Hepatoprotective and cytotoxic activities of *Delonix regia* flower extracts

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**ABSTRACT**

Fractionation of the ethanolic extract of the flowers of *Delonix regia* led to the isolation of three sterols, namely, stigmasterol (1.54 %), β-sitosterol and its 3-O-glucoside (6.93 %), a triterpene, namely, ursolic acid (3.61 %) and four flavonoids: quercetin (2.92 %), quercitrin (0.59 %), isoquercitrin (3.87 %) and rutin (5.12 %) in addition to the amino acid L-azeditine-2-carboxylic acid. The structures of the isolated compounds were established on the basis of physicochemical properties and spectral analysis (IR, UV, EI/MS, ¹H-NMR and ¹³C-NMR). The concentration of the isolated compounds was determined by HPLC technique. The ethanolic extract and its non-polar and flavonoid rich fraction as well as the isolated compounds evidenced cytotoxic activities against human liver cancer cell line (HEPG2) which were potent for ursolic acid (IC₅₀ 0.55 µg/ml) and L-azeditine-2-carboxylic acid (IC₅₀ 2.51 µg/ml). Meanwhile, rutin and isoquercitrin were inactive. Moreover, the ethanolic extract and its two fractions were tested for hepatoprotective activity against CCl₄ induced hepatic cell damage in rats at two dose levels (50 and 100 mg/kg), and the flavonoid rich fraction showed statistically significant hepatoprotection at 100 mg/kg. The presence of the aforementioned flavonoids with their efficient free radical scavenging properties may explain this liver protection ability. This could suggest the use of the ethanolic extract of the flowers of *D. regia* as a chemopreventive agent against the two main causes of liver damage; liver toxicity by chlorinated agents and liver cancer.

**Keywords:** *Delonix regia*, Hepatoprotective; Anticancer; Azeditine.

**INTRODUCTION**

*Delonix regia* (Hook.) Raf. (*Poinciana regia* Boj. ex Hook., Royal Poinciana, Gul mohar, Flame tree or Flamboyant, Fabaceae –Caesalpinioideae) is a large ornamental tree (10-18 m hight) with fern-like bipinnately compound leaves native to Madagascar and carrying the attractive red peacock flowers. It flowers in April and lasts for several months [1,2]. It is widely grown in Egypt lining the streets and gardens with its beauty, especially in Cairo, North coast and Sinai. The ethanolic extract of the flowers inhibited β- lactamase producing methicillin- resistant *Staphylococcus aureus* (MRSA) and methicillin-sensetive *S. aureus* (MSSA) and evidenced antioxidant property [3,4]. On the other hand, the ethyl acetate extract of the flowers showed a molluscidal activity [5]. Chemically, anthocyanins were reported in the flowers [6-10]. Carotenoids were isolated from the non-polar fraction of the flowers [11], keto and imino acids were detected in the flowers of *Delonix regia* [12]. The flower extract also was reported as a useful natural colour and as an acid-base indicator [7,9].

The aim of this study is to undergo a phytochemical investigation of the flowers in an attempt to isolate the major compounds and evaluate their possible hepatoprotective and cytotoxic activity to justify their use as chemopreventive agents. Accordingly, the ethanolic extract, as well as its different fractions (rich in these compounds) are traced for the same biological activities.

**MATERIALS AND METHODS**

**Plant Material**

The flowers of *Delonix regia* (Hook.) Raf. were obtained from the trees growing in the North coast (at the 24th km...
During spring 2007/2008. The plant was kindly identified at the Botany Department, Faculty of Science, Cairo University, Giza, Egypt. A voucher specimen (No.: D-3) is kept in the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

**General**

Authentic flavonoids, sterols and triterpenes were obtained from E. Merck, Darmstadt, Germany. Silica gel H (E-Merk, Darmstadt, Germany) for vacuum liquid chromatography (VLC), silica gel 60 (Fluka, 70-230 mesh ASTM, Germany) for column chromatography (CC) and sephadex LH 20 (Pharmacia) were used. Thin-layer chromatography (TLC) was performed on silica gel GF254 precoated plates (Fluka, Germany) using solvent systems S1: n-hexane-ethyl acetate (90:10); S2: n-hexane-ethyl acetate (80:20), S3: chloroform-methanol (95:5), S4: chloroform-methanol (90:10), S5: chloroform-methanol (80:20) and S6 ethyl acetate-methanol-water (100:16.5:13.5). The chromatograms were visualized using CHCl3-d6 and DMSO-d6 and chemical shifts were given from VX-300 NMR instrument. The NMR spectra were recorded in Germany using solvent systems S1: methanol (95:5) S2: chloroform-methanol (50:50), S3: water (v/v) 3% containing 20:80:1 v/v/v, while a solvent system acetonitrile-water-acetic acid (20:80:1 v/v/v) was used as a mobile phase for sterols. The flow rate was 1 ml/min. UV wavelength was 280 nm for flavonoids and 205 nm for sterols. Calculations for percentages were done considering standards and samples AUC. Sample preparation for HPLC: For sterols: the dried flowers (10 gm) were exhaustively extracted with petroleum ether (60-80°C). Unsaponifiable matters were separated from saponified petroleum ether extract. The unsaponifiable fraction was quantitatively used for sample preparation. For flavonoids: the dried flowers (10 gm) were exhaustively extracted with methanol and quantitatively used for sample preparation.

**Material for biological study**

Silymarin (Sedico Pharmaceutical Co., 6 October City, Egypt) and carbon tetrachloride (analar, El-Gomhoreya Co., Cairo, Egypt). Transaminase Kits (Bio-Meriéux Co.): biochemical kits for assessment of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase enzymes (ALP). Adult male albino rats of Sprague Dawely Strain weighing 100-150 g and albino mice (20 - 25g) were used. All animals were kept on standard laboratory diet and under hygienic conditions.

**Determination of LD50**

The LD50 of the ethanolic extract of the flowers was calculated according to Karber.[14]

**In vitro screening for cytotoxic activity**

The ethanolic extract of the flowers of *Delonix regia* (Hook.) Raf., its different fractions and the isolated compounds were tested for their cytotoxicity at the National Cancer Institute, Cairo, Egypt at different concentrations in DMSO (0-10 mg/ml), against human liver cancer cell line (HEPG2), according to the method of Skehan et al.[15]. The IC50 values were calculated and the results are shown in Table 1.

**Assessment of hepatoprotective activity**

The ethanolic extract of the flowers as well as the non-polar fraction A and the flavonoid rich fraction B were tested for...
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their hepatoprotective activity. The tested extracts were administered at two dose levels 50 and 100 mg/kg body weight for one month before induction of liver damage by intraperitoneal injection of 5ml/kg of 25% carbon tetrachloride (CCl4) in liquid paraffin according to the method of Klassan and Plaa [16], silymarin 25 mg / kg body weight was used as a reference drug. The extracts as well as the reference drug were continued to be administered to the rats for another month after liver damage. The levels of aspartate aminotransferase (AST) [17], alanine aminotransferase (ALT) [17] and alkaline phosphatase (ALP) [18] enzymes were measured in the blood of each group at zero time, after one month of receiving the tested drug, 72 hours after induction of liver damage and after one month of treatment with the tested samples. Results are shown in (Table 2).

RESULTS

Spectral data of the isolated compounds

Nine compounds were isolated from the ethanolic extract of the flowers of D. regia. The structures of the isolated compounds were established on the basis of physicochemical properties and spectral analysis (IR, UV, EI/MS, 1H-NMR and 13C-NMR).

Compound 1 (β-sitosterol)

White needle crystals (n-hexane)

m.p. 140-141°C

Rf: 0.41 in S2

MS (EI, 70 eV): m/z (%) = 414 [M]+ (100 %), 396 (51 %), 329 (42 %), 303 (44 %), 273 (60 %) and 255 (80 %).

1H-NMR: δ (300 MHz, CDCl3) 0.70 (3H, d, J=5.4,Me-21), 0.84 (3H, t, J=6.3,Me-29), 0.91 (3H, d, J=6.3, Me-26), 0.95 (3H,d, J=6.3, Me-27), 1.04 (3H, s, Me-18), 1.27 (3H, s, Me-19), 3.51 (1H, m, H-3), 5.38 (1H, br.s, H-6) ppm.

13C-NMR: δ (75 MHz, DMSO) 15.76(C-24), 16.68(C-25), 0.75(H, s,Me-24), 0.80 (3H, d, J=6.6,Me-29), 0.86 (3H, s, Me-26), 0.91 (3H, s,Me-27), 0.91 (3H, d, J=6.6,Me-30), 1.04 (3H, s, Me-23), 3 (1H, m, H-18), 4.25 (1H, d, J=5.4,H-3) and 5.13 (1H, br.s, H-12) ppm.

Table 1. In-vitro Cytotoxicity of the flowers Delonix regia (Hook.) Raf. On human liver cancer cell line (HEPG2).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 (µg/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The ethanolic extract</td>
<td>9.06</td>
</tr>
<tr>
<td>Fraction A</td>
<td>2.67</td>
</tr>
<tr>
<td>Fraction B</td>
<td>5.34</td>
</tr>
<tr>
<td>Compound 1</td>
<td>3.23</td>
</tr>
<tr>
<td>Compound 2</td>
<td>8.09</td>
</tr>
<tr>
<td>Compound 3</td>
<td>0.55</td>
</tr>
<tr>
<td>Compound 4</td>
<td>5.44</td>
</tr>
<tr>
<td>Compound 5</td>
<td>3.96</td>
</tr>
<tr>
<td>Compound 6</td>
<td>9.32</td>
</tr>
<tr>
<td>Compound 7</td>
<td>-</td>
</tr>
<tr>
<td>Compound 8</td>
<td>-</td>
</tr>
<tr>
<td>Compound 9</td>
<td>2.51</td>
</tr>
<tr>
<td>Doxorubicin®</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Fraction A, the non polar fraction of the ethanolic extract. Fraction B, the flavonoid rich fraction. Doxorubicin, an anticancer reference drug.

Compound 2 (Stigmasterol)

White needle crystals (n-hexane)

m.p. 169-170°C.

Rf 0.41 in S2.

EIMS (70 eV rel. int.), m/z at 412 [M]+ (100 %), 399 (43 %), 396 (65 %), 369 (73 %), 329 (17 %), 271 (26 %) and 255 (52 %).

1H-NMR: δ (300 MHz, CDCl3) 0.69 (3H, d, J=5.4 Hz, Me-21), 0.80 (3H, t, J=6.3, Me-29), 0.89 (3H, d, J=6.4 Hz, Me-26), 0.92 (3H, d, J=6.3 Hz, Me-27), 1.04 (3H, s, Me-18), 1.27 (3H, s, Me-19), 3.49 (1H, m, H-3), 5.15 (dd, 1H, J=8.3, 15.4, H-22), 5.21 (1H, dd, J=8, 15.2, H-23) and 5.4 (1H, br.s, H-6) ppm.

Compound 3 (Ursolic acid)

White microcrystalline powder.

m.p. 288-290°C.

Rf 0.39 in S2.

EIMS: (70 eV rel. int.), m/z at 456 [M]+ (12 %), 438 (33 %), 411 (62 %), 248 (100 %), 208 (42 %), 203 (31 %) and 190 (37 %).

1H-NMR: δ (300 MHz, DMSO) 0.68 (3H, s,Me-25), 0.75(3H, s,Me-24), 0.80 (3H, d, J=6.6,Me-29), 0.86 (3H, s,Me-26), 0.91 (3H, s,Me-27), 0.91 (3H, d, J=6.6,Me-30), 1.04 (3H, s, Me-23), 3 (1H, m, H-18), 4.25 (1H, d, J=5.4,H-3) and 5.13 (1H, br.s, H-12) ppm.

13C-NMR: δ (75 MHz, DMSO) 15.76(C-24), 16.68(C-25), 17.32(C-26), 18.09(C-29), 21.24(C-30), 23.06(C-11), 23.43(C-27), 24.26(C-16), 28.22(C-2), 28.30(C-15), 28.75(C-23), 30.45(C-21), 33.39(C-7), 36.67(C-22), 38.13(C-10), 38.44(C-1), 38.77(C-4), 38.95(C-20), 39.05(C-19), 40.88(C-8), 41.39(C-14),47.02(C-17), 51.23(C-18), 55.87(C-5), 77.44(C-3), 125(C-12), 138.92 (C-13) and 179.16(C-28).
**Table 2. Effect of Delonix regia (Hook.) Raf. on the serum AST, ALT and ALP level on adult main albino rats.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>AST (U/L) Zero</th>
<th>AST (U/L) 30 days</th>
<th>AST (U/L) 72h</th>
<th>ALT (U/L) Zero</th>
<th>ALT (U/L) 30 days</th>
<th>ALT (U/L) 72h</th>
<th>ALP (KAU) Zero</th>
<th>ALP (KAU) 30 days</th>
<th>ALP (KAU) 72h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>29.6 ± 0.9</td>
<td>29.2 ± 0.8</td>
<td>139.8 ± 5.3*</td>
<td>33.6 ± 0.7</td>
<td>31.9 ± 0.8</td>
<td>163.7 ± 6.2*</td>
<td>6.2 ± 0.1</td>
<td>6.3 ± 0.1</td>
<td>35.1 ± 1.1*</td>
</tr>
<tr>
<td>Silymarin</td>
<td>25 mg/kg</td>
<td>31.8 ± 0.9</td>
<td>30.4 ± 0.4</td>
<td>42.6 ± 1.3**</td>
<td>27.3 ± 0.7*</td>
<td>29.1 ± 0.9</td>
<td>26.3 ± 0.6</td>
<td>37.5 ± 1.4**</td>
<td>24.8 ± 0.5**</td>
<td>7.3 ± 0.1</td>
</tr>
<tr>
<td>Ethanoic extract</td>
<td>50 mg/kg</td>
<td>34.1 ± 1.3</td>
<td>33.5 ± 1.2</td>
<td>98.2 ± 3.1</td>
<td>71.4 ± 2.7</td>
<td>31.1 ± 1.2</td>
<td>29.8 ± 0.9</td>
<td>79.2 ± 3.1</td>
<td>52.7 ± 2.9</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>33.5 ± 1.2</td>
<td>32.9 ± 1.1</td>
<td>88.9 ± 3.2</td>
<td>76.8 ± 2.6</td>
<td>29.7 ± 0.6</td>
<td>29.4 ± 0.5</td>
<td>81.3 ± 2.6</td>
<td>59.9 ± 1.7</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>33.8 ± 1.2</td>
<td>33.1 ± 0.8</td>
<td>73.4 ± 2.4</td>
<td>64.3 ± 2.4</td>
<td>30.5 ± 0.9</td>
<td>30.1 ± 1.1</td>
<td>68.9 ± 2.4</td>
<td>58.2 ± 1.4</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>Ethanoic extract</td>
<td>100 mg/kg</td>
<td>31.4 ± 1.1</td>
<td>31.1 ± 1.3</td>
<td>73.6 ± 2.9*</td>
<td>41.8 ± 1.7*</td>
<td>29.5 ± 0.9</td>
<td>29.1 ± 0.7</td>
<td>58.3 ± 2.1*</td>
<td>39.8 ± 1.4*</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>27.3 ± 0.6</td>
<td>27.1 ± 0.9</td>
<td>61.2 ± 2.4*</td>
<td>41.8 ± 2.3**</td>
<td>29.8 ± 0.7</td>
<td>28.2 ± 0.4</td>
<td>71.4 ± 2.3*</td>
<td>43.6 ± 1.3*</td>
<td>7.1 ± 0.1</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>31.6 ± 1.2</td>
<td>30.4 ± 1.1</td>
<td>56.8 ± 1.6*</td>
<td>39.4 ± 1.2**</td>
<td>28.9 ± 0.8</td>
<td>29.1 ± 0.7</td>
<td>51.5 ± 1.9*</td>
<td>49.3 ± 1.2**</td>
<td>7.3 ± 0.1</td>
</tr>
</tbody>
</table>

Fraction A is the non polar fraction of the ethanoic extract, Fraction B is the flavonoid rich fraction.

*Statistically significant from zero time p < 0.01.<br>
• Statistically significant from 72 hours after CCl₄ at p < 0.01.<br>
□ Statistically significant from the control group at 72 hours after CCl₄ (>20% difference).
**Compound 4 (β-sitosterol-3-O-β-D-glucopyranoside)**

White microcrystalline powder.

m.p. 290°C.

Rf 0.37 in S6.

MS (EI, 70 eV): m/z (%): 414 [M]+ (100 %), 396 (46 %), 329 (36 %), 303 (39 %), 273 (66 %) and 255 (65 %).

1H-NMR (Me-SO, DMSO) δ ppm: 0.66 (3H, d, J = 5.5 Hz, Me-21), 0.78 (3H, t, J = 6.3 Hz, Me-29), 0.83 (3H, d, J = 6.2 Hz, Me-26), 0.90 (3H, d, J = 6.3 Hz, Me-27), 0.92 (3H, s, Me-18), 0.96 (3H, s, Me-19), 3.03 (1H, m, H-3), 4.21 (1H, d, J = 7.5 Hz, H-1’), 5.33 (1H, br.s, H-6) ppm.

**Compound 5 (Quercetin)**

Yellow microcrystalline powder.

m.p. 314-316 °C.

Rf 0.45 in S4.

UV λ max nm (MeOH): 256, 272, 302 (sh.), 372; NaOMe: 248, 272, 308 (sh.), 335, 450; AlCl₃/HCl: 270, 350, 395; NaOAc: 273, 324, 380; NaOAc/H₂BO₃: 262, 298, 377.

1H-NMR δ ppm (Me-SO, DMSO) δ ppm (Me-SO, DMSO) 7.76 (1H, d, J = 2.1 Hz, H-2’), 7.53 (1H, d, J = 8.1 Hz, H-6’), 6.71 (1H, d, J = 8.4 Hz, H-5’), 6.30 (1H, broad singlet, H-8), 6.19 (1H, d, J = 1.2 Hz, H-6), 5.41 (1H, d, J = 1.2 Hz, H-6’), 1.1 (3H, d, J = 6.3 Hz, Me).

13C-NMR δ ppm (Me-SO, DMSO) 175.8 (C-4), 164.0 (C-7), 160.6 (C-5), 156.1 (C-9), 156.0 (C-2), 147.7 (C-4’), 145.0 (C-3’), 135.7 (C-3), 121.9 (C-1’), 119.9 (C-6’), 115.6 (C-5’), 115.0 (C-2’), 102.9 (C-10), 98.2 (C-6), 93.6 (C-8), 77.5 (C-5’), 76.8 (C-3’), 74.3 (C-2’), 68.0 (C-4’), 61.3 (C-6’).

**Compound 6 (Quercetin-3-O-α-L-rhamnoside)**

Yellow microcrystalline powder.

m.p. 182-184 °C.

Rf 0.7 in S6.

UV λ max nm (MeOH): 257, 272, 302 (sh.), 372; NaOMe: 273, 324, 380; NaOAc/H₂BO₃: 262, 298, 377.

1H-NMR δ ppm (Me-SO, DMSO) 7.60 (1H, d, J = 2.1 Hz, H-2’), 7.54 (2H, dd, J = 2.1 & 8.4 Hz, H-2’, H-6’), 6.71 (1H, d, J = 8.4 Hz, H-5’), 6.30 (1H, broad singlet, H-8), 6.1 (1H, broad singlet, H-6’), 5.41 (1H, d, J = 1.2 Hz, H-1’), 1.1 (3H, d, J = 6.3 Hz, Me).

13C-NMR δ ppm (Me-SO, DMSO) 177.4 (C-4), 164.0 (C-7), 161.1 (C-5), 156.5 (C-9), 156.4 (C-2), 148.4 (C-4’), 144.7 (C-3’), 133.6 (C-3), 121.5 (C-1’), 121.1 (C-6’), 115.8 (C-5’), 115.2 (C-2’), 103.7 (C-10), 101.9 (C-1’), 98.9 (C-6), 93.6 (C-8), 71.5 (C-5’), 70.1 (C-5’), 70.5 (C-3’), 70.4 (C-2’), 17.3 (C-6’).

**Compound 7 (Quercetin-3-O-β-D-glucopyranoside)**

Yellow microcrystalline powder.

m.p. 242-243 °C.

Rf 0.55 in S6.

UV λ max nm (MeOH): 257, 362; NaOMe: 272, 327, 409; AlCl₃: 275, 305, 438; AlCl₃/HCl: 270, 350, 395; NaOAc: 273, 324, 380; NaOAc/H₂BO₃: 262, 298, 377.

1H-NMR δ ppm (Me-SO, DMSO) 7.61 (1H, d, J = 2.1 Hz, H-2’), 7.55 (2H, dd, J = 2.1 & 8.4 Hz, H-2’, H-6’), 6.83 (1H, d, J = 8.4 Hz, H-5’), 6.39 (1H, d, J = 1.2 Hz, H-8), 6.18 (1H, d, J = 1.2 Hz, H-6), 5.41 (1H, d, J = 6.9 Hz, H-1’).

13C-NMR δ ppm (Me-SO, DMSO) 177.5 (C-4), 164.0 (C-7), 160.6 (C-5), 156.5 (C-9), 156.4 (C-2), 148.4 (C-4’), 144.8 (C-3’), 133.9 (C-3), 121.4 (C-1’), 121.6 (C-6’), 116.5 (C-5’), 115.2 (C-2’), 104.2 (C-10), 101.4 (C-1’), 98.8 (C-6), 93.6 (C-8), 77.5 (C-5’), 76.8 (C-3’), 74.3 (C-2’), 68.0 (C-4’), 61.3 (C-6’).

**Compound 8 (Quercetin-3-O-rhamnoglucoside)**

Yellow microcrystalline powder.

m.p. 190-192 °C.

Rf 0.42 in S6.

UV λ max nm (MeOH): 258, 300, 358; NaOMe: 268, 328, 410; AlCl₃: 275, 305, 438; AlCl₃/HCl: 270, 350, 395; NaOAc: 273, 324, 380; NaOAc/H₂BO₃: 262, 298, 377.

1H-NMR δ ppm (Me-SO, DMSO) 7.70 (1H, d, J = 2.1 Hz, H-2’), 7.55 (2H, dd, J = 2.1 & 8.4 Hz, H-2’, H-6’), 6.83 (1H, d, J = 8.4 Hz, H-5’), 6.39 (1H, d, J = 1.2 Hz, H-8), 6.18 (1H, d, J = 1.2 Hz, H-6), 5.41 (1H, d, J = 6.9 Hz, H-1’).

13C-NMR δ ppm (Me-SO, DMSO) 177.5 (C-4), 164.0 (C-7), 161.2 (C-5), 156.5 (C-9), 156.4 (C-2), 148.4 (C-4’), 144.8 (C-3’), 133.9 (C-3), 121.4 (C-1’), 121.6 (C-6’), 116.5 (C-5’), 115.2 (C-2’), 104.2 (C-10), 101.4 (C-1’), 98.8 (C-6), 93.6 (C-8), 77.5 (C-5’), 76.8 (C-3’), 74.3 (C-2’), 68.0 (C-4’), 61.3 (C-6’).
THE BIOLOGICAL STUDY

Determination of LD50

The acute toxicity study of the ethanolic extract of the flowers of *D. regia* revealed no mortality up to a dose level of 7.5 g/kg b.w. thus the anticancer and hepatoprotective activities were assessed.

DISCUSSION

*Delonix regia* (Hook.) Raf. Flowers contain variety of sterols, triterpenes and flavonoids to be first reported. The isolated compounds were β-sitosterol (1), stigmasterol (2), ursolic acid (3), β-sitosterol-3-O-β-D-glucopyranoside (4), quercetin (5), quercetin-3-O-α-L-rhamnopyranoside (quercitrin) (6), quercetin-3-O-β-D-glucopyranoside (Isoquercitrin) (7) and quercetin-3-O-rhamnosylglucoside (rutin) (8). Their structures were confirmed by comparing their chromatographic, chemical and spectroscopic data (EI/MS, UV, 1H-NMR and 13C-NMR) with the published data [19-29]. The above compounds were not traced in the literature for the genus *Delonix*. Compound 9 was identified as L-azeditine-2-carboxylic acid, its structure was confirmed by comparing its chromatographic data (IR, 1H-NMR and 13C-NMR) with that reported in the literature[30]. Compound 9 was isolated before from the legumes of the same plant[31]. The concentrations of the isolated compounds were determined using HPLC technique it was noticed that β-sitosterol and

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Compound 1" /></td>
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<tr>
<td>2</td>
<td><img src="image2.png" alt="Compound 2" /></td>
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<tr>
<td>3</td>
<td><img src="image3.png" alt="Compound 3" /></td>
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**Figure 1. Structures of the isolated compounds**
quercetin-3-O-rhamnoglucoside (rutin) were the major compounds in the flowers (6.93 % and 5.12 %, respectively).

As previous studies had shown that chlorinated hydrocarbons had been demonstrated to induce oxidative stress (imbalance in the pro-oxidant and oxidative status) this oxidative stress and oxidative damage in turn may be responsible for hepatic tumor promoting activity of these compounds[32,53]. Thus, compounds or treatments that lower the oxidative stress can also inhibit tumour promotion[54]. Recent studies also showed that the antioxidant dietary supplementation can result in ablation of the hepatic lesion growth through an increase in apoptosis in hepatic lesion[55]. On the other hand, the presence of significant proportions of quercetin, isoquercitrin and rutin with their reported free radical scavenging, anti-lipid peroxidation[36,37] and hepatoprotective activity[37,20,58], in addition to, the reported antioxidant activity of the ethanolic extract of the flowers under investigation[41] added a support for the authors to evaluate the cytotoxic activity against liver cancer cell line and the hepatoprotective activity of the ethanolic extract and its fractions against CCl4 induced liver damage.

The ethanolic extract revealed a cytotoxic activity against the human liver cancer cell line (HEPG2) (Table 1), as it had an IC50 = 9.06 µg/ml. The two main fractions A and B showed higher potency displayed by lower IC50 values (2.67 and 5.34, respectively). The isolated compounds were also tested for their cytotoxic activity where ursolic acid (2.67 and 5.34, respectively). The isolated compounds were also tested for their cytotoxic activity where ursolic acid (2.67 and 5.34, respectively) had an IC50= 9.06 µg/ml. The two main fractions A and B showed higher potency displayed by lower IC50 values (2.67 and 5.34, respectively). The isolated compounds were also tested for their cytotoxic activity where ursolic acid (2.67 and 5.34, respectively) had an IC50= 9.06 µg/ml. The two main fractions A and B showed higher potency displayed by lower IC50 values (2.67 and 5.34, respectively) had an IC50= 9.06 µg/ml. The two main fractions A and B showed higher potency displayed by lower IC50 values (2.67 and 5.34, respectively).

Since the ethanolic extract of the flowers as well as its non-polar fraction (A) and the flavonoid rich fraction (B) showed neither significant change in AST, ALT and ALP levels after one month of administration nor/or weak hepatoprotection against CCl4 induced liver damage at 50 mg/kg b. wt. (Table 2). Therefore, the extract and its fractions were tested at a higher dose level (100 mg/kg). A daily dose of the ethanolic extract and the two fractions (A and B) (100 mg/kg b. wt.) showed no significant change in AST, ALT and ALP levels after one month of administration (Table 2). On comparing the increase in liver enzymes in the control group at 72 hours after induction of liver damage (by 25% CCl4) it was observed that the ethanolic extract, its non-polar fraction A as well as its flavonoid rich fraction B prevented the increase in the level of AST enzyme by 47.30, 56.20 and 59.80%, respectively and 64.38, 56.38 and 68.54% in ALT enzymatic level and 44.4, 28.20 and 45.29% in that of ALP level, respectively. This protective effect (especially the effect of the ethanol extract and its flavonoid rich fraction B) was comparable to that of silymarin which prevented the rise in the levels of AST, ALT and ALP 69.5, 77.09 and 70.1% of, respectively. Furthermore, Administration of the extracts for another one month after induction of liver damage (by 25% CCl4) led to a significant decrease in the enzyme levels regarding their respective normal values which indicates stabilization of the hepatocyte cell membrane as well as repairing of hepatic tissue damage caused by CCl4[57]. Since, the preventive action of the liver damage induced by CCl4 has widely been used as a marker of hepatoprotective activity of drugs in general[59] and as the liver cancer is one of the death leading diseases in worldwide and particularly in Egypt, therefore, the present study has established that the flowers of D. regia are holding a great expectation for food and pharmaceutical applications.

CONCLUSION

Our results could justify the use of the ethanolic extract of the flowers of D. regia as a chemopreventive agent against the two causes of liver damage which are liver toxicity by chlorinated agents and liver cancer.

REFERENCES


Aly M. El-Sayed, et al.: Hepatoprotective and cytotoxic activities of Delonix regia flower extracts


