ (MeOH) = 253, 266, 294 sh, 367; (NaOMe) 278, 316, 416; ($AlCl_3$) 260 sh, 268, 303 sh, 424; ($AlCl_3$/HCl) 256 sh, 269, 303 sh, 424; (NaOAc) 274, 303, 387; ($NaOAc/H_3BO_3$) 267, 297 sh, 372 nm. – $^1H$ NMR (300 MHz, DMSO): $\delta_H$ = 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`). – $^{13}C$ NMR (75 MHz, DMSO): $\delta_C$ = 94.02 (C-6), 98.75 (C-8), 102.33 (C-3), 104.00 (C-10), 116.21 (C-3`), 116.21 (C-5`), 121.50 (C-1`), 131.30 (C-2`), 131.30 (C-6`), 156.46 (C-2), 157.26 (C-9), 160.10 (C-4`), 161.18 (C-5), 167.80 (C-7), 177.77 (C-4).

*Eriodictyol* (**6**): Orange microcrystalline powder. – M.p. 257–258 °C. – $R_f$ 0.79 [chloroform/methanol (95:5, v/v)]. – UV/Vis: $\lambda_{max}$ (MeOH) = 289, 324 sh; (NaOMe) 242 sh, 323; ($AlCl_3$) 272, 306; ($AlCl_3$/HCl) 307, 375; (NaOAc) 289 sh, 324; ($NaOAc/H_3BO_3$) 290, 333 sh nm. – $^1H$ NMR (300 MHz, DMSO): $\delta_H$ = 2.64 (1H, *dd*, *J* = 3.3 Hz eq* ax, *J* = 17.1 Hz gem coupling, H-3 eq), 3.12 (1H, *dd*, *J* = 12.4 Hz ax* ax, *J* = 17.1 Hz gem coupling, H-3ax), 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`). – $^{13}C$ NMR (75 MHz, DMSO): $\delta_C$ = 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. – $R_f$ 0.61 [chloroform/methanol (80:20, v/v)]. – UV/Vis: $\lambda_{max}$ (MeOH) = 268, 332; (NaOMe) 248 sh, 269, 385; ($AlCl_3$) 275, 300, 348, 386; ($AlCl_3$/HCl) 273, 300, 350, 385; (NaOAc) 256 sh, 267, 355, 388; ($NaOAc/H_3BO_3$) 267, 340 nm. – $^1H$ NMR (300 MHz, DMSO): $\delta_H$ = 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`). – $^{13}C$ NMR (75 MHz, DMSO): $\delta_C$ = 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-7-O-glucoside* (**8**): Yellow microcrystalline powder. – M.p. 237–239 °C. – $R_f$ 0.59 [chloroform/methanol (80:20, v/v)]. – UV/Vis: $\lambda_{max}$ (MeOH) = 255, 266 sh, 348; (NaOMe) 264, 300 sh, 394; ($AlCl_3$) 274, 298 sh, 329, 432; ($AlCl_3$/HCl) 371, 294 sh, 357, 387; (NaOAc) 259, 266 sh, 364 sh, 405; ($NaOAc/H_3BO_3$) 259, 372 nm. – $^1H$ NMR (300 MHz, DMSO): $\delta_H$ = 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'). – $^{13}C$

NMR (75 MHz, DMSO): $\delta_C$ = 61.33 (C-6``), 70.28 (C-4``), 73.82 (C-2``), 77.10 (C-3``), 77.80 (C-5``), 95.41 (C-8), 100.22 (C-6), 100.62 (C-1``), 103.71 (C-3), 106.03 (C-10), 114.10 (C-2`), 116.68 (C-5`), 119.87 (C-6`), 121.76 (C-1`), 146.59 (C-3`), 151.01 (C-4`), 157.61 (C-9), 161.81 (C-5), 163.6 (C-2), 165.21 (C-7), 182.51 (C-4).

*Acacetin-7-O-galacturonide* (**9**): Yellow microcrystalline powder. – M.p. 228–230 °C. – $R_f$ 0.55 [chloroform/methanol (80:20, v/v)]. – UV/Vis: $\lambda_{max}$ (MeOH) = 267, 324; (NaOMe) 245 sh, 287, 355; ($AlCl_3$) 278, 300, 345, 383; ($AlCl_3$/HCl) 278, 301, 338, 381; (NaOAc) 268, 324; (NaOAc/$H_3BO_3$) 267, 328 nm. – $^1$H NMR (300 MHz, DMSO): $\delta_H$ = 3.85 (3H, *s*, $OCH_3$), 6.44 (1H, *br.s*, H-6), 6.85 (1H, *br.s*, H-8), 6.91 (1H, *s*, H-3), 7.09 (2H, *d*, *J* = 7.8 Hz, H-3`,5`), 8.02 (2H, *d*, *J* = 7.8 Hz, H2`,6`). $^{13}$C NMR (75 MHz, DMSO): $\delta_C$ = 55.55 ($OCH_3$), 71.59 (C-4``), 72.85 (C-3``), 74.64 (C-5``), 77.1 (C-2``), 93.91 (C-8), 98.79 (C-6), 102.80 (C-3), 103.64 (C-10), 115.91 (C-3`), 115.91 (C-5`), 121.14 (C-1`), 128.39 (C-2`), 128.39 (C-6`), 157.25 (C-4`), 161.09 (C-5), 161.39 (C-9), 163.68 (C-2), 164.09 (C-7), 175.00 (C-6``), 181.85 (C-4).

*Kaempferol-3-O-rutinoside* (**10**): Yellowish brown powder. – M.p. 223–224 °C. – $R_f$ 0.56 [ethyl acetate/methanol/water (100:16:12, v/v/v)]. – UV/Vis: $\lambda_{max}$ (MeOH) = 266, 316 sh, 350; (NaOMe) 245, 273, 309 sh, 388; ($AlCl_3$) 255 sh, 275, 296, 347, 396; ($AlCl_3$/HCl) 275, 294 sh, 347, 397; (NaOAc) 273, 322 sh, 352, 388; (NaOAc/$H_3BO_3$) 266, 315 sh, 353 nm. – $^1$H NMR (300 MHz, DMSO): $\delta_H$ = 7.89 (1H, *d*, *J* = 8.4 Hz, H-2`,6`), 6.87 (1H, *d*, *J* = 8.4 Hz, H-3`,5`), 6.35 (1H, *br. s*, H-8), 6.14 (1H, *br. s*, H-6); sugar: 5.60 (1H, *d*, *J* 1``*2``ax = 6 Hz, H-1``), 4.31 (1H, *d*, *J* 1```*2```eq = 1.5 Hz, H-1```), 3.94–3.55 (*m*, other protons of sugar), 0.87 (3H,CH3). – $^{13}$C NMR (75 MHz, DMSO): $\delta_C$ = 177.2 (C-4), 164.9 (C-7), 161.1 (C-5), 159.8 (C-4`), 156.8 (C-9), 156.5 (C-2), 133.3 (C-3), 130.9 (C-2`,6`), 120.9 (C-1`), 115.1 (C-3`,5`), 103.7 (C-10), 101.4 (C-1``), 100.7 (C-1```), 98.9 (C-6), 93.8 (C-8), 76.5 (C-3``), 75.8 (C-5``), 74.2 (C-2``), 72.1 (C-4```), 70.5 (C-3```), 70.3 (C-2```), 69.9 (C-4``), 68.2 (C-5``), 66.9 (C-6``), 17.6 (C-6```).

## 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 $CCl_4$ 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 $CCl_4$-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 $IC_{50}$ value of 200 $\mu$g/ml; consequently, its four fractions were also tested. The ethyl acetate fraction showed the highest free radical scavenging activity of all tested fractions ($IC_{50}$ = 65 $\mu$g/ml), followed by the *n*-butanol fraction ($IC_{50}$ = 150 $\mu$g/ml), whereas the *n*-hexane and chloroform fractions showed negligible activities.

The biochemical mechanism of $CCl_4$ 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 $CCl_4$; for example, the trichloromethyl radical ($CCl_3$·) or the trichloroperoxy radical ($CCl_3O_3$·). These radical species induce lipid peroxidation, disturbance in $Ca^{2+}$ 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-methoxyapigenin (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, $^{1}$H NMR and $^{13}$C NMR spectra with published data (Fig. 1).

The new compound **9**, showed the characteristic 15 signals of an acacetin aglycone in the $^{1}$H NMR and $^{13}$C NMR spectra, in addition to 6 extra signals of $\beta$-D-galacturonic acid (Agrawal, 1989) which is characterized by the presence of C-6`` at $\delta_C$ 175.00 ppm and C-3`` and C-5`` at $\delta_C$ 72.85 and 74.64 ppm (cf. $\beta$-D-glucuronic acid in which C-3`` and C-5`` appear at about $\delta_C$ 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 $\delta_H$ 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.

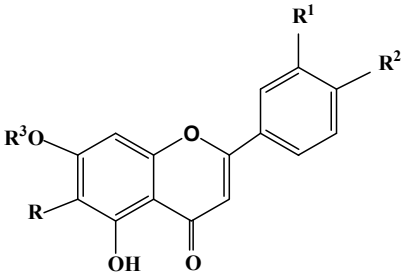
Table I. Effect of *Onopordum alexandrinum* L. on serum AST, ALT, and ALP levels of adult male albino rats.

| Treatment | | AST [U/L] | | | | ALT [U/L] | |
|---|---|---|---|---|---|---|---|
| | | Zero time | 15 d | 72 h after liver damage | 15 d after liver damage | Zero time | 15 d |
| | Control | 29.2 ± 1.1 | 28.6 ± 0.4 | 138.4 ± 5.1 | 149.7 ± 5 .9[a] | 31.6 ± 1.1 | 30.2 ± 1.1 |
| 25 mg/kg | Silymarin | 32.4 ± 1.1 | 29.8 ± 0.9 | 48.7 ± 1.3 | 27.3 ± 0.6[b] | 27.8 ± 0.5 | 26.5 ± 0.4 |
| 50 mg/kg | Ethanolic extract | 33.4 ± 1.2 | 33.5 ± 1.3 | 89.3 ± 2.8 | 69.6 ± 2.4[ab] | 27.3 ± 0.4 | 27.1 ± 0.3 |
| | *n*-Hexane fraction | 28.7 ± 0.1 | 28.9 ± 0.8 | 110.2 ± 4.8 | 81.6 ± 3.2 | 27.8 ± 0.7 | 27.6 ± 0.7 |
| | Chloroform fraction | 29.8 ± 0.7 | 29.4 ± 0.7 | 108.2 ± 4.7 | 76.2 ± 2.9 | 31.5 ± 1.1 | 31.6 ± 1.2 |
| | Ethyl acetate fraction | 31.9 ± 1.1 | 31.6 ± 0.9 | 66.3 ± 2.4 | 58.7 ± 2.3[ab] | 28.9 ± 0.6 | 28.5 ± 0.8 |
| | *n*-Butanol fraction | 32.6 ± 1.3 | 31.5 ± 0.8 | 102.7 ± 4.9 | 91.4 ± 4.3 | 33.4 ± 1.2 | 33.3 ± 1.1 |
| 10 mg/kg | Compound **9** | 33.1 ± 1.2 | 32.6 ± 1.1 | 76.4 ± 1.7 | 58.6 ± 1.3[ab] | 28.3 ± 0.8 | 27.9 ± 0.6 |
| 100 mg/kg | Ethanolic extract | 31.6 ± 1.1 | 31.2 ± 1.3 | 72.4 ± 1.8 | 48.3 ± 1.7[ab] | 34.1 ± 1.2 | 32.6 ± 1.3 |
| | *n*-Hexane fraction | 38.4 ± 1.4 | 39.9 ± 1.1 | 107.2 ± 2.3 | 88.4 ± 1.9 | 41.4 ± 1.4 | 39.9 ± 1.1 |
| | Chloroform fraction | 39.4 ± 1.5 | 41.9 ± 1.1 | 108.2 ± 2.4 | 89.4 ± 1.5 | 44.4 ± 1.5 | 43.9 ± 1.1 |
| | Ethyl acetate fraction | 32.8 ± 1.4 | 29.7 ± 0.5 | 61.6 ± 2.9 | 39.2 ± 1.3b | 28.6 ± 0.7 | 28.1 ± 0.4 |
| | *n*-Butanol fraction | 33.4 ± 1.2 | 31.9 ± 1.1 | 98.2 ± 3.4 | 82.4 ± 2.1 | 31.9 ± 1.1 | 32.4 ± 1.3 |
| 25 mg/kg | Compound **9** | 30.4 ± 1.1 | 29.8 ± 0.7 | 52.9 ± 1.8 | 42.5 ± 1.2[ab] | 29.2 ± 0.7 | 28.9 ± 0.4 |

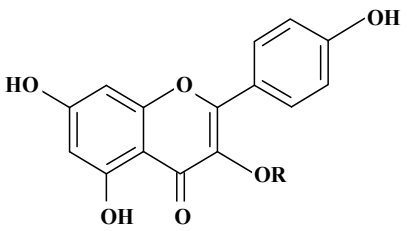
[a] Statistically significant from zero time at $p < 0.01$.
[b] Statistically significant from 72 h after $CCl_4$ at $p < 0.01$.

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 $^{1}$H 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 $^{13}$C 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 $^{1}$H 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 $^{13}$C 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 ($\delta_H$ 5.60 ppm) and presence of the anomeric carbon atom C-1`` at $\delta_C$ 101.4 ppm (Agrawal, 1989; Harborne *et al.,* 1975;

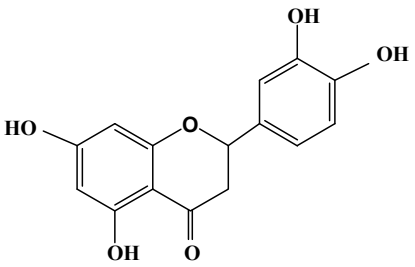
| ALT [U/L] | | ALP [U/L] | | | |
|---|---|---|---|---|---|
| 72 h after liver damage | 15 d after liver damage | Zero time | 15 d | 72 h after liver damage | 15 d after liver damage |
| 143.5 ± 6.8 | 148.4 ± 6.2[a] | 6.9 ± 0.1 | 7.1 ± 0.1 | 57.2 ± 1.8 | 62.3 ± 2.1[a] |
| 53.6 ± 1.8 | 29.2 ± 0.8[b] | 7.3 ± 0.1 | 6.9 ± 0.1 | 15.1 ± 0.6 | 6.8 ± 0.1[b] |
| 81.3 ± 3.2 | 62.4 ± 2.7[ab] | 7.2 ± 0.1 | 6.9 ± 0.1 | 42.3 ± 1.8 | 36.7 ± 1.6[ab] |
| 98.9 ± 4.5 | 91.3 ± 4.2 | 7.3 ± 0.1 | 7.2 ± 0.1 | 43.1 ± 2.6 | 46.5 ± 1.7 |
| 114.3 ± 5.2 | 94.5 ± 3.6 | 6.9 ± 0.1 | 7.0 ± 0.1 | 51.2 ± 2.7 | 47.3 ± 2.5 |
| 73.4 ± 2.7 | 58.6 ± 2.9[ab] | 7.1 ± 5.1 | 6.8 ± 0.1 | 45.4 ± 1.9 | 38.4 ± 1.5 |
| 121.6 ± 5.9 | 103.2 ± 4.8 | 7.4 ± 0.1 | 7.5 ± 0.1 | 55.3 ± 2.4 | 49.2 ± 2.3 |
| 68.4 ± 2.9 | 43.6 ± 2.3[ab] | 7.5 ± 0.1 | 7.4 ± 0.1 | 31.2 ± 0.8 | 26.9 ± 0.7[ab] |
| 65.2 ± 3.4 | 41.7 ± 1.6 | 7.5 ± 0.1 | 7.3 ± 0.1 | 18.3 ± 0.4 | 14.3 ± 0.2[ab] |
| 117.2 ± 2.3 | 98.4 ± 1.9 | 8.4 ± 1.4 | 9.9 ± 1.1 | 39.2 ± 2.3 | 36.4 ± 1.9 |
| 109.2 ± 2.4 | 99.4 ± 1.5 | 39.4 ± 1.5 | 41.9 ± 1.1 | 108.2 ± 2.4 | 89.4 ± 1.5 |
| 58.9 ± 1.8 | 44.2 ± 1.6[ab] | 7.3 ± 0.1 | 7.1 ± 0.1 | 21.8 ± 0.4 | 16.1 ± 0.2[ab] |
| 87.5 ± 2.2 | 78.5 ± 2.2 | 7.2 ± 0.1 | 7.4 ± 0.1 | 38.9 ± 1.3 | 31.7 ± 1.2 |
| 63.4 ± 2.7 | 51.6 ± 2.3[ab] | 7.5 ± 0.1 | 7.2 ± 0.1 | 27.2 ± 0.8 | 18.2 ± 0.3 [ab] |




**1**: R =, $R^1$= $R^3$ = H, $R^2$= OH
**2**: R = $R^1$ = $R^3$ = H, $R^2$=$OCH_3$
**3**: R = $R^1$ = $R^3$ = H, $R^2$ = OH
**4**: R = $R^3$ = H, $R^1$ = $R^2$ = OH
**7**: R = $R^1$ = H, $R^2$ = OH, $R^3$ = β-glucose
**8**: R = H, $R^1$ = $R^2$ = OH, $R^3$ = β-glucose
**9**: R = $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = β-galacturonic acid




**5**: R = H
**10**: R = 3-*O*-rhamnoglucoside




**6**

Fig. 1. Chemical structures of the isolated compounds.

Markham, 1982). 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 μg/ml) relative to silymarin ($IC_{50}$ = 45 μg/ml), but less than shown by ascorbic acid and quercetin (7.5 and 0.09 μg/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.

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