Anticancer potential of furanocoumarins and flavonoids of Heracleum persicum fruit

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Abstract

Heracleum persicum Desf. ex Fischer (Umbelliferae) is a herbaceous perennial plant distributed in Iran and Turkey. The aromatic fruits of this plant are commonly used as food additive, carminative, antiseptic and tonic. The present study was designed to isolate non-volatile constituents of *H. persicum* fruits and evaluate their anti-tumor potentials against different cancer cells. Phytochemical analysis using chromatography on silica gel and Sephadex LH-20 columns resulted in the isolation of phellopterin (**1**), angelicin (**2**), pimpinellin (**3**), bergapten (**4**), isopimpinellin (**5**) and xanthotoxin (**6**) from dichloromethane fraction along with apterin (**7**), isorhamnetin 3-O-glucoside (**8**), isorhamnetin 3-O-rutinoside (narcissin) (**9**), and quercetin 3-O-rutinoside (rutin) from *n*-butanol fraction of *H. persicum* fruits extract. ^{1}H-NMR and ^{13}C-NMR spectral analyses were applied to characterize the chemical structures. In MTT assay all of the tested compounds demonstrated preferential cytotoxic activity against cancer cells (IC_{50}; 40–370 µg/mL) in comparison with HUVEC normal cells (IC_{50}; > 1000 µg/mL). Among the compounds phellopterin (**1**) showed the highest anti-tumor activity toward U-266, SK-MM-1 and RPMI-8226 multiple myeloma cells with IC_{50} values of 44.3 ± 1.4, 69.1 ± 1.2 and 85.7 ± 1.8 µg/mL, respectively.

Introduction

Heracleum persicum Desf. ex Fischer from Umbelliferae family is one of ten member of the genuse *Heracleum* L. represented in flora of Iran [1]. This species grows as a perennial plant with up to 1.5 m height, large compound basal leaves bearing leaflets pinnately divided serrate dentate lobes, white flowers arranged in large compound umbels composed by 30–50 unequal rays and ovate-oblong fruits characterized by 4 and 2 clavate vittae on dorsal and commissural sides of mericarps, respectively [1]. *H. persicum* is known as "Golpar" in Iran and its aromatic fruits are commonly used as spice to flavor pickles, dairies and local foods [2]. In Iranian traditional medicine the fruits *H. persicum* have been mentioned useful as carminative, digestive, tonic, aphrodisiac, anthelmintic, analgesic, emmenagogue and for the treatment of memory impairment [2, 3]. Previous studies have shown antioxidant [4], antimicrobial [5, 6], antileishmanial [7], insecticidal [8], anti-inflammatory [9], analgesic [9], antidiabetic [10], antihyperlipidemic [11], hepatoprotective [12], anticonvulsant [13] and immunomodulatory [14] activity of different extracts of *H. persicum.*

There are also some reports on cytotoxic potentials of *H. persicum* fruits [15–20]. In 2009, Moshafi *et al*. reported the considerable general toxicity for the essential oil (IC_{50}: 0.007 µg/mL), petroleum ether extract (IC_{50}: 38.4 µg/mL), chloroform extract (IC_{50}: 33.8 µg/mL) and methanol extract (IC_{50}: 103.5 µg/mL) of the fruits of *H. persicum* in brine shrimp lethality test [16]. Bagheri *et al*. showed hydroalcoholic extract of *H. persicum* fruits decreased cell viability of MCF7 breast cancer cells (IC_{50}: 117.3 µg/mL), with higher selectivity as compared to HU02 normal fibroblast cell line (IC_{50}: 507.1 µg/mL) [17]. In another study, the ethanolic extract of *H. persicum* fruits was found to possess potent cytotoxicity against human lymphocytes by 60 to 100% reduction in cell viability, at concentrations ranging 0.25-10 mg/mL [18]. Despite of these interesting findings, there is not comprehensive knowledge regarding about the anti-

tumor activity of phytochemical ingredients of *H. persicum* fruits. Hence, the present study was designed to isolate non-volatile constituents of *H. persicum* fruit extract from its both non-polar and polar fractions and evaluate their anti-tumor activity against some selected cancer cell lines.

Experimental

Plant material

The dried fruits of *Heracleum persicum* were purchased from a herbal market at Tehran Grand Bazaar (Tehran, Iran) in September 2020. The botanical identity of the fruits was authenticated and a sample was deposited in the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

Extraction and fractionation

The grinded fruits (500 g) were macerated by 70% methanol in water (4×5 L) to get a total hydroalcoholic extract. The dried extract (114 g) was dispersed in water (1 L) and fractionated successively with dichloromethane and *n*-butanol (each 3×4 L). The obtained main fractions were concentrated using a rotary evaporator at 45°C under the reduced pressure and then dried in a vacuum oven at 45°C.

Isolation and purification of the compounds

The dichloromethane fraction (44 g) was moved on a normal phase silica gel column (mesh 230–400, Merck) (5×50 cm) and eluted using a gradient mixture of *n*-hexane:EtOAc (100:0 to 50:50) to get twenty six subfractions (D1-26). Silica gel column chromatography (mesh 230–400, 2×40 cm) of subfraction D9 (126 mg) with *n*-hexane:EtOAc (100:0 to 80:20) resulted in the isolation of compound **1** (21 mg). Subfractions 10 (0.3 g), 12 (2.7 g), 16 (1.4 g), 20 (0.8 g) and 24 (0.7 g) were individually recrystallized in ethanol to get compounds **2** (16 mg), **3** (165 mg), **4** (85 mg), **5** (43 mg) and **6** (57 mg), respectively.

A portion of *n*-butanol fraction (3 g) was chromatographed on a sephadex LH-20 column (GE Healthcare) (3×50 cm) using methanol to obtain fourteen subfractions (B1-14). Compound **7** (38 mg) was isolated from subfraction B2 (325 mg) through chromatography on a silica gel column (mesh 230–400, 2×30 cm) with $CHCl_3$:MeOH:H_2O (80:15:5). Subfractions B6 (162 mg), B9 (248 mg) and B14 (186 mg) were individually subjected to chromatography on sephadex LH20 column (2×70 cm) to isolate compounds **8** (85 mg), **9** (43 mg) and **10** (57 mg), respectively.

Thin layer chromatography (Pre-coated silica gel GF_{254} plates, Merck) visualized by UV light (254 and 366 nm) was used for the monitoring of the column chromatography. The chemical structures of the isolated compounds were elucidated by ^{1}H-NMR and ^{13}C-NMR (Bruker Avance 300-DRX, 300 MHz for ^{1}H and 75 MHz for ^{13}C) spectral analysis, either by comparing with reference data represented in the literature.

MTT assay

The cytotoxic activities of the isolated compounds were evaluated on some selected cancer and normal cell lines (Table 1) by MTT technique according to Dehnoee et al. [21]. After obtaining 80% cell density, the sample was exposed to 1% trypsin-EDTA solution and after 3 minutes of incubation at 37°C in a cell culture incubator with 5% CO2 and observation of cells removed from the bottom of the plate, the sample was centrifuged at 5000 rpm for 5 minutes and then the cell precipitate was decrypted by adding trypsin culture medium. Immediately sample was centrifuged for 5 minutes at 5000 rpm, Then the cell sediment was decoded by adding a trypsin culture medium. Next, adding trypan blue, and the cell suspension was measured via neobar slide, and also cytotoxicity test was performed with the MTT technique.

At first, cells that were previously seeded in the culture medium were treated with concentrations of 1 to 1000 µg/mL of each compound for 24 h. Then the cells were exposed to 20 µL of MTT under certain conditions (at 37 ° C with 5% CO2) for 5 hours. Next, DMSO was added and absorption rate read at 570 nm via ELISA reader (ELISA Teknika Oraganon reader, Netherlands), then cell viability rate was calculated. To report the inhibitory concentration of 50% (IC_{50}), the absorbance of each well was compared with the absorbance of the control solution.

Results and discussion

Phytochemical investigation of hydroalcoholic extract obtained from the fruits of *H. persicum* using chromatography on normal and sephadex LH-20 columns resulted in the isolation of six compounds (**1**–**6**) from dichloromethane fraction and four compounds (**7**–**10**) from *n*-butanol fraction. The structures of the isolated compounds were characterized as four linear furanocoumarin derivatives; phellopterin (**1**), bergapten (**4**), isopimpinellin (**5**) and xanthotoxin (**6**), three angular furanocoumarin derivatives; angelicin (**2**), pimpinellin (**3**), and apterin (**7**), along with three flavonol glycosides; isorhamnetin 3-O-glucoside (**8**), isorhamnetin 3-O-rutinoside (narcissin) (**9**), and quercetin 3-O-rutinoside (rutin) (**10**) (Fig. 1) on the basis of ^{1}H-NMR and ^{13}C-NMR spectral analyses comparing with those published in the literature [22–25].

Spectroscopic data of isolated compounds

Compound **1**: *Phellopterin* ($C_{17}H_{16}O_5$): ^{1}H-NMR (300 MHz, $CDCl_3$); δ 8.09 (1H, *d*, *J* = 9.8 Hz, H-4), 7.65 (1H, *d*, *J* = 2.1 Hz, H-2'), 7.07 (1H, *d*, *J* = 2.1 Hz, H-3'), 6.36 (1H, *d*, *J* = 9.8 Hz, H-3), 5.57 (1H, *t*, *J* = 7.2 Hz, H-2"), 4.81 (2H, *d*, *J* = 7.2 Hz, H-1"), 4.04 (3H, *s*, 5-OCH_3), 1.76 (3H, *s*, H-4"), 1.70 (3H, *s*, H-5"). ^{13}C-NMR ($CDCl_3$, 75 MHz); δ 160.98 (C-2), 150.46 (C-7), 145.67 (C-5), 145.43 (C-2'), 143.25 (C-9), 140.07 (C-4), 139.64 (C-3"), 133.85 (C-8), 119.61 (C-2"), 113.51 (C-6), 112.76 (C-3), 109.42 (C-10), 104.24 (C-3'), 70.04 (C-1"), 62.31 (5-OCH_3), 25.80 (C-5"), 17.97 (C-4") [22].

Compound **2**: *Angelicin* ($C_{11}H_6O_3$): ^{1}H-NMR (300 MHz, $CDCl_3$); δ 7.81(1H, *d*, *J* = 9.6 Hz, H-4), 7.71 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.44 (1H, *d*, *J* = 8.5 Hz, H-6), 7.39 (1H, *d*, *J* = 8.5 Hz, H-5), 7.14 (1H, *d*, *J* = 2.0 Hz, H-3'), 6.39 (1H, *d*, *J* = 9.6 Hz, H-3). ^{13}C-NMR (75MHz, $CDCl_3$); 160.8 (C-2), 157.3 (C-7), 148.5 (C-9), 145.9 (C-2'), 144.5 (C-4), 123.8 (C-5), 116.9 (C-8), 114.1 (C-3), 113.5 (C-10), 108.8 (C-6), 104.1 (C-3') [23].

Compound **3**: *Pimpinellin* ($C_{13}H_{10}O_5$): ^{1}H-NMR (300 MHz, $CDCl_3$); δ 8.06 (1H, *d*, *J* = 9.7 Hz, H-4), 7.64 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.05 (1H, *d*, *J* = 2.0 Hz, H-3'), 6.35 (1H, *d*, *J* = 9.7 Hz, H-3), 4.12 (3H, *s*, OCH_3), 4.01 (3H, *s*, OCH_3).^{13}C-NMR (75 MHz, $CDCl_3$); δ 160.84 (C-2), 149.75 (C-7), 144.37 (C-2'), 144.39 (C-5), 143.13 (C-9), 139.92 (C-4), 135.08 (C-6), 114.06 (C-8), 113.68 (C-3), 109.40 (C-10), 104.27 (C 3'), 62.36 (OCH_3) 61.20 (OCH_3) [24].

Compound **4**: *Bergapten* ($C_{12}H_8O_4$): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.11(1H, *d*, *J* = 9.8 Hz, H-4), 7.57 (1H, *d*, *J* = 2.2 Hz, H-2'), 7.08 (1H, *s*, H-8), 7.00 (1H, *d*, *J* = 1.7 Hz, H-3'), 6.23 (1H, *d*, *J* = 9.8 Hz, H-3), 4.25 (3H, *s*, 5-OCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.23 (C-2), 158.30 (C-7), 152.60 (C-9), 149.50 (C-5), 144.74 (C-2'), 139.27 (C-4), 112.51 (C-3), 112.38 (C-6), 106.24 (C-10), 105.03 (C-3'), 93.66 (C-8), 59.99 (5-OCH_3) [22].

Compound **5**: *Isopimpinellin* ($C_{16}H_{14}O_4$): δ 8.11 (1H, *d*, *J* = 9.8 Hz, H-4), 7.62 (1H, *d*, *J* = 2.3 Hz, H-2'), 6.99 (1H, *d*, *J* = 2.3 Hz, H-3'), 6.27 (1H, *d*, *J* = 9.8 Hz, H-3), 4.16 (3H, *s*, 8-OCH_3), 4.15 (3H, *s*, 5-OCH_3).^{13}C-NMR (75 MHz, $CDCl_3$): δ 160.49 (C-2), 150.00 (C-7), 145.11 (C-2'), 144.28 (C-5), 143.66 (C-9), 139.42 (C-4), 128.15 (C-8), 114.74 (C-6), 112.80 (C-3), 107.57 (C-10), 105.10 (C-3'), 61.70 (5-OCH_3), 60.79 (6-OCH_3) [24].

Compound **6**: *Xanthotoxin* ($C_{12}H_8O_4$): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.74 (1H, *d*, *J* = 9.6 Hz, H-4), 7.68 (1H, *d*, *J* = 1.7 Hz, H-2'), 7.01 (1H, *d*, *J* = 1.7 Hz, H-3'), 6.76 (1H, *s*, H-5), 6.38 (1H, *d*, *J* = 9.6 Hz, H-3), 4.02 (3H, *s*, 8-OCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 161.01 (C-2), 146.88 (C-7), 145.97 (C-2'), 144.37(C-4), 143.04 (C-9), 142.96 (C-8), 118.48 (C-6), 114.41 (C-10), 113.56 (C-3), 104.51 (C-5), 103.65 (C-3'), 56.43 (8-OCH_3) [22].

Compound **7**: *Apterin* ($C_{12}H_8O_4$): ^{1}H-NMR (300 MHz, DMSO-d_6); δ 8.01 (1H, *d*, *J* = 9.6 Hz, H-4), 7.61 (2H, *d*, *J* = 8.4 Hz, H-5), 6.93 (1H, *d*, *J* = 8.4 Hz, H-6), 6.26 (1H, *d*, *J* = 9.6 Hz, H-3), 5.44 (1H, *d*, *J* = 6.2 Hz, H-3'), 4.55 (1H, *d*, *J* = 7.2 Hz, H-1"), 4.51 (1H, *d*, *J* = 6.2 Hz, H-2'), 2.5–3.5 (2H, *overlapped signals*, H-2" to H-6"), 1.77 (3H, *s*, H-5'), 1.47 (3H, *s*, H-6'). ^{13}C-NMR (75 MHz, DMSO-d_6); δ 162.95 (C-7), 160.03 (C-2), 151.43 (C-9), 144.99 (C-4), 130.95 (C-5), 116.78 (C-8), 112.83 (C-10), 111.77 (C-3), 107.57 (C-6), 97.36 (C-1"), 92.00 (C-2'), 77.24 (C-4'), 76.85 (C-3"), 76.66 (C-5"), 73.38 (C-2"), 69.91 (C-4'), 68.10 (C-4"), 60.70 (C-3'), 24.40 (C-6'), 22.90 (C-5') [24].

Compound **8**: *Isorhamnetin 3-O-glucoside* ($C_{22}H_{22}O_{12}$): ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.94 (1H, *d*, *J* = 1.9 Hz, H-2'), 7.48 (1H, *dd*, *J* = 8.5, 1.9 Hz, H-6'), 6.91 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.44 (1H, *d*, *J* = 1.9 Hz, H-8), 6.20 (1H, *d*, *J* = 1.9 Hz, H-6), 5.56 (1H, *d*, *J* = 7.4 Hz, H-1"(, 3.82 (3H, s, 3'-OCH_3), 3.1–3.6 (6H, *overlapped signals*, H-2" to H-6") [25].

Compound **9**: *Isorhamnetin 3-O-rutinoside* (narcissin) ($C_{28}H_{32}O_{16}$): ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.85 (1H, *d*, *J* = 1.9 Hz, H-2'), 7.49 (1H, *dd*, *J* = 8.4, 1.9 Hz, H-6'), 6.90 (1H, *d*, *J* = 8.4 Hz, H-5'), 6.42 (1H, *d*, *J* = 2.0 Hz, H-8), 6.19 (1H, *d*, *J* = 2.0 Hz, H-6), 5.42 (1H, *d*, *J* = 7.3 Hz, H-1"), 4.42 (1H, *br s*, H-1'"), 3.82 (3H, *s*, 3'-OCH_3), 3.04–3.72 (9H, *overlapped signals*, H2"-H6" and H2'"-H5'"), 0.96 (3H, *d*, *J* = 6.1 Hz, H-6'"). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 177.36 (C-4), 164.27 (C-7), 161.22 (C-5), 156.52 (C-9), 156.50 (C-2), 149.42 (C-4'),

146.90 (C-3'), 133.00 (C-3), 122.28 (C-6'), 121.06 (C-1'), 115.26 (C-5'), 113.23 (C-2'), 104.01 (C-10), 101.19 (C-1"), 100.96 (C-1'"), 98.78 (C-6), 93.85 (C-8), 76.38 (C-5"), 75.94 (C-3"), 74.30 (C-2"), 71.78 (C-2'"), 70.32 (C-3'"), 70.08 (C-4'"), 70.51 (C-4"), 68.43 (C-5'"), 55.64 (C-6'"), 17.76 (3'-OCH_3) [25].

Compound **10**: *Quercetin 3-O-rutinoside* (rutin) ($C_{27}H_{30}O_{16}$): ^{1}H-NMR (300 MHz, DMSO-d_6): δ 7.55 (1H, *br d*, *J* = 8.1 Hz, H-6'), 7.52 (1H, *br s*, H-2'), 6.86 (1H, *d*, *J* = 8.1 Hz, H-5'), 6.38 (1H, *br s*, H-8), 6.18 (1H, *br s*, H-6), 5.33 (1H, *d*, *J* = 6.8 Hz, H-1"), 4.37 (1H, *br s*, H-1'"), 3.0-3.8 (11H, *overlapped signals*, H-2" to H-6" and H-2'" to H-5'"), 0.98 (3H, *d*, *J* = 6.1 Hz, H-6'"). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 177.37 (C-4), 164.07 (C-7), 161.23 (C-5), 156.61 (C-9), 156.44 (C-2), 148.46 (C-4'), 144.78 (C-3'), 133.29 (C-3), 121.60 (C-6'), 121.16 (1'), 116.26 (C-5'), 115.25 (C-2'), 103.92 (C-10), 101.20 (C-1'"), 100.77 (C-1"), 98.73 (C-6), 93.62 (C-8), 76.44 (C-3"), 75.20 (C-5"), 74.07 (C-2"), 71.84 (C-4'"), 70.99 (C-3'"), 70.38 (C-2'"), 69.99 (C-4"), 68.26 (C-5'"), 66.50 (C-6"), 17.76 (C-6'") [25].

A literature review shows that there are two reports on furanocoumarin content of the fruits of *H. persicum*. In 2004, Souri *et al*. investigated the ethyl acetate extract of *H. persicum* fruits, as the extract having the highest antioxidant activity in lipid peroxidation method, and isolated four furanocoumarins; pimpinellin, isopimpinellin, bergapten, and bakuchicin, of which pimpinellin, isopimpinellin were found to have potent antioxidant activity [26]. Xanthotoxin (8-methoxypsoralen), a linear furanocoumarin with phototoxic potential, was also isolated by Sajjadi and Noroozi (2007) from *H. persicum* fruits [27].

The present study, we report the isolation of phellopterin (**1**) angelicin (**2**), apterin (**7**), isorhamnetin 3-O-glucoside (**8**), isorhamnetin 3-O-rutinoside (**10**) and quercetin 3-O-rutinoside (**9**) from the fruits of *H. persicum* for the first time. Moreover, this is the first report on isolation of isorhamnetin 3-O-glucoside (**8**), isorhamnetin 3-O-rutinoside (**10**) and quercetin 3-O-rutinoside (**9**) from the genus *Heracleum* L..

Among the isolated compounds, angelicin (**2**), bergapten (**4**), isopimpinellin (**5**) and xanthotoxin (**6**) have also been previously isolated from the root of *H. persicum* [28]. In 2017, the mentioned compounds (**2**, **4**, **5** and **6**) along with psoralen, angelicin, isobergapten, sphondin, heratomin, 5-methoxyheratomin, moellendorffiline and fraxetin were isolated from *n*-hexane extract of *H. persicum* roots, of which moellendorffiline (a pimpinellin-dimer) demonstrated a potent α-glucosidase inhibitory effect with IC_{50} value of 17.9 ± 2.9 nM [28].

Regarding to the previous studies on cytotoxic activity of *H. persicum* fruits [16–18], anti-tumor potentials of the isolated compounds were assessed in the present study on different cancer cell lines in comparison with HUVEC normal cells using MTT assay.

As shown in Table 1, all of the tested compounds deceased the viability of cancer cell lines with IC_{50} values ranging 44–367 µg/mL, without significant toxicity on examined HUVEC normal cells (IC_{50}: > 1000).

Table 1
Cytotoxic activity of the isolated compounds from *H. persicum* fruits on different cell lines.

Compounds	**Cancer type**	**Cancer cell lines (IC_{50} (μg/mL))**				**Normal cells (IC_{50})**
Phellopterin (**1**)	*multiple myeloma*	SK-MM-1 (69.1 ± 1.2)	RPMI-8226 (85.7 ± 1.8)	U-266 (44.3 ± 1.4)	-	HUVEC (> 1000)
Pimpinellin (**3**)	*oral cancer*	HSC-4 (196.9 ± 5.2)	HSC-3 (184.3 ± 2.7)	HSC-2 (159.7 ± 5.3)	Ca9-22 (250.2 ± 1.7)	HUVEC (> 1000)
Bergapten (**4**)	*breast cancer*	600MPE (109.4 ± 3.9)	AMJ13 (91.8 ± 4.0)	AU565 (93.5 ± 5.7)	Evsa-T (249.6 ± 5.3)	HUVEC (> 1000)
Isopimpinellin (**5**)	*liver cancer*	SNU-423 (124.9 ± 5.1)	SK-HEP-1 (146.0 ± 4.7)	PLC/PRF/5 (169.6 ± 8.4)	SNU-475 (139.4 ± 6.9)	HUVEC (> 1000)
Xanthotoxin (**6**)	*breast cancer*	MCF7 (223.1 ± 7.1)	Hs 578Bst (273.7 ± 6.2)	Hs 319.T (304.3 ± 4.9)	MDA-MB-453 (249.9 ± 7.3)	HUVEC (> 1000)
Apterin (**7**)	*lung cancer*	HT144 (249.8 ± 4.7)	SKMEL2 (366.0 ± 7.3)	WM266-4 (272.6 ± 4.9)	IPC-298 (322.4 ± 6.0)	HUVEC (> 1000)
Isorhamnetin 3-O-glucoside (**8**)	*lung cancer*	H69 (125.2 ± 4.5)	COR-L47 (119.8 ± 6.2)	DMS53 (125.3 ± 4.4)	DMS79 (89.8 ± 3.2)	HUVEC (> 1000)
Narcissin (**9**)	*ovarian carcinoma*	PA-1 (273.7 ± 8.1)	Caov-3 (347.1 ± 4.9)	SW 626 (266.6 ± 6.0)	SK-OV-3 (225.7 ± 7.7)	HUVEC (> 1000)
Rutin (**10**)	*bone cancer*	A-673 (86.4 ± 2.0)	CADO-ES1 (124.6 ± 1.9)	HOS (106.0 ± 4.3)	SW-1353 (129.7 ± 2.8)	HUVEC (> 1000)

According to the results, the most potent cytotoxic effects were observed for phellopterin (**1**) against U-266, SK-MM-1 and RPMI-8226 multiple myeloma cells with IC_{50} values of 44.3 ± 1.4, 69.1 ± 1.2 and 85.7 ± 1.8 µg/mL, respectively.

Phellopterin is a prenylated linear furanocoumarin which has been previously isolated from *H. mantegazzianum* and *H. sibiricum* [29, 30]. In 2018, BAO *et al*. reported the potent dose-dependently cytotoxic activity of phellopterin with concentration ranging from 1 to 40 µM against SMMC-7721 Human Hepatoma cells caused by apoptosis mediated by the inhibition of PI3K/Akt/caspase signaling pathway [31]. Further *in vivo* study revealed that administration of phellopterin at the doses of 10 and 25 mg/kg in rat model of DEN-induced hepatoma significantly suppressed the tumor growth with low toxicity [31].

The structure-activity relationship study of isolated fuanocoumarin derivatives suggests that the increased cytotoxicity of phellopterin might be related to the presence of prenyl group in its structure. This is in agreement with results published by Yang *et al*. (2004) about higher toxicity of osthol (7-Methoxy-8-prenylcoumarin) and imperatorin (8-prenylpsoralene), toward HL-60 and P-388 cell lines rather than other tested coumarin derivatives (bergapten, isopimpinellin and xanthotoxin) [32]. Furthermore, antiproliferative activity of phellopterin against Vero cells (IC_{50}: 14.61 ± 2.90 µg/mL) has been reported more potent than its demethyl analogue, imperatorin (IC_{50}: 58.51 ± 16.51 µg/mL) [33].

In our study bergapten also exhibited considerable cytotoxic effects on AMJ13 and AU565 breast cancer cells with IC_{50} values of 91.8 ± 4.0 and 93.5 ± 5.7 µg/mL, respectively. In a mechanistic study on cytotoxicity of bergapten against MCF7 and ZR-75 breast cancer cells, De Amicis *et al*. (2015) showed that bergapten induced autophagic process by the up-regulation of PTEN expression following the activation of p38MAPK/NF-Y signaling [34]. Increased susceptibility of breast cancer cells to apoptosis followed by induction of p53 gene expression and SMAD4 mediated depletion of estrogen receptor alpha (ERα) in breast cancer cells are the other mechanisms suggested for bergapten cytotoxicity on breast cancer cells [35, 36].

Among flavonoid glycosides tested in present study, antiproliferative effects of isorhamnetin 3-O-glucoside (**8**) on DMS79 (IC_{50}: 93.5 ± 5.7 µg/mL) and rutin (quercetin 3-O-glucoside) (**10**) on A-673 (IC_{50}: 86.4 ± 2.0 µg/mL) were found greater than other compounds.

Isorhamnetin and quercetin have previously shown cytotoxic activity toward different human cancer lines [37–40]. Quercetin has recently received special attention as a natural compound with interesting anticancer potential in cancer chemotherapy drug development researches [41–43]. It has been demonstrated that quercetin induces growth inhibition, apoptosis and authophagy in cancer cells via modulation of Wnt/β-catenin, PI3K/Akt/mTOR, and MAPK/ERK1/2 pathways [43].

Rutin (quercetin 3-O-glucoside), the major flavonoid isolated from *H. persicum* fruits in the present study, has also been documented for its considerable anti-cancer potentials [44]. Guon and Ghung (2016) showed rutin induces caspase-dependent mitochondrial apoptosis in HT-29 human colon cancer cells

[45]. In an *in vivo* study, intraperitoneal administration of 20 mg/kg/day of rutin into nu/nu mice bearing tumors of SW480 colon cancer cells for 32 days resulted in the significant decrease in growth and tumor weight, without any toxic effect on body weight and relative organs weight [46]. In 2016, Ben Sghaier *et al*. studied the effects of rutin on A549 human lung, along with HT29 and Caco-2 colon cancer cell lines. Beside observed inhibitory effects on viability of cancerous cells, rutin showed to have significant ability to attenuate the superoxide production in HT29 cells, as well as to decrease the adhesion and migration of A549 and HT29 cells [47].

Conclusion

The results of present study revealed that *H. persicum* fruits are rich in furanocoumarin derivatives and flavonoid glycosides (**1**–**10**) with preferential cytotoxic activity against cancer cells. Among the tested compounds phellopterin (**1**), bergapten (**4**), isorhamnetin 3-O-glucoside (**8**) and quercetin 3-O-glucoside (rutin) (**10**) were found to have potent anti-tumor effects (IC_{50}: <100 µg/mL) on some cancer cells. Thus, based on our findings, these compounds could be considered as appropriate candidates for new natural anti-cancer drug development research. Further toxicological, pharmacodynamic and pharmacokinetic studies is needed to clarify the potentials of these natural compounds to use in cancer chemotherapy.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article and its additional information files.

Competing interests

The authors declare no competing interests.

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Author's contributions

A.D. collaborated with the preparation of extracts, and isolation of compounds. S.T. collaborated in the characterization of compounds. M.Z. and A.Z. collaborated in biological assays. R.J. and M.D. wrote the manuscript and supervised all phases of the project. All authors read and approved the final manuscript.

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Figures

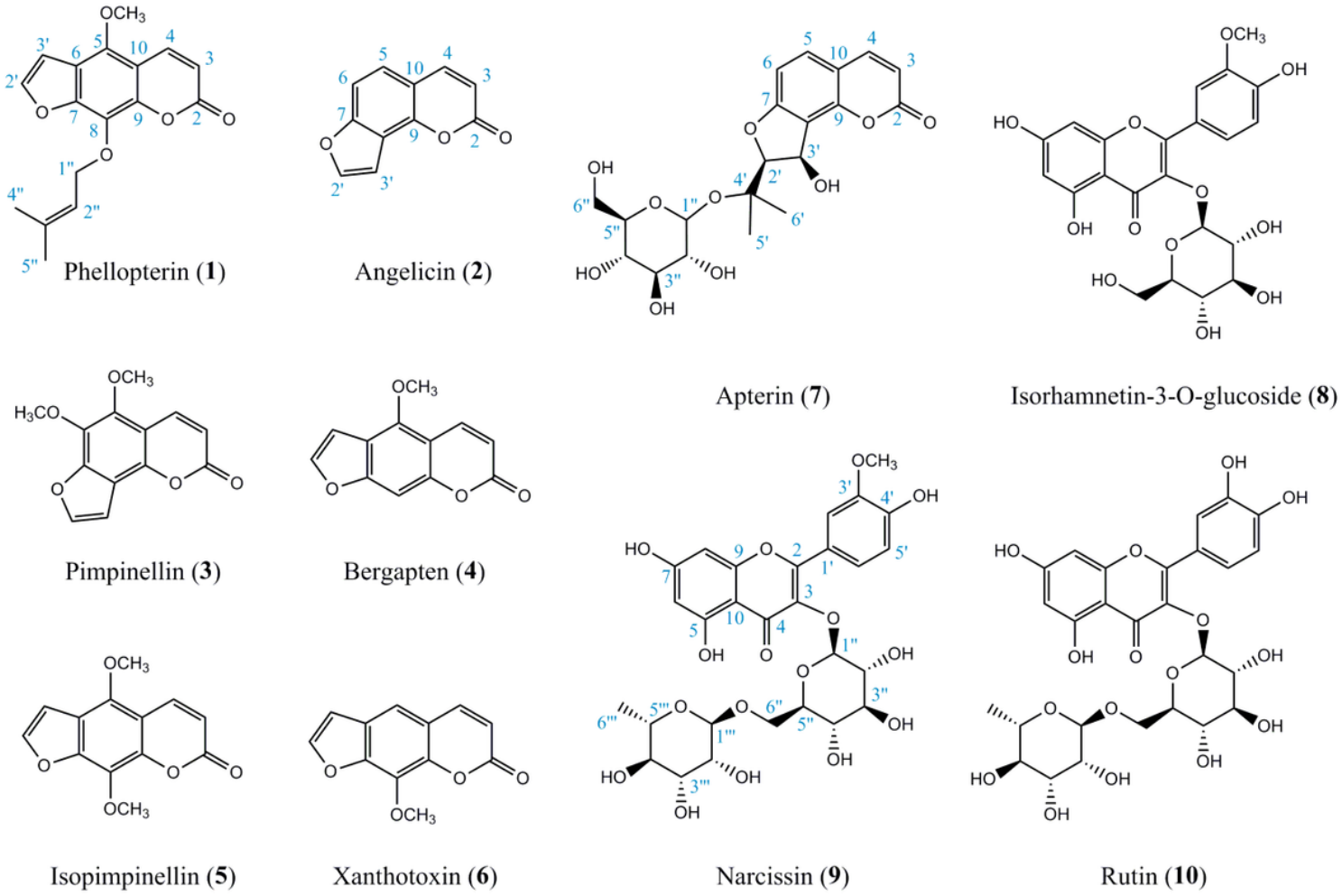

Figure 1

Structures of the isolated compounds from *H. persicum* fruits