Chemical Constituents and Cytotoxic Effect of the Main Compounds of *Lythrum salicaria* L.

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*Lythrum salicaria* L. (Lythraceae), a herbaceous plant growing widely in Iran, has been well known for many centuries for its astringent and styptic properties. A phytochemical investigation of this plant, based on spectroscopic analysis, identified fourteen compounds: 5-hydroxy-2-pyrrolidinone (1), umbelliferone-6-carboxylic acid (2), 3,3',4'-tri-O-methyl-ellagic acid-4-O-β-D-glucopyranoside (3), 3,3',4'-tri-O-methyl-ellagic acid-4-O-β-D-p-glucopyranoside (4), daucosterol (5), phytol (6), dodecanoic acid (7), oleanolic acid (8), 3,3',4'-tri-O-methyl-ellagic acid (9), corosolic acid (10), β-sitosterol (11), peucedanin (12), buntansin (13), and erythrodiol (14). All compounds, except for 8 and 11, have been isolated from *L. salicaria* for the first time. Cytotoxic activities of the compounds were examined against three cancerous cell lines, colon carcinoma (HT-29), leukemia (K-562), and breast ductal carcinoma (T47D), and Swiss mouse embryo fibroblast (NIH-3T3) cells using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and methotrexate as positive control. Compounds 5, 10, 11, and 14 were the most active against the HT-29 cell line with IC\(_{50}\) values of 192.7, 36.8, 38.2, and 12.8 μg/mL, respectively. Compounds 14, 11, 5, and 10 were 6.4, 2.8, 2.6, and 1.4 times, respectively, more selective than methotrexate. Compound 5 was the most active against the K-562 cell line (IC\(_{50}\) = 50.2 μg/mL), with a selectivity exceeding that of methotrexate 13.3 times. The results of the cytotoxic assay confirmed that growth and proliferation of the cancer cell lines are predominantly influenced by triterpene derivatives and sterols of this plant.

**Key words:** *Lythrum salicaria*, Phytochemistry, Cytotoxic Effect

### Introduction

Cancer has become one of the most devastating diseases of humankind in the world (Ma *et al.*, 2009). Natural products or their related components constitute more than half of the drugs used in cancer therapy (Newman and Cragg, 2007). Discovery of efficacious anticancer agents has provided convincing evidence that plants could be a source of chemotherapeutic agents (Kostova, 2005).

*Lythrum salicaria* belongs to the plant family Lythraceae comprising 30 species, seven of which are found in Iran (Rechinger, 1968). The plant is known as “Turbinkwash” and “Surmankhal” in Persian (Soltani, 2011). Phytochemical investigations on this plant have revealed the presence of tannins, anthocyanins, phenolic aids, and flavon-C-glycosides (Rauha *et al.*, 2000, 2001; Zhou *et al.*, 2011). Additionally, biologically active compounds have been isolated from the plant such as oleanolic acid and ursolic acid as antifungal compounds and the hexahydroxydiphenoyl ester vescalagin as an antibacterial agent (Becker *et al.*, 2005). Antioxidant activity of various extracts of the plant has been tested, and polar extracts were found to be the most active ones (Coban *et al.*, 2000, 2001; Zhou *et al.*, 2011).
The plant extract decreases the blood glucose level in both hyperglycaemic and normoglycaemic rats and rabbits (Lamela et al., 1986; Torres and Suarez, 1980). Flowering aerial parts of the plant have been traditionally used for the treatment of diarrhea, chronic intestinal catarrh, haemorrhoids, eczema, varicose veins, bleeding of the gums, and vaginitis (Mantle et al., 2000; Rauha et al., 2000). Evaluation of the antidiarrheal effect of Salicairine®, a formulated medication, demonstrated that there was either a significant increase in the colon net fluid absorption or a decrease in the net fluid secretion (Brun et al., 1997).

We have previously analysed the extract and fractions of L. salicaria for cytotoxic activity against cancer cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Ethyl acetate and chloroform fractions of the plant exhibited cytotoxicity toward the T47D cancer cell line with IC_{50} values of 63.1 and 108.2 μg/mL, respectively (Khanavi et al., 2011). The objective of the present study was the isolation and characterization of major constituents of L. salicaria through chromatographic and spectroscopic methods, respectively. We also demonstrated cytotoxic effects of the purified compounds using the MTT assay against the three cancerous cell lines HT-29 (colon carcinoma), K-562 (leukemia), and T47D (breast ductal carcinoma), and the normal cell line NIH-3T3 (Swiss mouse embryo fibroblast).

Material and Methods

General

Column chromatography (CC) was carried out using silica gel (70–230 mesh; Merck, Darmstadt, Germany) and Sephadex LH-20 (Fluka, Buchs, Switzerland) as stationary phases. Pre-coated silica gel 60 F254 plates (Merck) were used for thin-layer chromatography (TLC). Spots on TLC plates were visualized under UV light (at 254 and 366 nm) and also after spraying the developed plates with anisaldehyde followed by heating for 5 min. All solvents used in this study were distilled prior to use. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker (Billerica, MA, USA) DRX 500 instrument (500 MHz for 1H NMR, 125 MHz for 13C NMR) with tetramethylsilane (TMS) as an internal standard. El mass spectra were recorded on an Agilent Technology instrument (New York, NY, USA) (HPTM) with a 5973 Network mass selective detector (MS model). UV spectra were measured on an Optizen (Daejeon, Korea) model 2021 UV plus, IR spectra on a Nicolet (New York, NY, USA) 550-A spectrometer using NaCl pellets, and melting points on a Büchi (Flawil, Switzerland) melting point B-545 apparatus.

Plant material

Aerial parts (stems, leaves, flowers) of L. salicaria were collected in May 2011 from Lahijan city, Guilan province (North of Iran) and deposited in the Central Herbarium of Medicinal Plants, Karaj, Iran (Ajani 313). They were cleaned and dried in the shade at room temperature.

Isolation of compounds

Aerial, shade-dried parts of the plant (1800 g) were crushed and extracted with 80% aqueous MeOH (3 × 48 h). The extracts were concentrated in a vacuum evaporator to give a crude extract (330 g), which was fractionated to three main fractions, i.e. chloroform, ethyl acetate, and methanol, yielding 1.17, 7.97, and 161.83 g, respectively.

The methanol fraction was subjected to column chromatography on silica gel using MeOH/CHCl_3 (2:8–10:0, v/v) as an eluent to yield eight fractions, M_1–M_8. Fraction M_3 (280 mg) was subjected to chromatography on a silica gel C_{18}-reverse phase (C_{18}-RP) column eluted with MeOH/H_2O (5:5–0:10), which yielded compound 1 (5 mg). Fraction M_4 (12 g) was subjected to silica gel column chromatography eluted with MeOH/CHCl_3 (1:19–0:20), which gave nine sub-fractions, M_{41}–M_{49}. On the basis of analytical TLC, sub-fraction M_{45} (761 mg) was chromatographed on a C_{18}-RP column, eluted with MeOH/H_2O (3:7–5:5), and then on a Sephadex LH-20 column using MeOH, yielding compound 2 (5.5 mg). Sub-fraction M_{46} (538 mg) was purified on a C_{18}-RP column eluted with MeOH/H_2O (3:7–10:0) to afford three new fractions, of which each for final purification was subjected to Sephadex LH-20 column chromatography using MeOH to produce compounds 3 (10 mg), 4 (1.4 mg), and 5 (10 mg).

For purification of the ethyl acetate fraction, it was applied to a silica gel column eluted with CHCl_3/CH_2OAc (7:3–0:10) and produced five fractions, E_1–E_5. Fraction E_1 (600 mg) was subjected to column chromatography over silica
gel eluted with n-hexane/EtOAc (9:1–0:10) and then with CHCl3/MeOH (10:0–2:8) to produce compounds 6 (4.2 mg) and 7 (12.4 mg). Fraction E2 (700 mg) was subjected to a silica gel column eluted with n-hexane/EtOAc (8:2–6:4) and re-chromatographed on a Sephadex LH-20 column eluted with CHCl3/MeOH (5:5) to produce compound 8 (73 mg). Fraction E3 (143 mg) was chromatographed on a silica gel column eluted with n-hexane/EtOAc (8:2–0:10) and then on a Sephadex LH-20 column using MeOH to produce compound 9 (4 mg). Fraction E4 (342 mg) was purified on a silica gel column eluted with CHCl3/EtOAc (7:3–0:10) to produce new sub-fractions. Sub-fraction E41 was chromatographed on a silica gel column eluted with CHCl3/MeOH (19:1–0:20) to yield compound 10 (12 mg).

Purification of the chloroform fraction was carried out using column chromatography on silica gel eluted with CHCl3/EtOAc (1:0–9:1), producing eight new fractions, C1–C8. Fraction C3 (800 mg) was re-chromatographed as above, using n-hexane/EtOAc (19:1–5:5), and produced new sub-fractions. Sub-fraction C34 was purified on a silica gel column eluted with n-hexane/EtOAc (9:1–7:3) to produce compounds 11 (8.1 mg), 12 (3.4 mg), and 13 (5 mg). Fraction C3 was subjected to column chromatography over silica gel eluted with n-hexane/EtOAc (8:2–0:10) and produced new sub-fractions. Sub-fraction C35 was subjected to a silica gel column eluted with n-hexane/EtOAc (19:1–5:5) and yielded compound 14 (8.2 mg).

5-Hydroxypropyrrolidin-2-one (1): 1H NMR (500 MHz, MeOD): δ = 5.5 (1H, dd, J5,6eq = 1.7 Hz, J5,6ax = 6.35 Hz, H-5), 2.5 (1H, m, J3ax,3ax = 17.15 Hz, J3ax,4ax = 9.15 Hz, J3ax,4eq = 8 Hz, H-3ax), 2.37 (1H, m, J4ax,4eq = 13.5 Hz, J4ax,4eq = 9.8 Hz, J4ax,3ax = 9.15 Hz, J4ax,5 = 6.35 Hz, H-4ax), 2.2 (1H, m, J2eq,3ax = 17.15 Hz, J2eq,4eq = 9.8 Hz, J2eq,4eq = 3.6 Hz, H-3eq), 1.90 (1H, m, J2eq,4ax = 13.5 Hz, J2eq,3eq = 8 Hz, J2eq,5 = 1.7 Hz, H-1). 13C NMR (125 MHz, MeOD): δ = 180.8 (C-2), 80.9 (C-5), 31.2 (C-4), 29.5 (C-3). – MS (ESI, 70 eV): m/z (%) = 101 (54) [M+], 84 (100), 83 (50), 81 (15), 77 (70), 55 (50), 53 (10).

Umbelliferone-6-carboxylic acid (2): M.p. 199.6–200.6 °C. – IR (NaCl): ν = 3420, 3296, 1743 cm⁻¹. – UV/Vis (MeOH): λmax (log e) = 250 (1.06), 305 (1.5, sh), 330 nm (2.32). – 1H NMR (500 MHz, DMSO-d6): δ = 8.3 (OH), 7.96 (1H, s, H-5), 7.92 (1H, d, J = 9.35 Hz, H-4), 6.45 (1H, s, H-8), 6.03 (1H, d, J = 9.35 Hz, H-3). – 13C NMR (125 MHz, DMSO-d6): δ = 169.86 (COOH), 169.58 (C-7), 160.74 (C-10), 157.2 (C-2), 157.2 (C-8a), 145.43 (CH, C-4), 130.47 (CH, C-5), 117.04 (C-4a), 109.06 (CH, C-3), 108.3 (C-6), 102.46 (CH, C-8). – MS (EI, 70 eV): m/z (%) = 206 (10) [M+], 111 (100), 110 (68), 109 (93), 83 (81).

3,3′,4′-Tri-O-methylallagic acid-4-O-β-D-(2-acetyl)-glucopyranoside (3): IR (NaCl): ν = 3394, 2925, 2854, 1739, 1456, 1252, 1032 cm⁻¹. – UV/Vis (MeOH): λmax (log e) = 237.5 (2.5, sh), 260 (2.68), 365 nm (0.88). – 1H NMR (500 MHz, DMSO-d6): δ = 7.86 (1H, s, H-5'), 7.65 (1H, s, H-5), 4.05 (3H, s, 3'-OMe), 4.01 (3H, s, 3-OMe), 3.97 (3H, s, 4'-OMe); sugar protons: 5.1 (1H, d, H-1'), 3.7 (1H, Ha-6'), 3.5 (1H, Hb-6'), 3.47 (1H, H-2'), 3.44 (1H, H-3'), 3.17 (1H, H-4'), 2.06 (s, 3H, 2'-COCH₃). – 13C NMR (125 MHz, DMSO-d6): δ = 158.35 (C-7), 158.12 (C-7'), 154.45 (C-4), 151.37 (C-4'), 141.47 (C-3'), 141.37 (C-2'), 140.9 (C-2), 140.8 (C-3), 114.16 (C-1'), 112.76 (C-1), 112.55 (C-6), 112.19 (C-6'), 112.19 (C-5'), 107.67 (C-5), 61.59 (3'-OMe), 61.39 (3'-OMe), 56.83 (4'-OMe); sugar carbon atoms: 169.47 (2′-COCH₃), 98.59 (C-1''), 77.38 (C-5''), 73.54 (C-3''), 73.46 (C-2''), 69.52 (C-4''), 60.33 (C-20''), 20.89 (2″-COCH₃).

3,3′,4′-Tri-O-methylallagic acid-4-O-β-D-glucopyranoside (4): UV/Vis (MeOH): λmax (log e) = 230 (2.5, sh), 267 (2.86), 350 nm (1.78). – 1H NMR (500 MHz, DMSO-d6): δ = 7.85 (1H, s, H-5'), 7.67 (1H, s, H-5), 4.1 (3H, s, 3'-OMe), 4.05 (3H, s, 3'-OMe), 4.02 (3H, s, 4'-OMe); sugar protons: 5.18 (1H, d, H-1'), 3.79 (1H, Ha-6'), 3.5 (1H, Hb-6'), 3.47 (1H, H-5'), 3.47 (1H, H-4'), 3.45 (1H, H-2'), 3.44 (1H, H-3'). – 13C NMR (125 MHz, DMSO-d6): δ = 158.23 (C-7), 157 (C-7'), 154 (C-4), 151.92 (C-4'), 141.2 (C-2'), 141 (C-2), 140.8 (C-3'), 140 (C-3), 113.73 (C-1'), 112.94 (C-6), 112.94 (C-11), 112.95 (C-6'), 108 (C-5), 61.72 (3'-OMe), 61.38 (3'-OMe), 56.82 (4'-OMe); sugar carbon atoms: 101 (C-1''), 77.29 (C-5''), 76.48 (C-3''), 73.34 (C-2''), 69.48 (C-4''), 60.53 (C-6').

Daucosterol (5): 1H NMR (500 MHz, DMSO-d6): δ = 5.3 (1H, bs, H-6), 1.2 (3H, s, H-19), 0.64 (3H, s, H-18); sugar protons: 4.2 (1H, bs, H-1'), 3.62 (1H, Ha-6'), 3.39 (1H, Hb-6'), 3.11 (1H, H-3'), 3.05 (1H, H-5'), 3 (1H, H-4'), 2.88 (1H, H-3'). – 13C NMR (125 MHz, DMSO-d6): δ = 140.32 (C-5), 121.26 (C-6), 76.95 (C-3), 56.22 (C-17), 55.46 (C-14), 49.63 (C-8), 45.17 (C-24), 41.89 (C-13), 40 (C-12), 39.83 (C-4), 38.33 (C-10), 36.25 (C-9), 35.53
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Corosolic acid (10): \(^{1}H\) NMR (500 MHz, pyridine-d$_{5}$): \(\delta = 5.44\) (1H, m, J = 3.5 Hz, H-12), 4.09 (1H, t, J = 9.4, 4.4 Hz, H-2), 3.39 (1H, d, J = 9.4 Hz, H-3), 2.61 (1H, d, J = 11.35 Hz, H-18), 1.26 (3H, s, H-27), 1.19 (3H, s, H-23), 1.03 (3H, s, H-25), 1 (3H, s, H-26), 0.97 (3H, d, J = 4.9 Hz, H-30), 0.96 (3H, s, H-24), 0.93 (3H, d, J = 5.5 Hz, H-29).

β-Sitosterol (11): \(^{1}H\) NMR (500 MHz, CDCl$_{3}$): \(\delta = 5.36\) (1H, bs, H-6), 3.5 (1H, m, J = 6.3, 4.5 Hz, H-3), 1.02 (3H, s, H-19), 0.93 (3H, d, J = 5.8 Hz, H-21), 0.84 (3H, t, J = 7.2 Hz, H-29), 0.83 (3H, d, J = 6.8 Hz, H-27), 0.81 (3H, d, J = 7.2 Hz, H-26), 0.69 (3H, s, H-18).

Peucedanin (12): \(^{1}H\) NMR (500 MHz, CDCl$_{3}$): \(\delta = 7.72\) (1H, d, J = 9 Hz, H-4), 7.5 (1H, s, H-5), 7.19 (1H, s, H-8), 6.30 (1H, d, J = 9 Hz, H-3), 3.87 (3H, s, 9-OMe), 3.18 (1H, sept, J = 7 Hz, H-1'), 1.28 (6H, J = 7 Hz, H-2', H-3').

Buntansin (13): \(^{1}H\) NMR (500 MHz, CDCl$_{3}$): \(\delta = 11.21\) (6-COOH, s), 8.04 (1H, s, H-5), 7.63 (1H, d, J = 9.5 Hz, H-4), 6.9 (1H, s, H-8), 6.30 (1H, d, J = 9.5 Hz, H-3), 3.99 (3H, s, 6-OMe).

3,3′,4′-Tri-O-methylellagic acid (9): UV/Vis (MeOH): \(\lambda_{max}\) (log \(\varepsilon\)) = 237.5 (2.6, sh), 267 (2.77), 372 (0.88), 402 nm (0.7, sh).

Dodecanoic acid (7): \(^{1}H\) NMR (500 MHz, pyridine-d$_{5}$): \(\delta = 2.5\) (2H, t, J = 7.35 Hz, H-2), 1.77 (2H, m, J = 7.35 Hz, H-3), 1.36 (2H, m, J = 4.4 Hz, H-18), 1.26 (3H, s, H-27), 1.22 (3H, s, H-23), 1 (3H, s, H-30), 1 (3H, s, H-24), 0.98 (3H, s, H-29), 0.92 (3H, s, H-25), 0.87 (3H, s, H-26).

Oleanolic acid (8): \(^{1}H\) NMR (500 MHz, pyridine-d$_{5}$): \(\delta = 5.47\) (1H, H-12), 3.42 (1H, dd, J = 10.3, 6.1 Hz, H-3), 3.29 (1H, dd, J = 13.8, 4.4 Hz, H-18), 1.26 (3H, s, H-27), 1.22 (3H, s, H-23), 1 (3H, s, H-30), 1 (3H, s, H-24), 0.98 (3H, s, H-29), 0.92 (3H, s, H-25), 0.87 (3H, s, H-26).

Phytol (6): \(^{1}H\) NMR (500 MHz, CDCl$_{3}$): \(\delta = 5.42\) (1H, d, J = 6.8, 1.3 Hz, H-2), 4.16 (2H, d, J = 6.8 Hz, H-1), 1.99 (2H, m, H-4), 1.67 (3H, bs, H-20).

\(^{13}C\) NMR (125 MHz, DMSO-d$_{6}$): \(\delta = 140.32\) (C, C-3), 123.06 (CH, C-2), 59.4 (CH$_{2}$, C-1), 39.58 (CH$_{3}$, C-4), 39.34 (CH$_{2}$, C-5), 37.4 (CH$_{3}$, C-9), 37.33 (CH$_{3}$, C-6), 37.26 (CH$_{2}$, C-8), 36.63 (CH$_{3}$, C-10), 32.78 (CH, C-11), 27.97 (CH, C-15), 25.12 (CH$_{3}$, C-12), 24.78 (CH$_{3}$, C-13), 24.77 (CH$_{3}$, C-19), 24.46 (CH$_{3}$, C-14), 24.44 (CH$_{3}$, C-20), 23.68 (CH, C-7), 22.6 (CH$_{3}$, C-18), 19.72 (CH$_{3}$, C-16), 16.15 (CH$_{3}$, C-17).

\(^{13}C\) NMR (500 MHz, pyridine-d$_{5}$): \(\delta = 180.16\) (C-28), 141.06 (C-2), 111.8 (C-5), 61.42 (3'-OMe), 56.79 (4'-OMe). The signals for the other carbon atoms could not be detected because of the small sample size.
Cytotoxicity assay

Cytotoxic effects of the compounds were assessed using the modified MTT assay (Rahman et al., 2008). Erythrodiol (14): 1H NMR (500 MHz, CDCl3): δ = 5.22 (1H, bs, H-12), 3.5 (1H, d, J = 11 Hz, H-28a), 3.22 (1H, d, J = 11 Hz, H-28b), 3.2 (1H, m, H-3), 1.17 (3H, s, H-27), 1 (3H, s, H-30), 0.94 (3H, s, H-29), 0.94 (3H, s, H-26), 0.89 (3H, s, H-25), 0.88 (3H, s, H-24), 0.79 (3H, s, H-23). – MS (EI, 70 eV): m/z (%): 221 (31) [M+ 1], 171 (53), 153 (92), 101 (38), 87 (53), 78 (100), 59 (69).

Erythrodiol (14): 1H NMR (500 MHz, CDCl3): δ = 5.22 (1H, bs, H-12), 3.5 (1H, d, J = 11 Hz, H-28a), 3.22 (1H, d, J = 11 Hz, H-28b), 3.2 (1H, m, H-3), 1.17 (3H, s, H-27), 1 (3H, s, H-30), 0.94 (3H, s, H-29), 0.94 (3H, s, H-26), 0.89 (3H, s, H-25), 0.88 (3H, s, H-24), 0.79 (3H, s, H-23). – MS (EI, 70 eV): m/z (%): 221 (31) [M+ 1], 171 (53), 153 (92), 101 (38), 87 (53), 78 (100), 59 (69).

Statistical analysis

IC50 values were calculated by sigma plot 10 software. Data representative of three independent experiments with similar results were presented as means ± standard deviation (SD).

Results and Discussion

Successive chromatographic separations of the methanol, ethyl acetate, and chloroform fractions of L. salicaria aerial parts yielded compounds 1–5, 6–10, and 11–14, respectively (Fig. 1). The structures of these compounds were determined on the basis of spectroscopic data compared with those reported in the literature. The compounds included three coumarins: umbelliferone-6-carboxylic acid (2) (Zhao et al., 2012), the furanocoumarin peucedanin (12) (Alavi et al., 2005), and dodecanoic acid (11) (Huang et al., 1989); three ellagic acid derivatives: 3,3’4’-tri-O-methyllellagic acid-4-O-β-D-(2”-acetyl)-glucopyranoside (3), 3,3’4’-tri-O-methyllellagic acid-4-O-β-D-glucopyranoside (4), and 3,3’4’-tri-O-methyllellagic acid (9) (Ndukwe et al., 2008; Li et al., 1999); three triterpenes: oleanolic acid (8), erythrodiol (14) (Mahato and Kundu, 1994), and the ursan-type triterpene corosolic acid (10) (Seo et al., 1975); two steroids: dau-costeryl (5) (Kim et al., 2006) and β-sitosterol (11) (Kim et al., 2005); together with 5-hydroxyppyrrolidin-2-one (1) (Chen et al., 2008), phytol (6) (Kim et al., 2005), and dodecanoic acid (7). Compounds 8 and 11 had been previously isolated from this plant but the others were found for the first time. To the best of our knowledge, coumarins and pyrrolidin derivatives have not previously been reported from this plant.

Three cancerous (HT-29, K-562, and T47D) and one normal (NIH-3T3) cell lines were exposed to various concentrations of the isolated compounds. In the MTT assay, 5, 10, 11, and 14 were the most active compounds against the HT-29 cell line with
IC₅₀ values of 192.7, 36.8, 38.2, and 12.8 μg/mL, respectively (Table I). Based on the SI values of these compounds against the HT-29 cancerous cell line, 14, 11, 5, and 10 were 6.4, 2.8, 2.6, and 1.4 times, respectively, more selective than the positive control, methotrexate.

Compound 5 was the most active against the K-562 cell line (IC₅₀ = 50.2 μg/mL) and was 13.3 times more selective than methotrexate. It was also active against the T47D cancerous cell line (IC₅₀ = 229.8 μg/mL) and was 1.5 times more selective than methotrexate (Table I). All other compounds were less cytotoxic to any of the tested cancer cell lines compared with the normal cell line (NIH-3T3).

Thus, erythrodiol (14), corosolic acid (10), and β-sitosterol (11) inhibited the HT-29 cell line most strongly, and of these, compound 14 affected the growth of the HT-29 cell line (colon carcinoma cells) with relatively high selectivity (SI 6.7). Our results corroborate an earlier finding holding erythrodiol (14) to be responsible for the cytotoxic effect of *Viscum coloratum* against HO-8910 and SMMC-7721 cells (Yang *et al*., 2009). Furthermore, its mechanism of action has revealed that it has antiproliferative and pro-apoptotic activity toward the HT-29 cell line (Juan *et al*., 2008). Corosolic acid (10) has also been found to have potent cytotoxic activity against several human cancer cell lines and to inhibit protein kinase C in a dose-dependent manner (Ahn *et al*., 1998). The major compound of *L. salicaria*, oleanolic acid (8), is a significant antitumour agent against the HCT15 cell line (Li *et al*., 2002). Finally, triterpene derivatives were found to inhibit the growth of HepG2 cells in a concentration-dependent manner (Ma *et al*., 2009). Antiproliferative and apoptosis-inducing activities of β-sitosterol (11) in human leuke-
mic U937 cells have been well documented (Park et al., 2007). In the present study daucosterol (5) showed a strong cytotoxicity against the K-562 cells with greatest tumour-specific cytotoxicity (SI 10.5). A cytotoxic effect of daucosterol at >200 μg/mL has been reported previously against the human gastric cancer cell lines AGS and SNU638 (Jeong et al., 2009). Moreover, this result suggested that compound 5, which possesses a sugar moiety, acts more selectively on the K-562, T47D, and HT-29 cell lines compared to its aglycone β-sitosterol (11). It seems that the sugar moiety is a determining factor for the higher selectivity and cytotoxicity of 5 toward the K-562 and T47D cell lines. An interesting phenomenon has been observed with 5-hydroxypyrrrolidin-2-one (1), i.e. a relatively weaker cytotoxicity to NIH-3T3 (normal cell line) as compared to the HT-29 and T47D cell lines.

Coumarin derivatives have been reported as cytotoxic agents (Kostova, 2005; Kawase et al., 2005), although the coumarins we isolated in our study had no significant cytotoxicity. However, peucedanin (12), with a furan ring, was more inhibitory to T47D than buntansin (13). A literature review revealed that some furanocoumarins exhibit a marked inhibitory effect on tumour cell lines (Kostova, 2005). The potential cytotoxic and antiproliferative activities of ellagic acid have been verified in human cancer cell lines. Prevention of tumour growth was attributed to the ability of ellagic acid to induce apoptosis through the intrinsic mitochondrial pathway (Sepulveda et al., 2011). Nevertheless, in our hands ellagic acid derivatives were not active against the cancerous cell lines (IC_{50} > 150 μg/mL), which is comparable to the inactivity of two ellagic acid derivatives from Turpinia formosana against MCF-7, Daoy, WiDr, and Hep2 cancer cell lines (Huang et al., 2012).

Based on the SI values, some of the compounds were more selective than the positive control, methotrexate. Triterpenes and sterols appear to be responsible for the anticancer activity of the nonpolar extracts. Nonpolar fractions of the plant had shown cytotoxicity against the T47D cancer cell line in our previous study (Khanavi et al., 2011). According to our results, polar extracts of the plant, which are used in traditional medicine, are not toxic, while potent cytotoxic metabolites are present in the ethyl acetate and chloroform fractions.

In conclusion, the L. salicaria triterpene derivatives and sterols, respectively, 10, 11, and 14, due to their low IC_{50} values against the HT-29 cell line, and compound 5 against K-562 cells, respectively, could protect against colon cancer development and leukemia.

Acknowledgement

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Table I. Cytotoxic activities of the L. salicaria compounds against three cancerous cell lines and the normal NIH-3T3 cell line determined by the MTT assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>NIH-3T3 IC_{50} [μg/mL]</th>
<th>T47D SI^b IC_{50} [μg/mL]</th>
<th>K-562 SI^b IC_{50} [μg/mL]</th>
<th>HT-29 SI^b IC_{50} [μg/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methotrexate</td>
<td>0.24 ± 0.013</td>
<td>1.5</td>
<td>0.16 ± 0.09</td>
<td>0.75</td>
</tr>
<tr>
<td>1</td>
<td>&gt; 1000</td>
<td>–</td>
<td>482.1 ± 7.98</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>–</td>
<td>551.7 ± 5.8</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>711.7 ± 34.1</td>
<td>1.5</td>
<td>473.2 ± 9.12</td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td>530.4 ± 6.7</td>
<td>2.3</td>
<td>229.7 ± 8.8</td>
<td>10.5</td>
</tr>
<tr>
<td>9</td>
<td>420.5 ± 10.7</td>
<td>1.1</td>
<td>374.5 ± 16.55</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>54.4 ± 9.0</td>
<td>0.4</td>
<td>116.2 ± 14.27</td>
<td>0.2</td>
</tr>
<tr>
<td>11</td>
<td>109.5 ± 6.9</td>
<td>0.3</td>
<td>359.4 ± 4.7</td>
<td>0.2</td>
</tr>
<tr>
<td>12</td>
<td>42.3 ± 15.2</td>
<td>0.5</td>
<td>85.0 ± 7.07</td>
<td>0.3</td>
</tr>
<tr>
<td>13</td>
<td>50.4 ± 8.7</td>
<td>–</td>
<td>&gt; 1000</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td>86.6 ± 1.2</td>
<td>0.5</td>
<td>164.9 ± 4.9</td>
<td>–</td>
</tr>
</tbody>
</table>

^a IC_{50}, concentration causing 50% growth inhibition ± standard deviation; it was calculated based on different concentrations (1.9, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500, and 1000 μg/mL).

^b SI (selectivity index) = IC_{50} for normal cell line/IC_{50} for cancerous cell line.

^c Not determined.


