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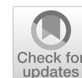

Phytochemical identification by LC-ESI MS/MS method of the *Iris barnumiae* methanolic extract and its antiproliferative and apoptosis-inducing effects

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Abstract
In vitro antiproliferative and apoptosis induction effects of the phytoextracts IrF (flower extract) and IrRz (rhizomes extract) against PC3, MCF-7, U-87 MG, and HT-29 cancer cells have been evaluated in the *Iris* plant. The LC/ESI–MS/MS was established to identify some biochemicals in the *Iris barnumiae* methanolic extract. Nineteen compounds were identified in IrF and IrRz. The most abundant compound was quinic acid (48.337 mg analyte/g extract) and (28.133 mg analyte/g extract) for IrF and IrRz, respectively. Both extracts (lrF and lrRz) are much more sensitive to anticancer activity on HT-29 compared to other cells. Especially the result of IrRz extract on HT-29 (78.22 ± 0.89 IC_{50} value (µg/mL)) which compared to the cis-platin (standard). The sensitivity of the PC3 cells to the apoptosis-inducing potential of the extract was also high compared to other cell lines with apoptotic-mediated programmed cell death. The antiproliferative activity of the two extracts was assayed against four human tumor cell lines. Both extracts showed cytotoxic effects, with IC_{50} (µg/mL) values order HT-29 < MCF-7 < PC3 < U-87MG (IrRz) and HT-29 < PC3 < MCF-7 = U-87MG (IrF). The current study will be beneficial for future research into the isolation of bioactive compounds.

Keywords Plant chemicals · LC–MS/MS · Quinic acid · Antiproliferative · Apoptosis

1 Introduction

In classical herbal medicine systems all across the globe, herbal plants have been extensively prescribed and used for thousands of years to cure a variety of illnesses and used in food industry [1]. Most of the medications used to treat cancer today were extracted from natural compounds, about 60% of them. [2]. In many nations, medicinal plants are a popular alternative to conventional cancer treatment. Currently, anticancer activities have been identified in more than 3000 plants across the globe. Between 10 and 40% of cancer patients worldwide use plant-derived medications for treatment, with individuals from Asia seeing a 50% incidence rate [3, 4]. The Kurdish people's knowledge of medicinal plants is based on observations, beliefs, and a long tradition of herbal medicine dating back hundreds or even thousands of years. [5]. In Kurdistan, traditional herbal therapy is still used as the primary treatment for many disorders, especially by those who cannot afford to purchase expensive contemporary pharmaceuticals, as is the case in other developing nations [6].

With over 389 species, *Iris* is a big genus in the Iridaceae family [7], of perennial plants (rhizomatous irises) that grow from spreading rhizomes or, in dry areas, from bulbs (bulbous irises). The gorgeous blossoms have a violet-like smell that distinguishes them. From Eurasia to North America, the Northern Hemisphere's temperate zones support the growth of the plants [8]. According to reports, there are 12 different species of *Iris* in Iraq; in the Kurdistan region, they are particularly common in hilly areas like Korek Mountain (Rawanduz district) and Halgurd Mountain (Choman district). These plants include *Iris barnumiae* Baker et Foster, *Iris aucheri* (Baker) Sealy, *Iris caucasica* Hoffm., *Iris germanica* L., *Iris gatesii* Foster, *Iris heylandiana* Boiss. et Reut. ex Boiss., *Iris hymenospatha* B. Mathew et Wendelbo, *Iris postii* Mouterde, *Iris persica* L, *Iris masia* Dykes, *Iris*

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pseudocaucasica Grossh., and *Iris reticulata* M. Bieb [9]. Many *Iris* species are ornamental plants and economically important. On one hand, they are used in various traditional medicines for the treatment of cancer, inflammations, bacterial and viral infections. In addition, according to studies on this genus (*Iris*), they exhibit potent antioxidant, anticancer, anti-inflammatory, hepatoprotective, neuroprotective, and anti-microbial properties [7], and they are used in the manufacture of luxury expensive perfumes [10]. On the other hand, the methanolic extract of *I. barnumiae* is used to prepared nanoparticles and applied for removal of Congo red in the aqueous solution [11]. The genus *Iris* is rich in bio-active polyphenolics and other compounds. Therefore, electrospray ionization mass spectrometry (ESI–MS) combined with high performance liquid chromatography (HPLC–ESI–MS/MS) provides a simple and versatile approach to identify the constituents in the *I. barnumiae* methanolic extract and anti-proliferative and apoptosis-inducing efficacy against PC3, MCF-7, U-87 MG, and HT-29 cancer cells.

2 Experimental sections

2.1 Collection of plant material

The plant components were gathered in May at Kodo Mountain in Haji Omran, Iraq's Kurdistan province. Permission was taken to collect the plants and be identified by the botanist and put on display in the Salahaddin University-Erbil/Iraq. The accession number for the voucher specimens (7706, Herbarium). *I. barnumiae* roots were dried by air in a dark, comfortable environment without any light. The dried plants were then finely pulverized in a lab grinding mill and sieved to produce a homogenous powder for examination. Then, in order to prevent contamination, it is kept in glass bottles in a dark environment.

2.2 Preparation of the plant extracts

Dried plant materials (20 g) of the *Iris* (flowers and rhizomes) separately mixed with absolute methanol (3 × 100 mL) were subjected to microwave-assisted extraction method (Panasonic P90N28AP-S3) at 800 W: the time consumed was 5 min using an irradiation cycle of 20-s intervals [12]. After the extracts were filtered, a rotary evaporator was used to evaporate the solvents at 35 °C while under vacuum. Prior to LC–MS/MS analysis, dry extracts were diluted to 1000 mg/L with methanol, spiked with ferulic acid D3 (20 mg/L), rutin D3 (1 mg/L), and quercetin D3 (5 mg/L), and then passed through a 0.2 m syringe filter. After that, it is kept in glass bottles in a dark location to prevent contamination [13].

2.3 Preparation of standard solutions

The analytical method was developed using real standards, including 53 natural phenolic compounds and three isotope-labeled phenolic compounds (used as internal standards). By dissolving their bought solid forms in methanol, 54 standard compounds (including ISs) were converted into 1000 mg/L main stock solutions. In an unusual move, 500 mg/L concentrations of the major stock solutions of the epicatechin and epigallocatechin standards were made. In order to speed up the procedure, middle stocks solutions of 53 phenolic standards were created. For the measurement of non-flavonoid molecules, flavonoid glycosides, and flavonoid aglycones, ferulic acid D3, rutin D3, and quercetin D3 were utilized, respectively. To create the calibration curve, eight calibration levels using a combination of 53 phenolic standards and three ISs were created. Each calibration level was spiked with ferulic acid D3 (20 mg/L), rutin D3 (1 mg/L), and quercetin D3 (5 mg/L), and each plant sample was analyzed [13].

2.4 Mass spectrometer and chromatography conditions

Fifty-three phytochemicals were quantitatively evaluated using UHPLC of the Shimadzu-Nexera model in tandem with a mass spectrometer. The autosampler (SIL-30AC model), column oven (CTO-10ASvp model), binary pumps (LC-30AD model), and degasser were all included in the reversed-phase UHPLC (DGU- 20A3R model). To get the best separation for 53 bioactive compounds and overcome the suppressive effects, the chromatographic conditions were improved. Different columns, including the Agilent Poroshell 120 EC-C18 model (150 mm, 2.1 mm, 2.7 m) and the RP-C18 Inertsil ODS-4 (100 mm, 2.1 mm, 2 m), as well as different mobile phase (B) additives, including ammonium formate, formic acid, ammonium acetate, and acetic acid, as well as different column temperatures, including 25 °C and 30°. Therefore, the reversed phase Agilent Poroshell 120 EC-C18 model analytical column (150 mm × 2.1 mm × 2.7 m) was used for the chromatographic separation. The temperature in the column was fixed to 40 °C. Eluent A (water + 5 mM ammonium formate + 0.1% formic acid) and Eluent B (methanol + 5 mM ammonium formate + 0.1% formic acid) made up the elution gradient; 20–100% B (0–25 min), 100% B (25–35 min), and 20% B were employed as the gradient elution profiles (35–45 min). Additionally, 0.5 mL/min and 5 L, respectively, were chosen as the solvent flow rate and injection volume. A Shimadzu LCMS-8040 tandem mass spectrometer outfitted with an electrospray ionization (ESI) source operating in both negative and positive ionization modes was used for the mass spectrometric detection. Lab

Table 1 Parameters for the LC–MS/MS technique's analytical method validation

No	Analytes	RT[a]	M.I. (m/z)[b]	F.I. (m/z)[c]	Ion. mode	Equation	r2[d]	RSD%[e]		Linearity range (mg/L)	LOD/LOQ (µg/L)[f]	Recovery (%)		U[g]	Gr. no
								Interday	Intraday			Interday	Intraday		
1	Quinic acid	3.0	190.8	93.0	Neg	y = −0.0129989 + 2.97989×	0.996	0.69	0.51	0.1–5	25.7/33.3	1.0011	1.0083	0.0372	1
2	Fumaric aid	3.9	115.2	40.9	Neg	y = −0.0817862 + 1.03467×	0.995	1.05	1.02	1–50	135.7/167.9	0.9963	1.0016	0.0091	1
3	Aconitic acid	4.0	172.8	129.0	Neg	y = −0.7014530 + 32.9994×	0.971	2.07	0.93	0.1–5	16.4/31.4	0.9968	1.0068	0.0247	1
4	Gallic acid	4.4	168.8	79.0	Neg	y = 0.0547697 + 20.8152×	0.999	1.60	0.81	0.1–5	13.2/17.0	1.0010	0.9947	0.0112	1
5	Epigallocatechin	6.7	304.8	219.0	Neg	y = 0.00494986 + 0.0483704×	0.998	1.22	0.73	1–50	237.5/265.9	0.9969	1.0040	0.0184	3
6	Protocatechuic acid	6.8	152.8	108.0	Neg	y = 0.211373 + 12.8622×	0.957	1.43	0.76	0.1–5	21.9/38.6	0.9972	1.0055	0.0350	1
7	Catechin	7.4	288.8	203.1	Neg	y = −0.00370053 + 0.431369×	0.999	2.14	1.08	0.2–10	55.0/78.0	1.0024	1.0045	0.0221	3
8	Gentisic acid	8.3	152.8	109.0	Neg	y = −0.0238983 + 12.1494×	0.997	1.81	1.22	0.1–5	18.5/28.2	0.9963	1.0077	0.0167	1
9	Chlorogenic acid	8.4	353.0	85.0	Neg	y = 0.289983 + 36.3926×	0.995	2.15	1.52	0.1–5	13.1/17.6	1.0000	1.0023	0.0213	1
10	Protocatechuic-aldehyde	8.5	137.2	92.0	Neg	y = 0.257085 + 25.4657×	0.996	2.08	0.57	0.1–5	15.4/22.2	1.0002	0.9988	0.0396	1
11	Tannic acid	9.2	182.8	78.0	Neg	y = 0.0126307 + 26.9263×	0.999	2.40	1.16	0.05–2.5	15.3/22.7	0.9970	0.9950	0.0190	1
12	Epigallocatechin gallate	9.4	457.0	305.1	Neg	y = −0.0380744 + 1.61233×	0.999	1.30	0.63	0.2–10	61.0/86.0	0.9981	1.0079	0.0147	3
13	1,5-Dicaffeoylquinic-acid	9.8	515.0	191.0	Neg	y = −0.0164044 + 16.6535×	0.999	2.42	1.48	0.1–5	5.8/9.4	0.9983	0.9997	0.0306	1
14	4-OH benzoic acid	10.5	137,2	65.0	Neg	y = −0.0240747 + 5.06492×	0.999	1.24	0.97	0.2–10	68.4/88.1	1.0032	1.0068	0.0237	1
15	Epicatechin	11.6	289.0	203.0	Neg	y = −0.0172078 + 0.0833424×	0.996	1.47	0.62	1–50	139.6/161.6	1.0013	1.0012	0.0221	3
16	Vanilic acid	11.8	166.8	108.0	Neg	y = −0.0480183 + 0.779564×	0.999	1.92	0.76	1–50	141.9/164.9	1.0022	0.9998	0.0145	1
17	Caffeic acid	12.1	179.0	134.0	Neg	y = 0.120319 + 95.4610×	0.999	1.11	1.25	0.05–2.5	7.7/9.5	1.0015	1.0042	0.0152	1
18	Syringic acid	12.6	196.8	166.9	Neg	y = −0.0458599 + 0.663948×	0.998	1.18	1.09	1–50	82.3/104.5	1.0006	1.0072	0.0129	1
19	Vanillin	13.9	153.1	125.0	Poz	y = 0.00185898 + 20.7382×	0.996	1.10	0.85	0.1–5	24.5/30.4	1.0009	0.9967	0.0122	1
20	Syringic-aldehyde	14.6	181.0	151.1	Neg	y = −0.0128684 + 7.90153×	0.999	2.51	0.77	0.4–20	19.7/28.0	1.0001	0.9964	0.0215	1
21	Daidzin	15.2	417.1	199.0	Poz	y = 9.45747 + 152.338×	0.996	2.25	1.32	0.05–2.5	7.0/9.5	0.9955	1.0017	0.0202	2
22	Epicatechin gallate	15.5	441.0	289.0	Neg	y = −0.0142216 + 1.06768×	0.997	1.63	1.28	0.1–5	19.5/28.5	0.9984	0.9946	0.0229	3
23	Piceid	17.2	391.0	135.1	Poz	y = 0.00772525 + 25.4181×	0.999	1.94	1.16	0.05–2.5	13.8/17.8	1.0042	0.9979	0.0199	1
24	p-Coumaric acid	17.8	163.0	93.0	Neg	y = 0.0249034 + 18.5180×	0.999	1.92	1.43	0.1–5	25.9/34.9	1.0049	1.0001	0.0194	1
25	Feruli acid-D3-ISh	18.8	196.2	152.1	Neg	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	0.0170	1
26	Ferulic acid	18.8	192.8	149.0	Neg	y = −0.0735254 + 1.34476×	0.999	1.44	0.53	1–50	11.8/15.6	0.9951	0.9976	0.0181	1
27	Sinapic acid	18.9	222.8	193.0	Neg	y = −0.0929932 + 0.836324×	0.999	1.45	0.52	0.2–10	65.2/82.3	1.0031	1.0037	0.0317	1
28	Coumarin	20.9	146.9	103.1	Poz	y = 0.0633397 + 136.508×	0.999	2.11	1.54	0.05–2.5	214.2/247.3	0.9950	0.9958	0.0383	1
29	Salicylic acid	21.8	137.2	65.0	Neg	y = 0.239287 + 153.659×	0.999	1.48	1.18	0.05–2.5	6.0/8.3	0.9950	0.9998	0.0158	1
30	Cynaroside	23.7	447.0	284.0	Neg	y = 0.280246 + 6.13360×	0.997	1.56	1.12	0.05–2.5	12.1/16.0	1.0072	1.0002	0.0366	2
31	Miquelianin	24.1	477.0	150.9	Neg	y = −0.00991585 + 5.50334×	0.999	1.31	0.95	0.1–5	10.6/14.7	0.9934	0.9965	0.0220	2
32	Rutin-D3-IS[h]	25.5	612.2	304.1	Neg	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	2
33	Rutin	25.6	608.9	301.0	Neg	y = −0.0771907 + 2.89868×	0.999	1.38	1.09	0.1–5	15.7/22.7	0.9977	1.0033	0.0247	2
34	Isoquercitrin	25.6	463.0	271.0	Neg	y = −0.111120 + 4.10546×	0.998	2.13	0.78	0.1–5	8.7/13.5	1.0057	0.9963	0.0220	2
35	Hesperidin	25.8	611.2	449.0	Poz	y = 0.139055 + 13.2785×	0.999	1.84	1.35	0.1–5	19.0/26.0	0.9967	1.0043	0.0335	2
36	o-Coumaric acid	26.1	162.8	93.0	Neg	y = 0.00837193 + 11.2147×	0.999	2.11	1.46	0.1–5	31.8/40.4	1.0044	0.9986	0.0147	1

Table 1 (continued)

No	Analytes	RT[a]	M.I. (m/z)[b]	F.I. (m/z)[c]	Ion. mode	Equation	r^{2d}	RSD%[e]		Linearity range (mg/L)	LOD/LOQ (μg/L)[f]	Recovery (%)		U[g]	Gr. no
								Interday	Intraday			Interday	Intraday		
37	Genistin	26.3	431.0	239.0	Neg	y = 1.65808 + 7.57459×	0.991	2.01	1.28	0.1–5	14.9/21.7	1.0062	1.0047	0.0083	2
38	Rosmarinic acid	26.6	359.0	197.0	Neg	y = − 0.0117238 + 8.04377×	0.999	1.24	0.86	0.1–5	16.2/21.2	1.0056	1.0002	0.0130	1
39	Ellagic acid	27.6	301.0	284.0	Neg	y = 0.00877034 + 0.663741×	0.999	1.57	1.23	0.4–20	56.9/71.0	1.0005	1.0048	0.0364	1
40	Cosmosiin	28.2	431.0	269.0	Neg	y = − 0.708662 + 8.62498×	0.998	1.65	1.30	0.1–5	6.3/9.2	0.9940	0.9973	0.0083	2
41	Quercitrin	29.8	447.0	301.0	Neg	y = -0.00153274 + 3.20368×	0.999	2.24	1.16	0.1–5	4.8/6.4	0.9960	0.9978	0.0268	2
42	Astragalin	30.4	447.0	255.0	Neg	y = 0.00825333 + 3.51189×	0.999	2.08	1.72	0.1–5	6.6/8.2	0.9968	0.9957	0.0114	2
43	Nicotiflorin	30.6	592.9	255.0	Neg	y = 0.00499333 + 2.62351×	0.999	1.48	1.23	0.05–2.5	11.9/16.7	0.9954	1.0044	0.0108	2
44	Fisetin	30.6	285.0	163.0	Neg	y = 0.0365705 + 8.09472×	0.999	1.75	1.19	0.1–5	10.1/12.7	0.9980	1.0042	0.0231	3
45	Daidzein	34.0	253.0	223.0	Neg	y = − 0.0329252 + 6.23004×	0.999	2.18	1.73	0.1–5	9.8/11.6	0.9926	0.9963	0.0370	3
46	Quercetin-D3-IS[h]	35.6	304.0	275.9	Neg	NA	NA	NA	NA	NA	NA	NA	NA	N.A	3
47	Quercetin	35.7	301.0	272.9	Neg	y = + 0.00597342 + 3.39417×	0.999	1.89	1.38	0.1–5	15.5/19.0	0.9967	0.9971	0.0175	3
48	Naringenin	35.9	270.9	119.0	Neg	y = − 0.00393403 + 14.6424×	0.999	2.34	1.69	0.1–5	2.6/3.9	1.0062	1.0020	0.0392	3
49	Hesperetin	36.7	301.0	136.0	Neg	y = + 0.0442350 + 6.07160×	0.999	2.47	2.13	0.1–5	7.1/9.1	0.9998	0.9963	0.0321	3
50	Luteolin	36.7	284.8	151.0	Neg	y = − 0.0541723 + 30.7422×	0.999	1.67	1.28	0.05–2.5	2.6/4.1	0.9952	1.0029	0.0313	3
51	Genistein	36.9	269.0	135.0	Neg	y = − 0.00507501 + 12.1933×	0.999	1.48	1.19	0.05–2.5	3.7/5.3	1.0069	1.0012	0.0337	3
52	Kaempferol	37.9	285.0	239.0	Neg	y = − 0.00459557 + 3.13754×	0.999	1.49	1.26	0.05–2.5	10.2/15.4	0.9992	0.9990	0.0212	3
53	Apigenin	38.2	268.8	151.0	Neg	y = 0.119018 + 34.8730×	0.998	1.17	0.96	0.05–2.5	1.3/2.0	0.9985	1.0003	0.0178	3
54	Amento-flavone	39.7	537.0	417.0	Neg	y = 0.727280 + 33.3658×	0.992	1.35	1.12	0.05–2.5	2.8/5.1	0.9991	1.0044	0.0340	3
55	Chrysin	40.5	252.8	145.0	Neg	y = − 0.0777300 + 18.8873×	0.999	1.46	1.21	0.05–2.5	1.5/2.8	0.9922	1.0050	0.0323	3
56	Acacetin	40.7	283.0	239.0	Neg	y = − 0.559818 + 163.062×	0.997	1.67	1.28	0.02–1	1.5/2.5	0.9949	1.0011	0.0363	3

[a]R.T.: retention time

[b]MI (m/z): molecular ions of the standard analytes (m/z ratio)

[c]FI (m/z): fragment ions

[d]r^2: coefficient of determination

[e]RSD: relative standard deviation

[f]LOD/LOQ (μg/L): limit of detection/quantification

[g]U (%): percent relative uncertainty at 95% confidence level ($k = 2$)

[h]IS: internal standard

[i]Gr. no: represents grouping of internal standards; these numbers indicate which IS stands for which phenolic compound

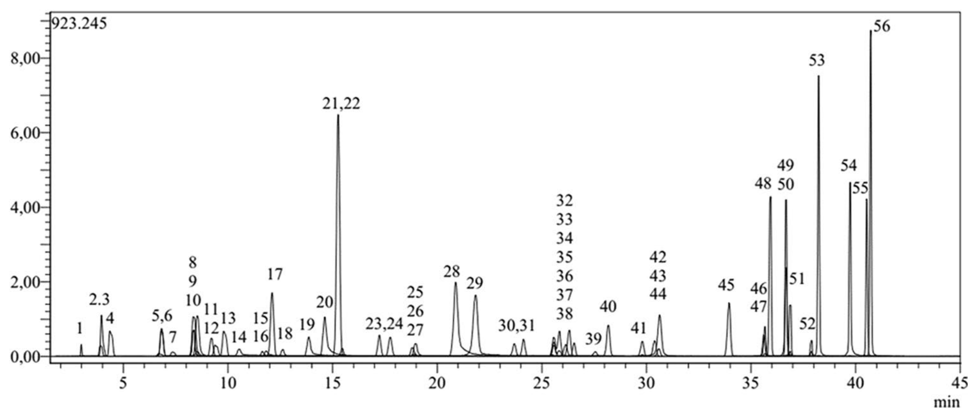

Fig. 1 Total ion chromatogram (TIC) for the mixture of the 53 phenolic standard compounds

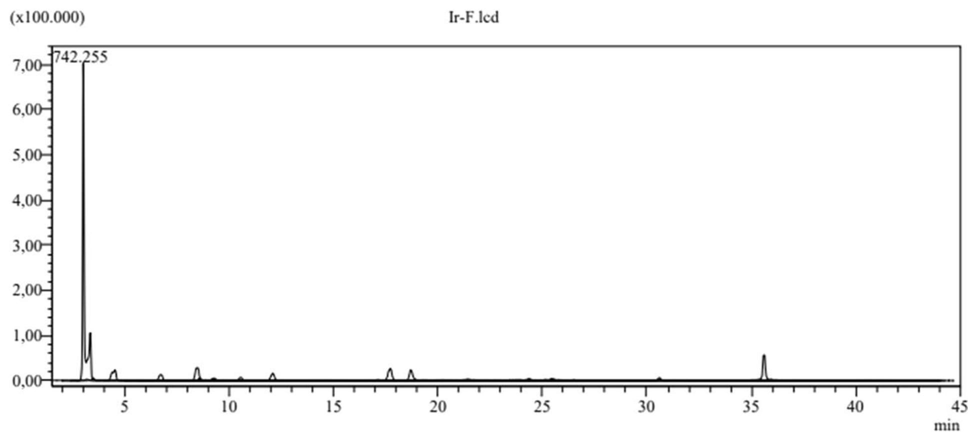

Fig. 2 LC–MS chromatogram of methanol extract of IrF

Solutions software was used to capture and process the LC–ESI–MS/MS. The chemical compounds were quantified using the MRM technique. Based on the screening of specific precursor phytochemical-to-fragment ion transitions, the MRM approach was developed to detect and quantify phytochemical substances only. In order to produce the best phyto-chemical fragmentation and the greatest transmission of the desired product ions, the collision energies (CE) were tuned. The MS was operated at the following temperatures: DL temperature, 250 °C, heat block temperature, 400 °C, and interface temperature, 350 °C. The drying gas (N2) flow rate was 15 L/min [14].

2.5 Cytotoxicity assay

Tests of cell viability were run against PC3, HT-29, MCF-7, and U-87MG cell lines [15, 16]. The cells were cultured in DMEM low glucose (U-87MG), RPMI 1640

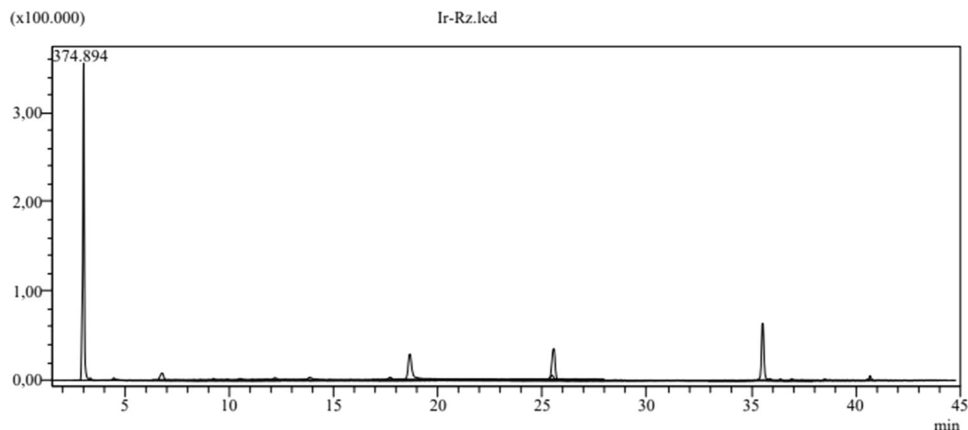

Fig. 3 LC–MS chromatogram of methanol extract of IrRz

(PC3), and DMEM high glucose (MCF-7 and HT-29) media with 10% fetal bovine serum (FBS) and 1% (V/V) penicillin–streptomycin in a humidified environment containing 95% medium and 5% CO^2. MTT assay was used to examine cell survival. In conclusion, 1.0 104 cells were pre-cultured in each well of a 96-well plate for 16 h in the incubator before being exposed to various concentrations of the complexes in fresh media for 72 h. Following the proper amount of time, each well received a fresh medium containing MTT solution at a final concentration of 0.50 mg/mL. This was then incubated for an additional 4 h under the same conditions. Finally, a solvent buffer containing the growth medium was eliminated, and the crystalline formazan was dissolved in 100 L of 100% DMSO. The BMG Spectro Nano Elizabeth Reader was then used to measure the absorbance of the samples at two wavelengths, 570 nm and 630 nm, which correspond to the formazan and background absorbances, respectively. The formula below was used to determine the percentage of living cells:

$$\text{Cell viability\%} = [A_{T(sample)}/A_{T(control)}] \times 100$$

where the AT is defined as $A_{570} - A_{630}$.

The IC_{50} concentration was estimated as mean ± standard deviation (STDEV) from three independent experiments using the GraphPad Prism 8 software.

2.6 Apoptosis/necrosis assay

A total of $2*10^5$ of four cell lines (PC3, MCF-7, HT-29, U-87MG) are pre-cultured for 16 h before 24 h of exposure to IrRz and IrF. The following day, Annexin V/PI staining using the Bioscience TM Annexin V apoptosis detection kit was carried out (Invitrogen). According to the technique, cells were washed twice: once with 1000 L 1X binding buffer and once with phosphate buffered saline (PBS). Following that, cells were suspended for 15 min in 100 L of binding buffer containing 5 L of Annexin V-fluorescein isothiocyanate. Cells were then resuspended in 200 L of the same buffer with 5 L of a propidium iodide (PI) solution after being washed once more with 1000 L of binding buffer. The rates of apoptosis were then measured using BD FACS Calibur TM flow cytometry (BD Biosciences, San Jose, CA, USA). The sum of early and late apoptosis was used to calculate the apoptosis rates [17].

3 Results and discussions

3.1 Method validation studies

The developed LC–MS/MS method for quantifying 53 phyto-chemical (majority flavonoids and phenolic acids) in plant species was analytically validated in terms of inter-day and intra-day precision (repeatability), accuracy (recovery), linearity, relative standard uncertainty (U% at 95% confidence level ($k = 2$)), and limits of detection/quantification (LOD/LOQ). Table 1 provides analytical parameters similar to those used to validate the LC–MS/MS technique.

3.2 LC–MS/MS quantification

Microwave-assisted extraction (MAE) method has been applied to extraction of phytochemicals [12]. Numerous studies have been done on LC–MS/MS-based quantitative analysis [18]. In our results, an accurate quantitative LC–MS/MS method was prepared for the identification of 19 compounds in the methanol extracts (lrF and lrRz). Figure 1 shows LC–MS/MS of 56 standard phytochemicals and prepared as standards, each of which has its own retention time including quinic acid, aconitic acid, fumaric acid, epigallocatechin, protocatechuic acid, gallic acid, catechin, chlorogenic acid, gentisic acid, tannic acid, protocatechuic aldehyde, 1,5-dicaffeoylquinic acid, epigallocatechin gallate, syringic acid, 4-hydroxybenzoic acid, vanilic acid, epicatechin, caffeic acid, ferulic acid D3, vanillin, syringic daidzin, aldehyde, epicatechin gallate, piceid, *p*-coumaric acid, ferulic acid, sinapic acid, coumarin, cynaroside, salicylic acid, miquelianin, rutin, rutin D3-IS, hesperidin, isoquercitrin, o-coumaric acid, genistin, rosmarinic acid, ellagic acid, cosmosiin, quercitrin, nicotiflorin,

Table 2 Quantitative screening of phytochemicals in methanol extracts of IrF and IrRz by LC–MS/MS (mg analyte/g extract)

RT	Phytochemicals	IrF	IrRz
1	Quinic acid	48.337	28.133
4	Gallic acid	0.924	0.022
6	Protocatechuic acid	0.502	0.296
9	Chlorogenic acid	0.042	N.D
10	Protocatechuic aldehyde	0.004	N.D
11	Tannic acid	0.731	0.016
14	4-hydroxybenzoic acid	0.718	0.222
17	Caffeic acid	0.109	0.022
19	Vanillin	N.D	0.11
24	*p*-Coumaric acid	1.036	0.119
33	Rutin	0.109	N.D
34	Isoquercitrin	N.D	0.057
35	Hesperidin	0.049	N.D
37	Genistin	N.D	0.044
43	Nicotiflorin	0.304	N.D
48	Naringenin	0.013	0.01
51	Genistein	N.D	0.009
55	Chrysin	0.006	0.005
56	Acacetin	0.008	0.028

RT retention time, *IrF* methanol flowers extract, *IrRz* methanol rhizomes extract

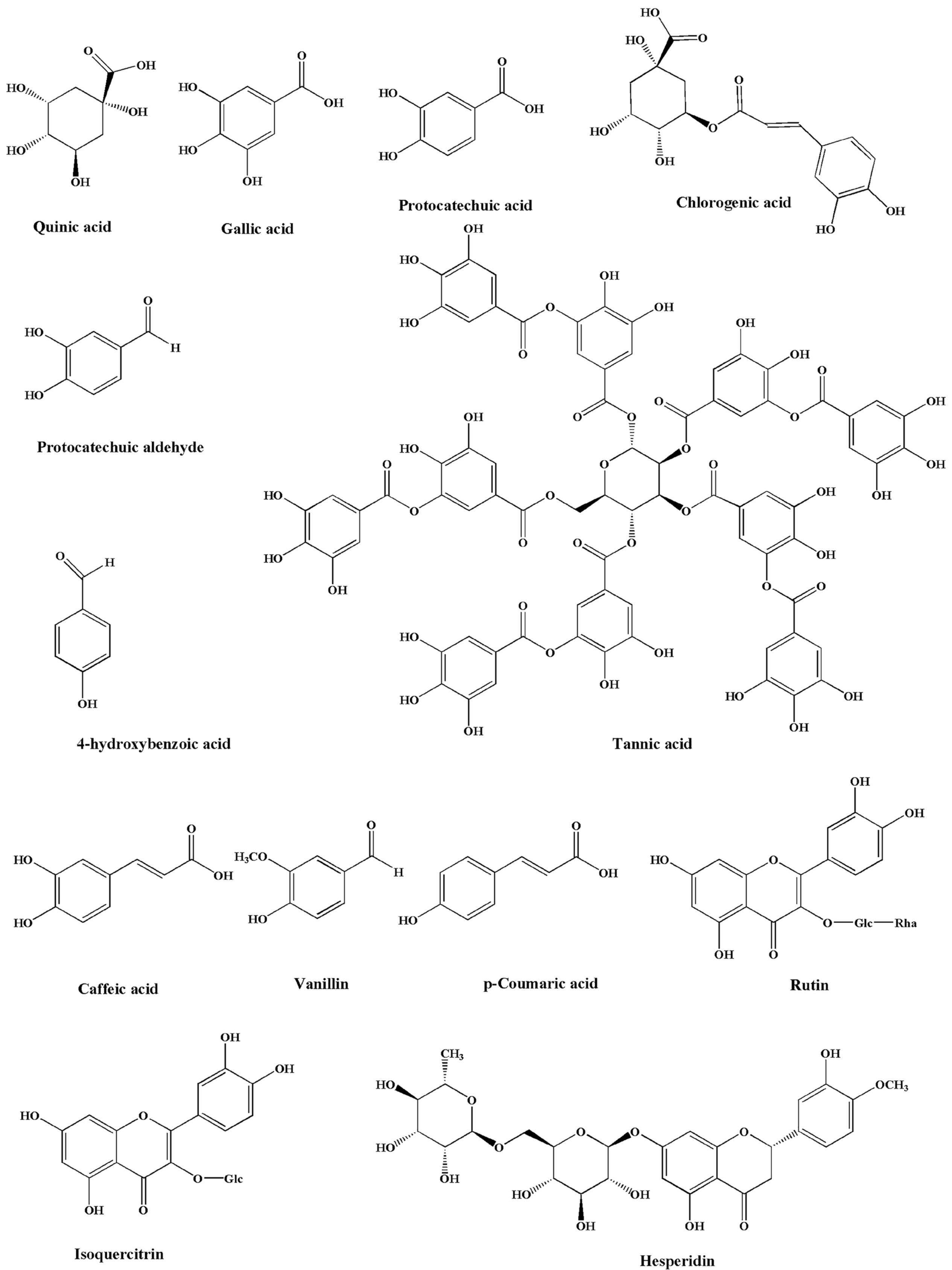

Fig. 4 Chemical structures of identified compounds in the flowers and rhizomes from *Iris barnumiae* methanolic extracts

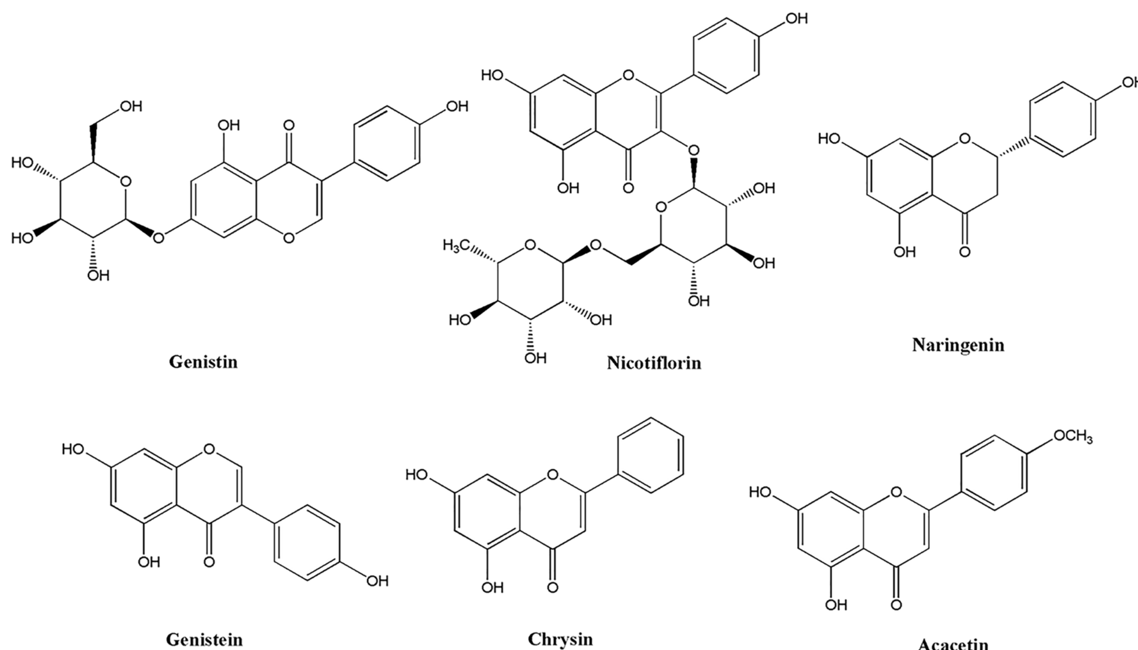

Fig. 4 (continued)

astragalin, fisetin, daidzein, quercetin D3-IS, kaempferol, hesperetin, naringenin, quercetin, genistein, luteolin, apigenin, amentoflavone, chrysin, and acacetin [13]. In our study, Figs. 2 and 3 and Table 2 show LC–MS/MS-TIC (total ion chromatogram) chromatograms and detected compound of IrF and IrRz respectively. In addition, the major compounds (bioactive) in both extracts of the *I. barnumiae* (lrF and lrRz) included quinic acid. However, the amount of lrF (48.337 mg analyte/g extract) was much higher than that in lrRz (28.133 mg analyte/g extract). The *p*-coumaric acid content of lrF (1.036 mg analyte/g extract) was also as much as 9 times that of IrRz (0.119 mg analyte/g extract). Also, in general, the compounds obtained from IrF were much more abundant than from IrRz, except for acacetin. Also, some compounds were found in lrF (chlorogenic acid, protocatechuic aldehyde, rutin, hesperidin, and nicotiflorin) that were not found in IrRz. Conversely, some compounds in IrRz, which are vanillin, isoquercitrin, genistin, and genistein, do not exist in lrF.

Table 3 Antiproliferative activity of the lrF and lrRz extracts against PC3, MCF-7, HT-29, and U87-MG cell lines

Cell line	IC_{50} value (μg/mL)		
	lrRz	lrF	Cis-platin
PC3	100.2 ± 1.16	184.2 ± 6.29	4.85 ± 0.32
MCF-7	95.11 ± 2.01	> 250	6.48 ± 0.26
HT-29	78.22 ± 0.89	128.1 ± 2.39	22.20 ± 0.72
U87-MG	146.8 ± 5.24	> 250	4.30 ± 0.233

According to a previous study on rhizome part for three *Iris* species and analyzed compounds by using HPLC–DAD-ESI–MS/MS method including *Iris crocea* (12 compounds), *Iris germanica* (10 compounds), and *Iris spuria* (13 compounds) [19]. But in this study results 19 compounds were identified from *I. barnumiae* by using HPLC–MS/MS. The chemical structures were identified from both extracts, as shown in Fig. 4.

3.3 Antiproliferative

One of the main causes of death, cancer is a multi-stage process that causes cells to divide suddenly and uncontrollably. The recorded incidents and the forecasts for the near

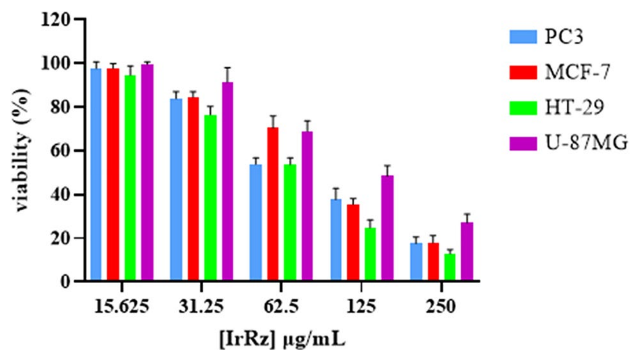

Fig. 5 The percentage of viability of PC3, MCF-7, HT-29, and U87-MG cell lines after treatment with concentration *lrRz* extract

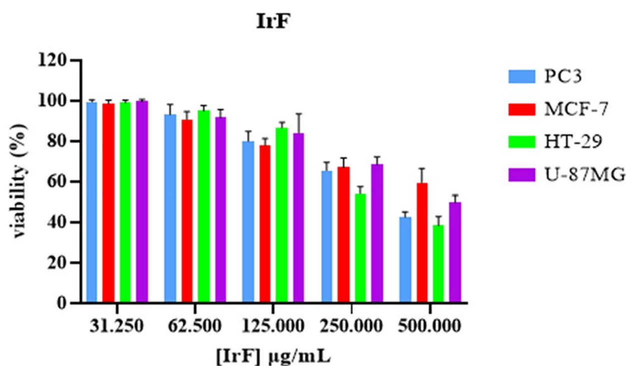

Fig. 6 The percentage of viability of PC3, MCF-7, HT-29, and U87-MG cell lines after treatment with concentration lrF extract

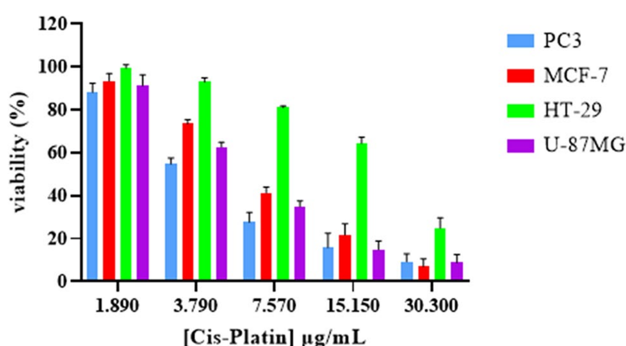

Fig. 7 The percentage of viability of PC3, MCF-7, HT-29, and U87-MG cell lines after treatment with concentration cis-platin

future are absurd [20]. The use of herbal treatments has become popular in recent years in many affluent nations as supplementary and alternative medicine, but only after strict regulation and oversight. [21]. Because they are thought to be more biologically friendly and hence more co-evolved with their target sites and less hazardous to normal cells, natural products have drawn growing attention in cancer treatment [22]. This accessible, non-toxic natural polyol has been discovered to be an effective anti-inflammatory, antiviral, antibacterial, antivascular, and anticancer drug. [23]. Quinic acid, a cyclitol, a cyclic polyol, and a cyclohexanecarboxylic acid, is being investigated as a powerful treatment for prostate cancer [24].

According to previous reports, the extracts of *Iris* species were used as anticancer on (IGR39, MDA-MB-231) cancer cells for rhizomes of *Iris hungary Waldst. & Kit. and Iris variegata* L. [25], as well as cell cancer (CORL-23, C32) for rhizomes and flowers of the *Iris pseudopumila* Tineo [26], and in addition, cancer cells (A549, Caco-2) for rhizomes of (*I. spuria* L., *Iris kashmiriana* Baker*, I. germanica* L.*, I. crocea* Jacquem. *ex Iris ensata* Thunb) [27]. More than the above, the cells (HCT116, HeLa, and HL-60) for rhizomes of the *Iris hungarica* [28]. In the present study, four types of cancer cells were selected that are very common in the world. Separately for both the IrF and IrRz parts of the *Iris barnumiae*, what was found here is that the rhizome performs effect better than the flower. As shown in Table 3 that includes cell cancers those PC3, MCF-7, HT-29, and U-87MG. According to the results available in Table 3 and compared to the cis-platin as a standard. Both extracts (lrF and lrRz) have anticancer activity on HT-29 that is much more sensitive compared to other cells. The anticancer results of the rhizomes part of the *Iris barnumiae* methanolic extract on the cell HT-29 (with IC_{50} value of 78.22 ± 0.89 µg/mL) and MCF-7 (with IC_{50} value of 95.11 ± 2.01 µg/mL) were more affected compared to PC3 (with IC_{50} value of 100.2 ± 1.16 µg/mL) and U87-MG (with IC_{50} value of 146.8 ± 5.24 µg/mL) cell lines as shown in Fig. 5. But the anticancer activity of flowers of the *Iris barnumiae* methanolic extract was tested to PC3, MCF-7, HT-29, and U-87MG, the results, HT-29 (with IC_{50} value of 128.1 ± 2.39 µg/mL), and PC3 (with IC_{50} value of 184.2 ± 6.29 µg/mL. The flower part of the *Iris barnumiae*

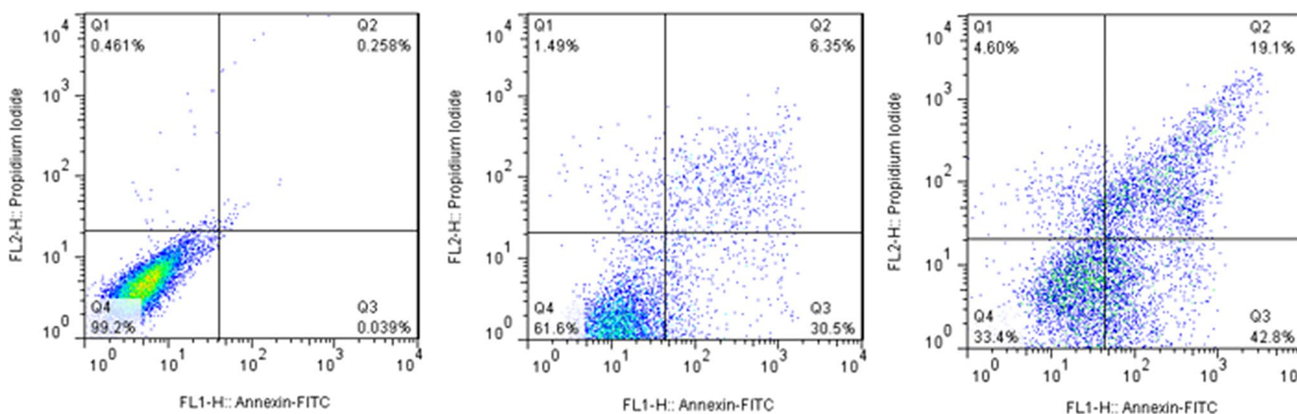

Fig. 8 Apoptotic effect of methanolic *lrRz* extract of *Iris barnumiae* on cell lines (PC3)

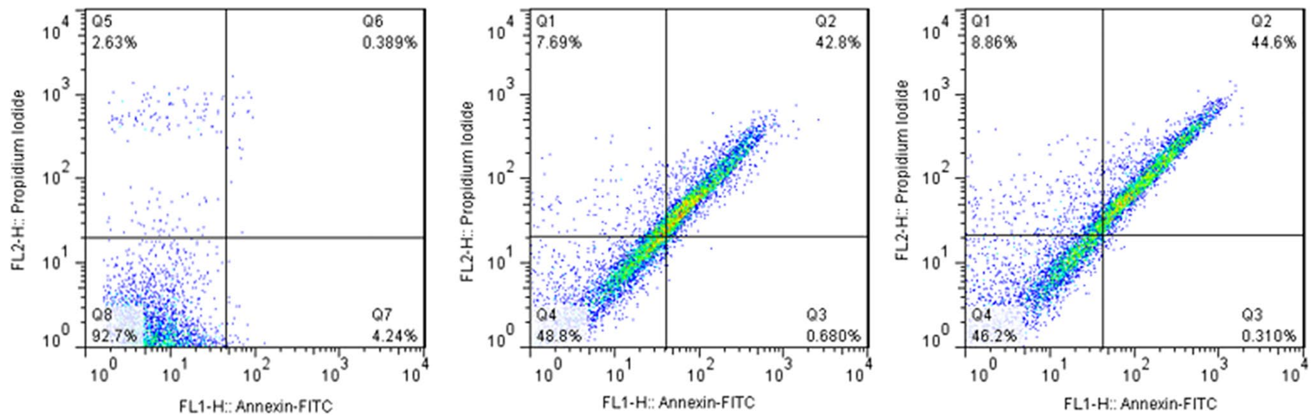

Fig. 9 Apoptotic effect of methanolic lrRz extract of *Iris barnumiae* on cell lines (U-87MG)

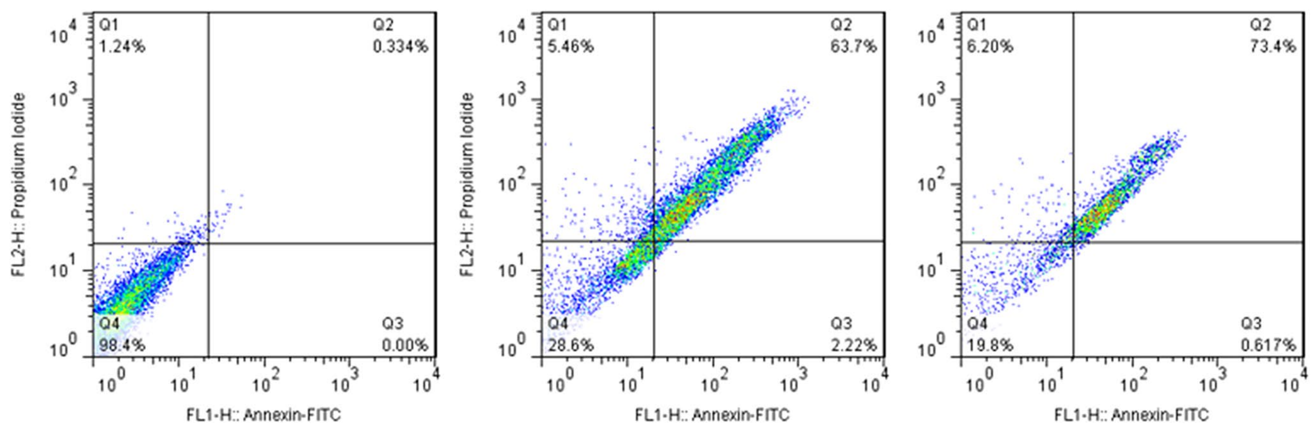

Fig. 10 Apoptotic effect of methanolic lrRz extract of *Iris barnumiae* on cell lines (MCF-7)

methanolic extract was more affected compared to MCF-7 and U87-MG (both cells with an IC_{50} value of > 250 μg/mL) cell lines (Fig. 6). However, both extracts of lrF and lrRz performed low effect on cell cancer compared to standard cis-platin (Fig. 7). Of note, the result from MeOH, lrRz extract of this study and effect on MCF-7 cancer cell was greater when compared with MeOH rhizomes of *Iris pseudopumila* (> 100 μg/mL) [29].

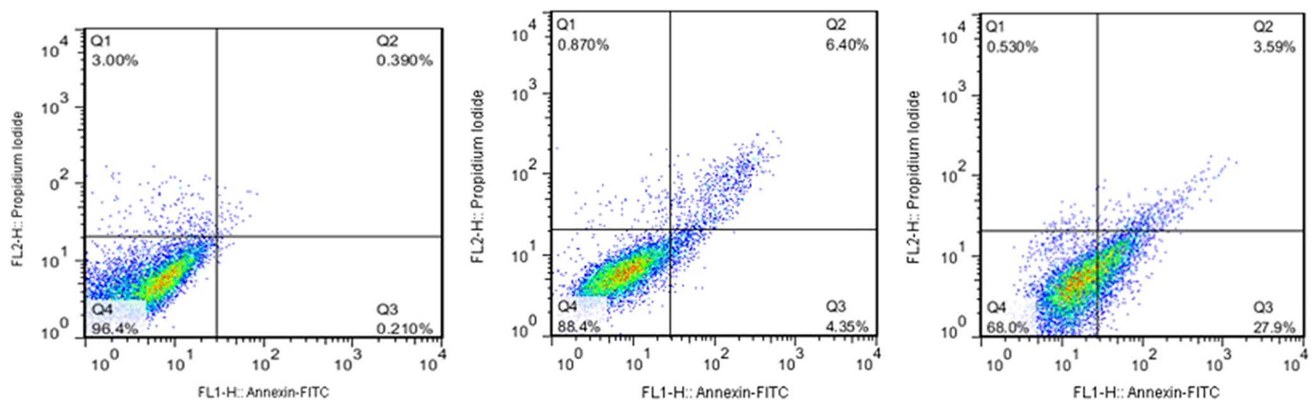

Fig. 11 Apoptotic effect of methanolic *lrRz* extract of *Iris barnumiae* on cell lines (HT-29)

Table 4 The percentage of cell populations in different stages (early apoptosis, late apoptosis, and necrosis)

PC3	Necrosis	Early apoptosis	Late apoptosis
Con	0.461	0.039	0.258
[IrRz] = 100 µg/mL	1.49	30.50	6.35
[IrRz] = 200 µg/mL	4.60	42.80	19.1
U-87MG	Necrosis	Early apoptosis	Late apoptosis
Con	2.63	4.24	0.389
[IrRz] = 146 µg/mL	7.69	0.68	42.80
[IrRz] = 292 µg/mL	8.86	0.31	44.60
MCF-7	Necrosis	Early apoptosis	Late apoptosis
Con	1.24	0.00	0.334
[IrRz] = 95.1 µg/mL	5.46	2.22	63.70
[IrRz] = 190.2 µg/mL	6.20	0.617	73.40
HT-29	Necrosis	Early apoptosis	Late apoptosis
Con	3.00	0.39	0.21
[IrRz] = 78.2 µM	0.87	6.40	4.35
[IrRz] = 156.4 µM	0.53	3.59	27.9

3.4 Apoptosis-inducing effects

The apoptosis-inducing potential of the extracts was further tested in cancer cells using the Annexin-FITC/propidium iodide double staining flow cytometric assay. The Annexin/PI assay also confirmed the ability of the extracts to induce early and late apoptosis. Unlike necrosis, apoptosis is an important cell death mechanism that does not trigger an inflammatory response that occasions collateral destruction of normal cells in the surrounding microenvironment [30].

The analysis was done on the death-inducing mechanisms of both lrF and lrRz plant crude extracts. The results showed a concentration-dependent apoptotic inducing ability of the extract only for lrRz; however, we did not get any results for lrF. As a necessary corollary of the results of the cytotoxicity assay. The results showed that the induction of cytotoxicity observed occurs through the mechanisms associated with apoptosis [31]. Generally, the extract induced apoptosis in a concentration-dependent manner. The PC3 cell line was directly sensitive to the concentration extract when compared with other cell lines as shown in Figs. 8, 9, 10, and 11. The sensitivity of the U-87MG cells to the apoptosis-inducing potential of the extract was also low compared to other cell lines. Table 4 shows that after increased concentration, PC3 had undergone early apoptosis, but MCF-7, HT-29, and U87-MG cell lines entered a late apoptotic stage after increased concentration. The total apoptosis (early + late) rate of IrRz is significantly elevated against PC3, U-87MG, and MCF-7 cells after treatment with 1xIC50 of IrRz (Fig. 12, $p = 0.0006$, $p = 0.0003$, and $p = 0.00004$) with no statistically significant necrosis, respectively. HT-29 treated cells showed a significant apoptosis rate in 2xIC50 value ($p = 0.006$).

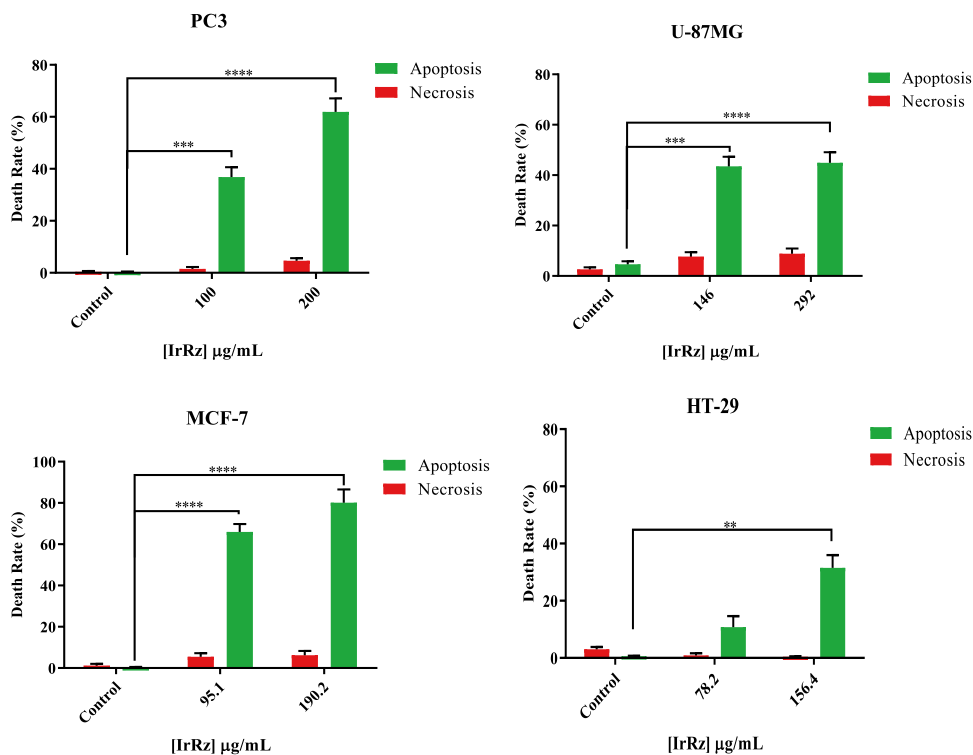

Fig. 12 Apoptosis and necrosis of *I. barnumiae* rhizome extract (IrRz) on cell lines PC3, U87MG, MCF-7, and HT-29. The analysis was performed in triplicate independent experiments with statistical threshold of $*p < 0.5$; $**p < 0.01$; $***p < 0.001$; and $****p < 0.0001$ using non-parametric one-way analysis of variance (ANOVA) test (Kruskal–Wallis)

4 Conclusion

Recently, several HPLC or LC–MS/MS techniques have been created and validated for the study of phytochemicals from various plant species. In our study, the investigation of methanol extracts of *lrF* and *lrRz* yielded the identification of 19 phytochemicals. The major compound secondary metabolic in both extracts of the *I. barnumiae* (lrF and lrRz) included quinic acid. However, the amount of lrF (48.337) was much higher than that in lrRz (28.133). which can be considered a source of quinic acid. Additionally, extracts of flower part (lrF) and the rhizome part (lrRz) of the *Iris barnumiae* against PC3, MCF-7, HT-29, and U-87MG as anticancer what found here is that the rhizome performs effect better than the flower but both extracts performed low effect on cell cancer compared to standard cis-platin. Generally, the extract induced apoptosis in a concentration-dependent manner. The results show that after increase in concentration, PC3 had undergone early apoptosis but MCF-7, HT-29, and U87-MG cell lines entered a late apoptotic stage after increase in concentration.

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Author contribution Fuad Abdullah: data curation, writing—original draft. Conceptualization, methodology, software investigation. The author read and approved the manuscript.

Data availability The data findings generated during this experiment are found within the published article. Details that back up the current outcomes are available upon request.

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Declarations

Ethical approval The study does not include human or animal subjects.

Conflict of interest The authors declare no competing interests.

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