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ORIGINAL ARTICLE

Flavonoid constituents and cytotoxic activity of *Erucaria hispanica* (L.) Druce growing wild in Egypt

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Abstract Thirteen flavonoid compounds were isolated for the first time from the aerial parts of *Erucaria hispanica* (L.) Druce growing in Egypt. Their structures were established on the basis of detailed chromatographic and spectroscopic techniques (UV, 1D and 2D NMR and ESIMS). The cytotoxic activity of the methanol extract as well as some isolated compounds against four human carcinoma cell lines; breast (MCF7), liver (HEPG2), cervix (HELA) and colon (HCT116) were evaluated.

1. Introduction

Brassicaceae is one of the largest angiosperm families with approximately 338 genera and more than 3709 species distributed worldwide (Al-Shehbaz et al., 2006). In the flora of Egypt, Brassicaceae is the fourth of eleven large families, widely distributed in all phytogeographic regions (Boulos, 1995). It includes economically important ornamentals, crops, and vegetables, as well as it is a source of cooking oils and forage (Judd et al., 1999). The plants of this family are used in the treatment of several diseases because of their anticancer, antibacterial, antifungal, antirheumatic, and antidiabetic properties (Kirtikar and Basu, 1975). The genus *Erucaria* is represented by four species that grow in Egypt; *Erucaria crassifolia*, *Erucaria microcarpa*, *Erucaria pinnata* and *Erucaria hispanica* (Täckholm, 1974; Boulos, 1999). Four flavonoid compounds: 3-*O*-galactopyranoside of quercetin and isorhamnetin, lucenin-1 (luteolin 6,8-di-*C*-β-glucopyranoside) and robinetin (3,7,3′,4′,5′-penta hydroxy flavone) were previously isolated from the aerial parts of *E. microcarpa* (Hashem, 2007). The flavonoid constituents and cytotoxic activity of *E. hispanica* have not yet been reported.

2. Experimental

2.1. General

1D and 2D NMR experiments (^{1}H, ^{13}C, HMQC and HMBC) were recorded on a Jeol EX-500 spectrometer: 500 MHz (^{1}H NMR), 125 MHz (^{13}C NMR). UV spectrophotometer (Shimadzu model 2401 PC), EIMS: Finnigan-Mat SSQ 7000 spectrometer, ESIMS: LCQ Advantage Thermo Finnigan

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spectrometer. CC Polyamide 6S (Riedel-De-Haen AG, Seelze Haen AG, Seelze Hanver, Germany) using MeOH/H_2O as eluent. CC Silica gel 60 (Merck, 0.063–0.2 mm) using CH_2Cl_2/MeOH (2:3). PC (descending) Whatman No. 1 and 3 MM papers, using solvent systems (1) H_2O, (2) 15% HOAc (H_2O–HOAc 85:15), (3) CAW ($CHCl_3$–HOAc–H_2O 90:45:6), (4) BAW (*n*-BuOH–HOAc–H_2O 4:1:5, upper layer), (5) BBPW (C_6H_6–*n*-BuOH–pyridine-H_2O 1:5:3:3, upper layer). Solvents 4 and 5 were used for sugar analysis, Sephadex LH-20 (Pharmazia). Authentic samples were obtained from the department of phytochemistry and plant systematics, NRC. Complete acid hydrolysis for *O*-glycosides (2 N HCl, 2 h, 100 °C) were carried out and followed by paper co-chromatography with authentic samples to identify the aglycones and sugar moieties. The sugar units of *C*-glycoside flavonoids were determined using ferric chloride degradation.

2.2. Plant material

Fresh samples of *E. hispanica* (L.) Druce were collected on the Alexandria–Cairo desert road near Bremely's cave (Egypt) in April 2008. The sample was collected and identified by Prof. Dr. S.A. Kawashty and Dr. M.M. Marzouk. A voucher specimen (No. 782) has been deposited in the herbarium of the National Research Centre (CAIRC).

2.3. Extraction and isolation

Air-dried, ground, aerial parts of *E. hispanica* (L.) Druce (750 g) were defatted with light petroleum ether (40–60 °C) and extracted three times at room temperature with 70% methanol/water. The methanol extract was evaporated under reduced pressure and temperature affording 85 g residue, then subjected to a polyamide column (100 × 5 cm) starting with water as eluent then decreasing the polarity by increasing the concentration of methanol. A total of 48 fractions were collected, each of about 500 mL. Similar fractions were combined according to their PC properties using H_2O, 15% HOAc, CAW and BAW as eluents to give six main fractions (A-F). Fraction A (20% MeOH/H_2O) was applied to a Silica gel column (35 × 2.5 cm) using CH_2Cl_2/MeOH (2:3). Fractionation gave rise to two major fractions. They were applied to PC using CAW then purified on a Sephadex LH-20 column (30 × 1.5 cm) using methanol yielded compounds **1** (16 mg), **2** (14 mg) and **3** (24 mg). Fraction B was rechromatographed on polyamide column (35 × 2.5 cm) using 50% H_2O yielded four major fractions. They were purified on a Sephadex LH-20 column using MeOH/H_2O (1:1) and repeated by using methanol yielded compounds **4** (23 mg), **5** (18 mg), **6** (12 mg), **7** (22 mg) and **8** (13.5 mg). Fraction C (60% MeOH/H_2O) yielded compound **9** (10 mg) by separation on PC using 15% HOAc followed by BAW. Fractions D and E (60–80% MeOH/H_2O) were chromatographed on PC using BAW to yield compounds **10** (12 mg) and **11** (6 mg). Fraction F (100% MeOH) was applied to PC using 15% HOAc followed by H_2O double solvent yielded compounds **12** (8 mg) and **13** (10 mg).

2.3.1. Kaempferol-3-O-β-sophoroside-7-O-β-glucopyranoside (*1*)

Yellow amorphous powder, mp 223–225 °C, R_f 0.22 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 265, 344 (+NaOMe), 266, 389, (+$AlCl_3$) 275, 299, 344, 394; (+$AlCl_3$ HCl) 275, 300, 344, 393; (+NaOAc) 265, 380; (NaOAc/H_3BO_3) 265, 345. ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm, *J*/Hz: 7.8 (2H, d, *J* = 9.0, H-2′,6′); 6.95 (2H, d, *J* = 9.0, H-3′,5′); 6.73 (1H, d, *J* = 2.0, H-8); 6.4 (1H, d, *J* = 2.0, H-6); 5.65 (1H, d, *J* = 7.2, H-1″); 5.07 (1H, d, *J* = 7.2, H-1‴), 4.46 (1H, d, *J* = 7.0, H-1⁗); 3–4 (m, sugar protons overlapped with -OH proton signals). ^{13}C NMR 125 MHz, DMSO-d_6, ppm, 177.5 (C-4), 164 (C-7), 161.4 (C-5), 159.7 (C-4′), 158.2 (C-2), 156.8 (C-9), 133.1 (C-3), 130.6 (C-2′), 130.6 (C-6′), 121.1 (C-1′), 116.2 (C-3′), 116.2 (C-5′), 105.2 (C-10), 104.1 (C-1⁗), 99.6 (C-1‴), 98.8 (C-6), 98.7 (C-1″), 94.5 (C-8), 82.5 (C-2″), 77.4 (C-5″), 77.1 (C-3⁗), 76.9 (C-5⁗), 76.6 (C-3″), 76.2 (C-3‴), 74.2 (C-2⁗), 73.9 (C-5‴), 73.1 (C-2‴), 70.1 (C-4‴), 69.7 (C-4⁗), 69.6 (C-4″), 60.9 (C-6‴), 60.8 (C-6⁗), 60.5 (C-6″). Negative ESIMS; *m*/*z* 771.2 $[M-H]^-$.

2.3.2. Kaempferol-3-O-β-sophoroside-7-O-β-2‴ feruloyl glucopyranoside (*2*)

Pale yellow amorphous powder, mp 187–190 °C, R_f 0.29 (BAW). UV spectral data, λ_{max} (nm): (MeOH) 267, 346, (+NaOMe) 268, 394, (+$AlCl_3$) 273, 302, 348, 399, (+$AlCl_3$/HCl) 274, 300, 345, 398, (+NaOAc) 266, 350; (+NaOAc/H_3BO_3) 264, 314, 348. ^{1}H NMR 500 MHz, DMSO-d_6, δ, ppm, *J*/Hz: 7.94 (2H, d, *J* = 8.5, H-2′,6′); 7.36 (1H, d, *J* = 15.6, H-7⁗); 7.12 (1H, d, *J* = 1.8, H-2⁗); 6.96 (1H, dd, *J* = 8.1, 1.8, H-6⁗); 6.89 (2H, d, *J* = 8.5, H-3′,5′); 6.7 (1H, d, *J* = 2.0, H-8); 6.66 (1H, d, *J* = 8.2, H-5⁗); 6.36 (1H, d, *J* = 2.0, H-6); 6.28 (1H, d, *J* = 15.8, H-8⁗); 5.65 (1H, d, *J* = 7.5, H-1″); 5.17 (1H, d, *J* = 7.2, H-1‴), 4.54 (1H, d, *J* = 7.0, H-1⁗); 4.12 (1H, t, H-2‴); 3.74 (3H, s, OCH_3); 3–4 (m, sugar protons overlapped with -OH proton signals). ^{13}C NMR 125 MHz, DMSO-d_6, ppm, 177.8 (C-4), 167.3 (C-9⁗), 163.4 (C-7), 162.3 (C-5), 161.8 (C-4′), 158.2 (C-2), 156.8 (C-9), 150.3 (C-4⁗), 147.8 (C-3⁗), 145.6 (C-8⁗), 133 (C-3), 131 (C-2′), 131 (C-6′), 126.2 (C-1⁗), 123.5 (C-6⁗), 120.2 (C-1′), 115.9 (C-5⁗), 116.3 (C-3′), 116.3 (C-5′), 115.1 (C-7⁗), 111.4 (C-2⁗), 104.6 (C-10), 103.9 (C-1⁗), 99.1 (C-1‴), 98.8 (C-1″), 98.7 (C-6), 94.5 (C-8), 82.2 (C-2″), 78.4 (C-2‴), 77.4 (C-5″), 77.2 (C-5‴), 76.9 (C-5⁗), 76.8 (C-3‴), 76.6 (C-3⁗), 76.3 (C-3″), 73.3 (C-2⁗), 70.2 (C-4‴), 69.9 (C-4″), 69.7 (C-4⁗), 60.9 (C-6‴), 60.8 (C-6⁗), 60.5 (C-6″), 56.8 (OCH_3). Negative ESIMS; *m*/*z* 947 $[M-H]^-$.

2.3.3. Kaempferol-3,7-di-O-α-rhamnopyranoside (*3*)

Whitish yellow needles, mp 201–205 °C. R_f 0.62 (BAW). Negative ESIMS; *m*/*z* 577.2 $[M-H]^-$.

2.3.4. Luteolin 6,8-di-C-β-glucopyranoside [Lucenin 1] (*4*)

Yellow amorphous powder, mp 182–184°C. R_f 0.31 (BAW). Negative ESIMS; *m*/*z* 609 $[M-H]^-$.

2.3.5. Kaempferol-3-O-β-glucopyranoside-7-O-α-rhamnopyranoside (*5*)

Yellow crystals, mp 186–188 °C. R_f 0.46 (BAW). Negative ESIMS; *m*/*z* 593.3 $[M-H]^-$.

2.3.6. Isorhamentin-3-O-β-glucopyranoside-7-O-α-rhamnopyranoside (*6*)

Yellow crystals, mp 202–204 °C. R_f 0.52 (BAW). Positive ESIMS; *m*/*z* 625.13 $[M+H]^+$.

2.3.7. Luteolin 8-C-β-glucoside [Orientin] (*7*)

Yellow amorphous powder, mp 176–178 °C. R_f 0.31 (BAW). Negative ESIMS; *m*/*z* 447 $[M-H]$.

*2.3.8. Apigenin 8-C-β-glucopyranoside [Vitexin] (**8**)*
Yellow crystals, mp 280–286 °C, R_f 0.39 (BAW). Negative ESIMS; *m/z* 431.17 $[M-H]^-$.

*2.3.9. Quercetin 7-O-β-glucopyranoside (**9**)*
Yellow crystals, mp 277–279 °C, R_f 0.32 (BAW). Negative ESIMS; *m/z* 463 $[M-H]^-$.

*2.3.10. Agathisflavone 7,7″,4′,4‴-tetra methyl ether (**10**)*
Yellow powder, mp 264–268 °C, R_f 0.79 (BAW). Negative ESIMS; *m/z* 595 $[M-H]^-$.

*2.3.11. Isorhamnetin (**11**)*
Pale yellow crystals, mp 246–248 °C, R_f 0.77 (BAW). EIMS; *m/z* 316 $[M^+]$.

*2.3.12. 3,7,4′-Trihydroxyflavone (**12**)*
Pale yellow amorphous powder, mp 203–205 °C, R_f 0.79 (BAW). EIMS; *m/z* 270 $[M^+]$.

*2.3.13. Apigenin (5,7,4′-trihydroxy-flavone) (**13**)*
Yellow crystals, mp 282–286 °C, R_f 0.72 (BAW). EIMS; *m/z* 270 $[M^+]$.

2.4. Cytotoxic activity

Potential cytotoxicity of the aqueous methanol extract of *E. hispanica* (L.) Druce as well as some pure compounds were tested using the method of Skehan et al. (1990). Cells were plated in a 96-multi well plate (104 cells/well) for 24 h before treatment with the extract to allow attachment of cells to the wall of the plate. Different concentrations of the extracts (0, 10, 25, 50 and 100 μg/mL) were added to the cell monolayer in triplicate. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with sulforhodamine B. Excess stain was washed with acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to obtain the survival curve of each tumor cell line as compared with Doxorubicin, the control anticancer drug.

3. Results and discussion

3.1. Identification of flavonoid compounds

The methanol extract of the aerial parts of *E. hispanica* was purified through chromatographic methods (Mabry et al., 1970; Markham, 1982) yielding 13 flavonoid compounds (Fig. 1). Their structures were established on the basis of detailed chemical and spectroscopic techniques (Markham, 1982; Mabry et al., 1982; Agrawal and Bansal, 1989; Markham and Geiger, 1994). Spectral data of the isolated flavonoids, were in a good accordance with those previously published ones (Nielsen et al., 1993; Ofman et al., 1995; Yuldashev and Karimov, 2001; Marzouk et al., 2009; Kim et al., 2010).

The structures of compounds **1** and **2** were elucidated and fully characterized by 1D and 2D NMR (HMQC and HMBC). On the other hand, compound **10** was reported for the first time in Brassicaceae.

Compound **1**: Kaempferol-3-*O*-β-sophoroside-7-*O*-β-glucopyranoside, was isolated as a yellow amorphous powder. UV spectral data with diagnostic shift reagents suggested the presence of a 3,7-disubstituted flavonol glycoside with free hydroxyl groups at the 5 and 4′ positions (Mabry et al., 1970; Markham, 1982). Two intermediate spots were detected upon mild acid hydrolysis with 0.1 N HCl, before yielding the aglycone. Complete acid hydrolysis (2 N HCl, 2 h, 100 °C) yielded glucose (Co-PC) and kaempferol (3,5,7,4′-tetrahydroxyflavone) (Co-PC, UV, EIMS and ^{1}H NMR). The negative-ion ESIMS showed a molecular ion peak $[M-H]^-$at *m/z* 771.2, corresponding to a molecular formula $C_{33}H_{40}O_{21}$. The ^{1}H NMR spectrum showed two pairs of doublets at δ 7.8 (J = 9.0) and δ 6.95 (J = 9.0) assigned to H 2′,6′ and H 3′,5′, respectively. The two meta coupled doublets at δ 6.7 (J = 2.0) and δ 6.4 (J = 2.0) are assigned to H-8 and H-6, respectively. This downfield chemical shift confirmed that C-7 is substituted in ring A (Mabry et al., 1970; Markham and Geiger, 1994]. The ^{1}H NMR spectrum also revealed three distinct anomeric proton resonances at δ 5.65 (J = 7.2 Hz), δ 5.07 (J = 7.2 Hz), and δ 4.46 (J = 7 Hz), attributed to H-1″, H-1‴ and H-1⁗ of three β-glucopyranose units (Markham and Geiger, 1994). The ^{13}C NMR spectrum displayed 33 carbon resonances; 15 of which were assigned to kaempferol as the aglycone moiety and 18 to three glucose moieties. The HMQC experiment showed the three anomeric protons of glucose moieties at δ 5.65, 5.07 and 4.46 were correlated with δ 98.7, 99.6 and 104.2, respectively. In the HMBC spectrum the anomeric proton of one glucopyranosyl unit (H-1″, δ 5.65) showed a correlation with C-3 (δ 134), and the second glucopyranosyl unit (H-1‴, δ 5.07) with C-7 (δ 162.4). The interglycosidic linkage at position 3 of the aglycone between two glucose moieties was found to be (1 → 2) as the signal at δ 82.5 is characteristic for C-2 in a 2-substituted glucose unit (Agrawal and Bansal, 1989; Mabry et al., 1982).

Chemical and spectral analysis of compound **2** (kaempferol-3-*O*-β-sophoroside-7-*O*-β-2‴ feruloyl glucopyranoside) showed consistence with the presence of compound **1** and ferulic acid. The negative-ion ESIMS showed a molecular ion peak $[M-H]^-$at *m/z* 947. In ^{1}H NMR spectrum, the anomeric proton of the glucose moiety at position 7 appeared at down field chemical shift (δ 5.17, d, J = 7.2 Hz) compared to that of compound 1, suggested that the 7-glucose moiety should be substituted at position 2 (Markham and Geiger, 1994), supported by the down filed shift of C-2‴ at 79.9 (Agrawal and Bansal, 1989; Mabry et al., 1982).

3.2. Cytotoxic activity

The cytotoxic activity of the methanol extract against Breast (MCF7), liver (HEPG2), cervix (HELA) and Colon (HCT116) human tumor carcinoma cell lines showed a moderate activity with IC_{50} values of 18, 20.8, 14.7 and 21.4 μg/mL, respectively (Fig. 2), while the pure isolated compounds; **4**, **7**, **9** and **10** showed an activity against cervix (HELA) cell line with IC_{50} values of 20.7, 15.6, 16.4 and 20.3 μg/mL, respectively (Fig. 3) and no activity evaluated against the remaining three cell lines.

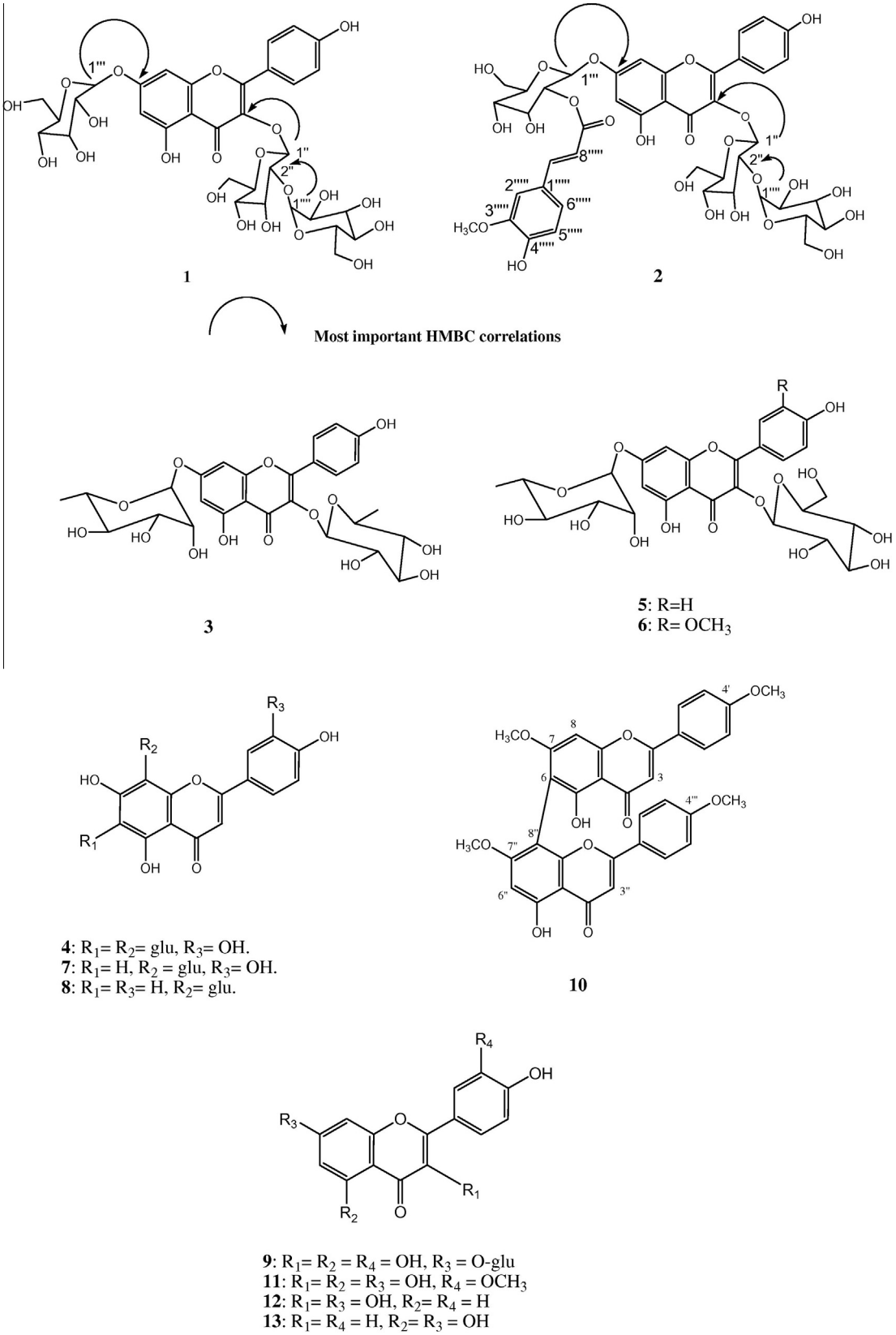

Figure 1 Chemical structures of flavonoid compounds (**1**–**13**) isolated from *Erucaria hispanica* (L.) Druce.

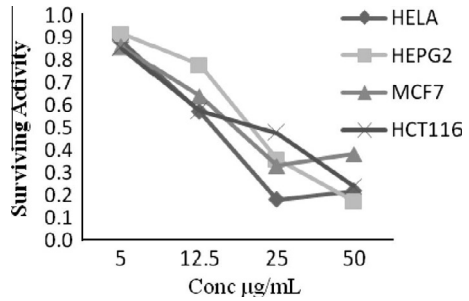

Figure 2 Cytotoxic activity of the methanol extract of *Erucaria hispanica* (L.) Druce against four human carcinoma cell lines: Cervix (HELA), liver (HEPG2), breast (MCF7) and colon (HCT116).

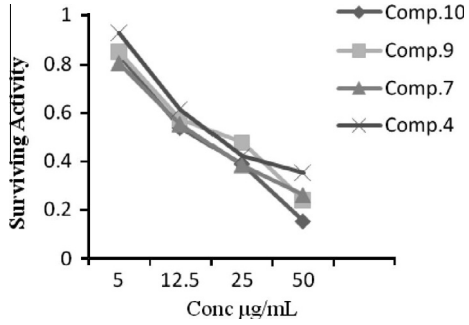

Figure 3 Cytotoxic activity of compounds **4**, **7**, **9** and **10** against Cervix carcinoma cells line (HELA).

4. Conclusion

This is the first report of the isolation of these flavonoid compounds from *E. hispanica* (L.) Druce and its cytotoxic activity evaluation. It is worthy to notice that *E. hispanica* is the first species of the family Brassicaceae to occurrence of biflavonoid.

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References

Agrawal, P.K., Bansal, M.C., 1989. Flavonoid glycosides. In: Agrawal, P.K. (Ed.), Carbon-13 NMR of Flavonoids. Elsevier, New York, pp. 283–364.

Al-Shehbaz, I.A., Beilstein, M.A., Kellogg, E.A., 2006. Systematics and phylogeny of the Brassicaceae (Cruciferae): an overview. Plant Syst. Evol. 259, 89–120.

Boulos, L., 1995. Flora of Egypt Checklist. Al-Hadara Publishing, Cairo, Egypt, pp. 38–48.

Boulos, L., 1999. Flora of Egypt, (Azollaceae–Oxalidaceae), vol. 1. Al Hadara Publishing, Cairo, Egypt.

Hashem, F.A., 2007. Phenolic compounds of *Erucaria microcarpa* Bioss and their effect as scavengers for singlet oxygen. J. Herb. Spic. Med. Plants 12 (3), 27–41.

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F., 1999. In: Plant Systematics. A Phylogenetic Approach. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

Kim, J.P., Lee, I.S., Seo, J.J., Jung, M.Y., Kim, Y.H., Yim, N.H., Bae, K.H., 2010. Vitexin, orientin and other flavonoids from *Spirodela polyrhiza* inhibit adipogenesis in 3T3-L1 cells. Phytother. Res. 24 (10), 1543–1548.

Kirtikar, R.L., Basu, L., 1975. Indian Medicinal Plants, vol. 1, second ed. Bishen Singh Mahendra Pal Singh, Dehra Dun, India.

Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer, Heidelberg.

Mabry, T.J., Markham, K.R., Chari, V.M., 1982. Carbon-13 NMR spectroscopy of the flavonoids. In: Harborne, J.B., Mabry, T.J. (Eds.), The Flavonoids Advances in Research. Chapman and Hall, London and New York, pp. 866–915.

Markham, K.R., 1982. Techniques of Flavonoid Identification. Academic Press, London.

Markham, K.R., Geiger, H., 1994. ^{1}H NMR spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In: Harborne, J.B. (Ed.), The Flavonoids Advances in Research Since 1986. Chapman and Hall, London, pp. 441–497.

Marzouk, M.M., Kawashty, S.A., Saleh, N.A.M., Al-Nowaihi, A.S.M., 2009. A new kaempferol trioside from *Farsetia aegyptia*. Chem. Nat. Compd. 45, 483–485.

Nielsen, J.K., Olsen, C.E., Petersen, M.K., 1993. Acylated flavonol glycosides in cabbage leaves. Phytochemistry 34 (2), 539–544.

Ofman, D.J., Markham, K.R., Vilain, C., Molloy, B.P.J., 1995. Flavonoid profiles of New Zealand Kauri and other species of *Agathis*. Phytochemistry 38 (5), 1223–1228.

Skehan, P., Storeng, R., Scudiero, D.A., Monks, A., McMahon, J., Vistica, D., Warren, J.T., Bokesch, H., Kenney, S., Boyd, M.R., 1990. New colorimetric cytotoxicity assay for anticancer-drug screening. J. Natl. Cancer Inst. 82, 1107–1112.

Täckholm, V., 1974. Student's Flora of Egypt. Cairo University, Cairo.

Yuldashev, M.P., Karimov, A., 2001. Flavonoids of *Scutellaria ocellata* and *S. nepetoides*. Chem. Nat. Compd. 37 (5), 431–433.