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Euphorbia granulata Forssk: Evaluation of antioxidant activity, cytotoxicity, and apoptosis induction in breast cancer cells

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ABSTRACT

Euphorbia is one of the six largest genera of flowering plants. The Euphorbia species are widely distributed throughout the world. So, the aim of this study was to investigate the phenolic compounds profile, antioxidant activity, cytotoxicity, and apoptosis induction of *Euphorbia. granulata* extract (EGE) on breast cancer cells. In this experiment, we used HPLC analysis to attain the phenolic and flavonoid compound profile. The total phenolic and flavonoid content (TPC and TFC) were evaluated by classic procedures. Besides, the antioxidant activity of the extract was assessed. MTT assay was performed and caspase-3 activity was also measured to detect the effect of *E. granulata* on the MCF-7 cell line. Rutin with the amount of 6.40 μg/mg extract was identified as the main component in EGE. The extract was rich in TPC and showed high free radical scavenging activity (IC_{50} = 22.96 ± 0.84 μg/mL). Based on MTT assay results, EGE exhibited anti-proliferative activity on MCF-7 cells with the IC_{50} value of 25.17 ± 0.95 μg/mL. EGE also affected MCF-7 cells by reducing cell viability via the induction of apoptosis. So, the treatment of the cells by EGE exhibited an increase in caspase-3 activity. These findings indicated that EGE has a rich source of phenolic compounds and the extract can be a potent anticancer agent in inducing apoptosis in breast cancer cells and probably other cancer cells.

1. Introduction

Euphorbia is one of the six largest genera of flowering plants. The genus belongs to Euphorbiaceae family, which is one of the largest families of higher plants. The Euphorbia species are widely distributed around the world (Vasas and Hohmann 2014; Ernst et al., 2015). The plants of Euphorbia genus have many medicinal uses that have been reported in the literature. For instance, *E. formosana* is used to treat liver cirrhosis, rheumatism, scabies, herpes zoster, and photoaging (Hsieh et al., 2013). *E. lunulata* is used to cure noncancerous growths, cancerous ailments (Fu et al., 2018). *E. macroclada* and *E. gaillardotii* are used for the treatment of rheumatism, swelling, and wart remover (Ertas et al., 2015). *E. caducifolia* possess antitumor properties (Bano et al., 2017).

Abbreviations: AscA, ascorbic acid; BHT, butylhydroxytoluene; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EDTA, ethylenediaminetetraacetic acid; EGE, *Euphorbia granulata* extract; FIC, ferrous ion chelating ability; IC_{50}, half maximal inhibitory concentration; MCF-7 cell line, Michigan cancer foundation-7 cell line; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RSA, radical scavenging activity; SD, standard deviation; TPC, total phenolic content; TFC, total flavonoid content; TOC, α-tocopherol

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Antitumor, antimicrobial, antiviral, immune-enhancing, sedative and analgesic activities have been reported as the pharmacological properties of *E. fischeriana* (Jian et al., 2018). *E. dracunculoides* is used to cure warts, snake bite, and epilepsy (Khattak et al., 2017). In traditional Chinese medicine, cancer is being treated using *E. fischeriana, E. ebracteolatais* and *E. tirucalli* (Wang et al., 2011; Yuan et al., 2016; Silva et al., 2019). *E. sororia* has been used for the treatment of abdominal pain and distention (Huang and Aisa 2010). *E. hirta* can be considered as the most famous species of the genus. Several medicinal uses for the plant are reported in the literature. The plant is used to cure of inflammation, respiratory system disorders, poisonings, circulatory system disorders, laryngeal spasms, digestive system disorders, genitourinary system disorders, infections/infestations, pain, skin/subcutaneous cellular tissue disorders, injuries, sterility, nutritional disorders, sensory system disorders, rheumatism, endocrine system disorders, pregnancy/birth/puerperium disorders, menstrual problems (Ernst et al., 2015; Kwan et al., 2016).

The various pharmaceutical uses of Euphorbia plants depend on their secondary metabolites. A few studies reported the phytochemical constituents of different Euphorbia species; briefly, tannins, flavonoids, alkaloids, phenolic compounds, coumarins, cyanogenic glycosides, sterols, triterpenes, and diterpenes were isolated from different plants of the genus (Awaad et al., 2017; Yu et al., 2008; Rozimamat et al., 2019).

For this study, *Euphorbia granulata* was chosen to evaluate a few phytochemical and biological properties of the plant extract. The research focused on the antioxidant activity, HPLC analysis to identify phenolic and flavonoid compounds, investigation on the cell mortality of breast cancer cell line, and induced apoptosis on MCF-7 cell line quantified by flow cytometry technique for the first time. So far, 70 species of Euphorbia have been reported from Iran that comprises *E. granulata* (Fallahian et al., 2017). The plant is variable densely growing to almost expose much-branched bowed low in nature annual or perennial herb (Anwer et al., 2015). The plant has several pharmaceuticals uses in the traditional medicine of different countries. For example, *E. granulata* possess antioxidant, antibacterial, antifungal, diuretic, anti-ulcerative colitis and spasmolytic properties (Ahmad et al., 2019), anti-ulcerative colitis (Awaad et al., 2013), antifungal (Ahmad et al., 2012). The latex of E. granulata is internally used to expel intestinal worms, its latex is also used as a purgative, anthelmintic and diuretic agent (Saleem et al., 2015).Some species of this genus are used in traditional Iranian medicine to cure skin diseases, gonorrhea, migraines, intestinal parasites, warts, and purgative (Ayatollahi et al., 2016). Thus, in this study, the antioxidant activity, cytotoxicity, and apoptotic influences of E. granulata extract on the breast cancer cells were investigated.

2. Materials and methods

2.1. Plant material

The plant, *Euphorbia granulata*, was collected in its natural habitat Sabzevar, Khorasan-Razavi province, Iran (GPS: 36°15′12″N, 57°40′36″E) at an elevation of 1085 m situated along Tohid Shahr road. The plant species was identified by a botanist in the biology department at Hakim Sabzevari University. The voucher specimen, designated 721, was deposited at the Hakim Sabzevari University Herbarium.

2.2. Preparation of plant extract

At first, the collected plant was dried. Then its chopped parts were macerated in methanol for 72 h. The solvent was filtered and then evaporated under low pressure in a Buchi evaporator machine. Finally, the pure extract (EGE) was dried in an oven at 50 °C.

2.3. Antioxidant activity assays

Determination of total phenolic content (TPC), total flavonoid content (TFC), radical scavenging activity (RSA), ferrous ion chelating (FIC) was carried out according to the previous studies (Mahdavi et al., 2017; Hosseinpoor Mohsen Abadi et al., 2016). The assays are explained briefly:

TPC: First, a mixture of Folin- Ciocalteu reagent (10% in distilled water), 1 mL of the extract (100 μg/mL in methanol), 3 mL of distilled water, and 4 mL of Na2CO3 (5%) was prepared and kept in dark for 120 min at room temperature. Next, the mixture absorbance was read at 760 nm. The analyses were run in triplicates. Finally, TPC was measured as gallic acid equivalent (GAE), that is, mg of gallic acid equivalent per gram of extract (mg GAE/g extract).

TFC: The absorbance of a mixture of 1 mL plant extract (100 μg/mL) and 1 mL of AlCl3 (2%) was measured at 415 nm. The analyses were run in triplicates. TFC amount was obtained using a standard curve of rutin. TFC was expressed in mg of rutin equivalent per gram of dried extract (mg RuE/g extract).

RSA: 2 ml of the plant extract at different concentrations within 1.5 mL of DPPH (0.1 mM) was shaken and kept for 90 min at room temperature in the dark. Then, the absorbance was read at 517 nm. The RSA was calculated using the following equation:

$$\mathrm{RSA}\% = \left[(\mathrm{Ac} - \mathrm{As})/\mathrm{Ac}\right] \times 100$$

Where “Ac” is the absorbance of the control and “As” is the absorbance of the extract.

FIC: Plant extract (1 ml at different concentrations), FeSO4 (100 μL, 2 mM), distilled water (2 mL), and ferrozine (200 μL, 5 mM) was prepared. After 10 min, the absorbance was read at 562 nm. All measurements were carried out in triplicates. FIC for the plant extract was determined using the following equation:

$$\%\ \mathrm{Inhibition} = \left[(\mathrm{Ac} - -\mathrm{As})/\mathrm{Ac}\right] \times 100$$

Where “Ac” is the absorbance of the control and “As” is the absorbance of the sample.

2.4. HPLC analysis and identification of the main compounds

The *Euphorbia granulata* extract with 10 mg/mL concentration in methanol was analyzed by HPLC method (Waters 2695 (USA) (Zengin et al., 2017). The chromatographic assay was performed on a 15 cm × 4.6 mm with pre-column, Eurospher 100–5 C_{18} analytical column provided by Waters (Sunfire) reversed-phase matrix (3.5 μm) (Waters). Elution was carried out in a gradient system with methanol as the organic phase (solvent A) and distilled water (solvent B) with the flow-rate of 1 mL min^{-1}. Peaks were monitored at 195–400 nm wavelength. Injection volume was 20 μL and the temperature was maintained at 25 °C. The identification of the compounds was achieved by comparison of their retention time and UV–Vis. spectral reference data with those of the standard controls. The levels of the different compounds were extrapolated from calibration standard curves.

2.5. MTT assay

Breast cancer cell line (MCF-7) was purchased from Pasteur Institute (Iran). The Dulbecco's modified Eagle's medium (GIBCO, England) supplemented with 10% fetal bovine serum (GIBCO, England) and 5% penicillin (Sigma Aldrich, USA) was used. In this study, cytotoxicity of EGE on MCF-7 cell proliferation was evaluated with MTT (3-(4, 5-

dimethylthi-azol-2-yl)−2, 5-diphenyltetrazolium bromide) assay according to the previous study with some modifications (Mahdavi et al., 2019). The cells were evenly distributed (5×10^3cells/well) in a 96-wells plate and incubated in a humidified incubator at 37 ˚C with 5% CO_2, overnight. Then the cells were treated with EGE at different concentrations (20–200 μg/mL) and incubated for 24, 48, and 72 h. In the next stage, the medium in each well was replaced with 20 μl MTT (5 mg/mL in PBS) and incubated for 4 h at 37 °C. The crystals of purple-blue formazan were dissolved in 100 μL dimethyl sulfoxide (DMSO) and the absorbance was measured at the wavelength of 570 and 630 nm (control wavelength) on a 96-well plate reader (Thermo Lab systems, Franklin, MA USA). IC_{50} (concentration of the extract that achieved a 50% of mortality) of EGE was calculated using the equation of mortality in term of concentration. The measurements were carried out in triplicate.

2.6. Annexin-V/PI dual staining assay

A quantitative assessment of apoptosis was carried out using fluorescein isothiocyanate (FITC) Annexin V Apoptosis Detection Kit I (BD Biosciences, Waltham, MA). Briefly, MCF-7 cells cultured (1.5×10^5 cells) overnight before the treatment with EGE. Then the cells treated with the IC_{50} value of the extract (25.00 μg/mL) for 24, 48, and 72 h. Next, cells collected and washed twice with cold PBS and trypsin. The cells re-suspended in binding buffer 10% (Bioscience USA) and then centrifuged at 1500 RPM for 15 Min. At the next step, the cells were transferred to a tube, 5 mL of FITC conjugated Annexin V (Annexin V-FITC) and 5 mL of propidium iodide (PI) were added and incubated for 15 min at room temperature in the dark. Samples were diluted with binding buffer and analyzed by a flow cytometer (CYTEK, DxP Athena CYTEK Biosciences). Data from 10^4 cells were collected in each data file. Four different populations of cells are easily categorized: viable cells (are unlabeled), early apoptotic (have only bound Annexin VFITC), necrotic (have been stained with PI), and late apoptotic/necrotic (have both bound Annexin V-FITC and been labeled with PI). The fluorescence distribution was shown as a two-color dot plot analysis, and the percent of fluorescent cells in each section was identified (Kwan et al., 2016).

2.7. Caspase-3 activity assay

The measuring of the caspase-3 activity test was carried out according to a previous study with some modifications (Zekri et al., 2015). Briefly, The MCF-7 cells were treated by different concentrations of EGE. Next, the cells were lysed, 10 μg of the supernatant was mixed with 90 μl of buffer and 20 μl of the caspase-3 substrate (acetyl-Asp-GluVal-Asp p-nitroanilide) in a 96-well. The mixture was incubated for 4 h at 37 ˚C. Fold changes in caspase-3 activity were evaluated by measuring the released p-nitroanilide concentrations from the substrate at 405 nm in an ELISA reader. The active caspase-3 concentrations were calculated from a standard curve constructed with known concentrations of p-nitroanilide.

3. Results

3.1. Total phenolic content (TPC) and total flavonoid content (TFC)

TPC and TFC results of EGE are exhibited in Table 1. According to the results, 372.2 mg GAE/g was calculated for TPC of the extract; while, 17.04 mgRuE/g was measured for TFC of E. granulata methanolic extract.

Table 1
Total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity (RSA), and ferrous ion chelating ability (FIC) of *Euphorbia granulata* extract.

	TPC mg GAE/g extract	TFC (mgRU/ extract)	RSA IC_{50} (μg/mL)	FIC IC_{50} (μg/mL)
EGE	458.33 ± 2.88	31.92 ± 0.19	22.96 ± 0.84	172.42 ± 0.32
BHT	–	–	17.42 ± 0.49	–
TOC	–	–	35.64 ± 0.56	–
EDTA	–	–	–	68.24 ± 1.21
AscA	–	–	–	1480.02 ± 3.20

Values are presented as means ± SD, (n = 3). DPPH: 2,2-diphenyl-1-picrylhydrazyl; EGE: *Euphorbia granulata* extrac; BHT: Butylhydroxytoluene; TOC: α-tocopherol; EDTA: Ethylenediaminetetraacetic acid; AscA: ascorbic acid.

3.2. Radical scavenging activity (RSA) and ferrous ion chelating (FIC)

The results are shown in Table 1. The radical scavenging activity of the extract with IC_{50} of 22.96 ± 0.84 μg/mL was more than the positive controls of α-tocopherol but less than BHT. For FIC assay E. granulated extract with IC_{50} of 166.01 ± 0.41 μg/mL was more active compared to the ascorbic acid, while the ability of EGE for chelating of ferrous ions was less than positive control of EDTA.

3.3. HPLC chromatogram of EGE

Fig. 1 illustrates the HPLC chromatogram of EGE. The results of the analysis are showed in the Table 2. Among the four selected standards including GA, Ru, Qu, and CA, the analysis approved the presence of GA, Ru, and Qu, while, CA was not found in the extract. Rutin with the amount of 6.40 μg/mg extract was the main compound followed by GA and Qu with a value of 4.10 and 0.28 μg/mg extract respectively.

3.4. Cytotoxic evaluation using MTT assay

The results of the cytotoxic assay are shown in Fig. 2. It displays concentration-dependent cytotoxicity on MCF-7 cell line at 72 h. The results show that the degree of inhibition of extract on cancer cells depends on the extract concentration treated on the cells. In this way, by increasing the concentration of extract, the biochemistry of cells decreases and in contrast, the toxicity of extract increases. The extract exhibited an acceptable cytotoxic effect on MCF-7 cells. The value of 25.17 ± 0.95 μg/mL was calculated for the extract IC_{50}.

3.5. Induced apoptotic activity using Annexin/PI dual staining

The induction of apoptosis by EGE was quantitatively determined by Annexin V-FITC and propidium iodide fluorescence staining (Fig. 3). For this assay, MCF-7 cells were treated by E. granulata extract with a concentration of 25.0 μg/mL close to the obtained IC_{50} in the MTT test. The treatment of MCF-7 with EGE significantly increased the percentage of Annexin V-staining positive cells (total apoptotic cells) as compared to the control. Table 3 tabulated the assay results for 24, 48, and 72 h. The maximum necrosis was 1.8% after 24 h (Q1), the highest late apoptosis (15.32%) was obtained after 72 h (Q2), and the highest early apoptosis (12.72%) was also calculated after 72 h. Overall, 28.04% was calculated for the induced apoptosis of *Euphorbia granulata* extract on breast cancer cells.

3.6. Caspase-3 activity

Caspases play a vital role in programmed cell death and caspase-3 as one of the most important members, has a significant effect on apoptosis by cleaving the cellular substrates (Abou-Hashem et al., 2019). Ac-

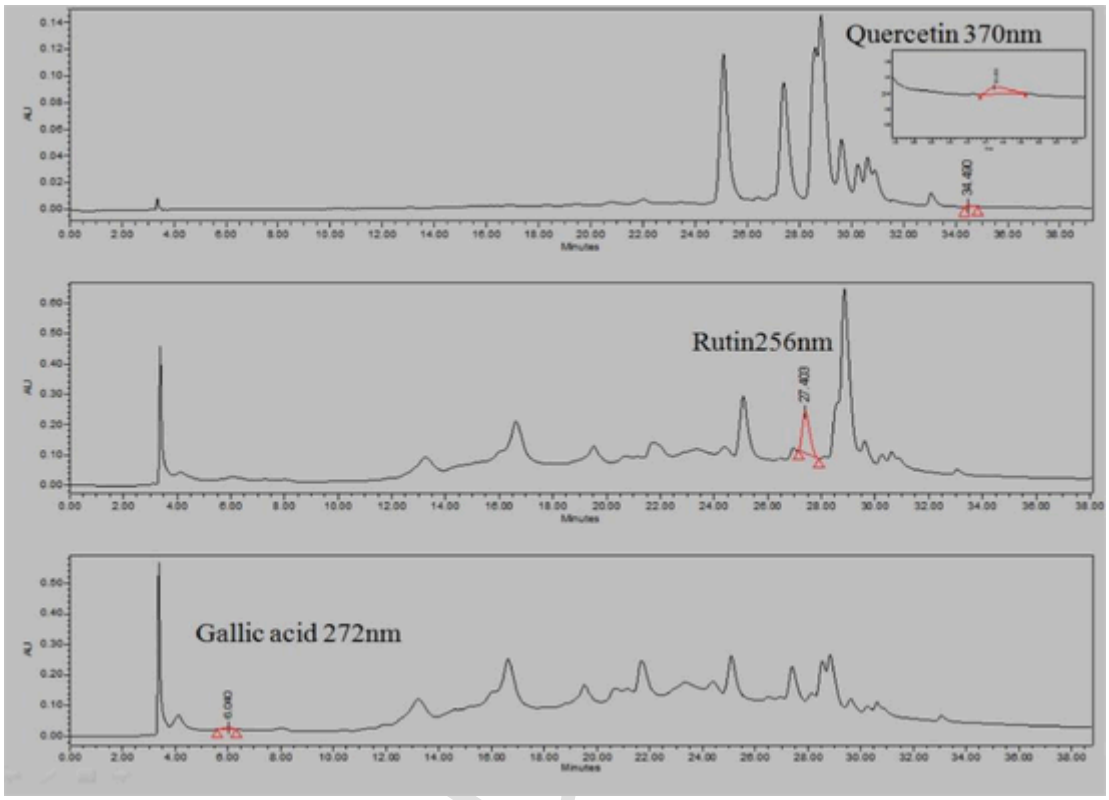

Fig. 1. HPLC chromatogram of *Euphorbia granulata* extract.

Table 2
High-performance liquid chromatography analysis of phenolic compounds content of *Euphorbia granulata* extract.

Compounds	UV λ_{Max} (nm)	RT(min)	µg/mg extract
Gallic acid	272	6.1	4.10
Caffeic acid	324	16.8	ND*
Rutin	256	27.0	6.40
Quercetin	370	34.37	0.28

1.1 *ND: not detected

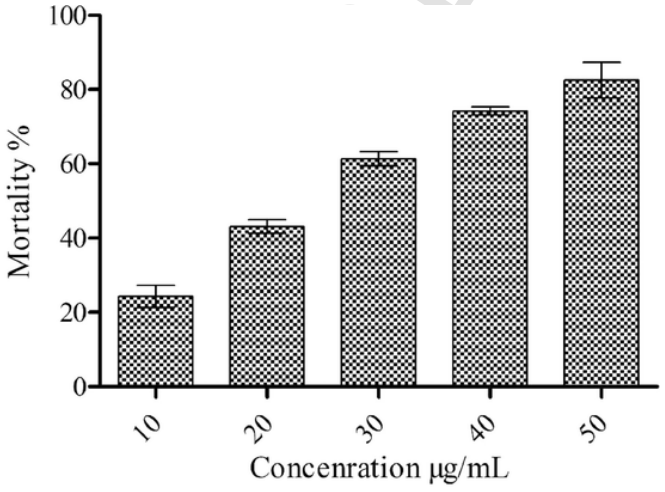

Fig. 2. Cytotoxic evaluation using MTT assay. Concentration-dependent effects of *Euphorbia granulata* extract on MCF-7 cells viability after cells were cultured with various concentrations of the extract. The error bars are ± Standard Deviation (n = 3).

cording to the results (Fig. 4). EGE increased enzymatic activity of the caspase-3. The activity of the released caspase-3 has increased after the treatment of the cells by different EGE concentrations from 14.8 ± 2.3 to 28.9 ± 2.2%.

4. Discussion

This study was carried out to investigate the protective and curative effects of *E. granulata* extract on breast cancer cells. We monitored the antioxidant activity, cytotoxicity, and apoptotic induction in cell lines. Many studies described the effect of medicinal herbs on breast cancer cells, and the corresponding mechanism, using cancer cell culture. Zengin et al. (2017) have reported 34.56 mgGAE/g and 25.41 mgRE/g for TPC and TFC of E. denticulate extract that is less than the selected plant in our research. The plant also showed acceptable FIC and RSA in the related assays. In a few kinds of research, similar to our funding the amount of TPC was more than TFC. For example, the methanolic extract of *E. gaillardotii* and *E. macroclada* extracts (Ertas et al., 2015). *E.spinidens* (Karimi et al., 2016), *E.retusa* (Lahmadi et al., 2019), *E. lathyris* (Zhang et al., 2017), *E. grandialata* (Ismaila et al., 2017), *E. hirta* (Basma et al., 2011; Asha et al., 2016), *E. paralias* and *E. terracina* (Jannet et al., 2017). In addition, the plants extracts were potent agents in radical scavenging activity. Based on HPLC or LC/MS analysis reports of different Euphorbia species, which have identified the amount of the selected phenolic and flavonoid compounds in this research, GA and Ru were more than Qu and CA. 2.77 and 0.45 µg/g have been reported for GA and CA in *E. denticulata* extract, but Qu was not detected for the plant extract (Zengin et al., 2017). For the extract of *E. gaillardotii* and *E. macroclada.*, the value of 6.80 and 1.97 µg/mg were reported for GA; 0.07 and 0.01 µg/mg for Ru; trace and 0.06 µg/mg for CA respectively; and the value of 0.12 µg/mg for Qu in *E. macroclada* extract (Ertas et al., 2015). Lahmadi et al. have reported the presence of Qu in the extract of *E. retusa* (Lahmadi et al., 2019).

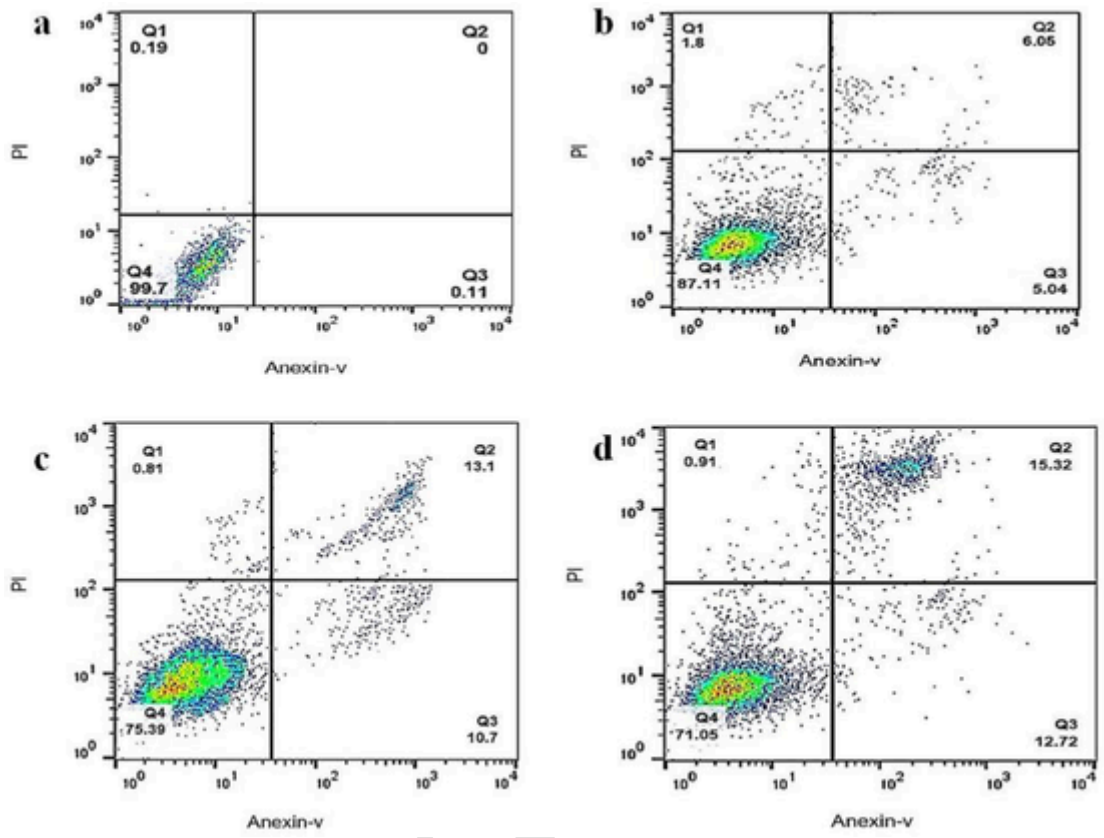

Fig. 3. Induced apoptotic activity using Annexin/PI dual staining. *Euphorbia granulata* extract induced apoptosis in MCF-7 cells. Flow cytometric evaluation of induced apoptosis using Annexin-V and Propidium iodide (PI) staining. (a: control; b: after 24 h treatment; c: after 48 h treatment; d: after 72 h treatment). Q1: necrosis; Q2: late apoptosis, Q3: early apoptosis, Q4: living cells.

Table 3
The apoptosis results of *Euphorbia granulata* extract on MCF-7 cells.

	Time (h)	Q1 (%)	Q2 (%)	Q3 (%)	Q4 (%)
Control		0.19	0	0.11	99.70
Sample	24	1.80	6.05	5.04	87.11
	48	0.81	13.10	10.70	75.39
	72	0.91	15.32	12.72	71.05

Q1: necrosis; Q2: late apoptosis, Q3: early apoptosis, Q4: living cells.

In our review of the literature, we found several studies on cytotoxicity of Euphorbia species. for instance *E. Tirucalli* inhibited the proliferation of MCF-7 with concentration more than 30 μg/mL (Choene and Motadi 2016); *E. caducifolia* prevented the growth of MCF-7 with IC_{50} of 61 μg/mL (Bano et al., 2017); *E. hirta* exhibited the toxic ability against the breast cell cancer with IC_{50} value of 25.26 μg/mL (Kwan et al., 2016). A comparison of our funding in this research with the previous studies, those mentioned above, it is revealed that *E. granulata* extract is more potent antitumor agent than the other Euphorbia species against breast cancer cells of MCF-7.

Previous studies have reported induction of apoptosis of Euphorbia species extracts on different cancer cells. Our literature review indicates the following activity of Euphorbia species: *E. lunulata* extract against human gastric tumor (Fu et al., 2018); *E. cheiradenia* against leukemic cells (Amirghofran et al., 2006); *E. microciadia, E. osyridea*, and *E. heteradenia* against uterine cervical cancer cells, myelogenous leukemia, and Fen bladder carcinoma (Shekofteh et al., 2017); hot-water extract of *E. formosana* against leukemia cell (Hsieh et al., 2013); *E. esula* extract against human gastric carcinoma cells (Fu et al., 2016); *E. hirta* against MCF-7 cell line. Also, the effect of other plant extracts, including Polygonatum odoratum extract and ionic liquid extract of Graviola fruit, on

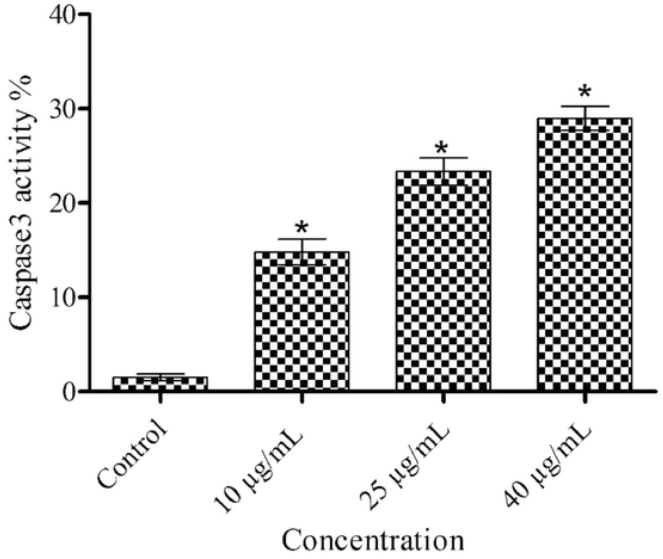

Fig. 4. The effect of *Euphorbia granulata* extract on caspase-3 activity. The activity was determined by measuring the concentrations of p-nitroanilide released from the substrate due to enzymatic activity of caspase-3. *$P < 0.05$ compared to the control. The error bars are ± SD ($n = 3$).

apoptosis in breast cancer cells has been presented (Tai et al., 2016; Daddiouaissa et al., 2019).

Apoptosis is a form of cell death. Both physiological (including programmed cell destruction, physiologic involution, and normal destruction of cells) and pathological (including Anticancer drug, Progressive cell death, and Pathologic atrophy of organs and tissues) factors could

be involved in the apoptosis (Wong 2011). Since resisting death and avoiding apoptosis is one of the ten cancer hallmarks , conducting a study on the process of apoptosis could be beneficial for oncology researches (Jian et al., 2018). It is obvious that the apoptosis ability of the plant extracts depends on the chemical constituents of their extract. Some research teams have reported the apoptosis potent of the isolated compounds from Euphorbia plants. Euphol from *E. tirucalli* on gastric cancer cells (Lin et al., 2012); Two isolated lathyrane diterpenes from *E. lathyris* on human lung carcinoma cells (A549) (Lin et al., 2017; Zhang et al., 2011); a tetrahydroingenol diterpene on human melanoma cell (Fallahian et al., 2017). The apoptosis ability of EGE against MCF-7 can be contributed to the presence of phenolic and flavonoids compounds in EGE, which was approved by HPLC analysis. According to many previous studies, gallic acid (Hsu et al., 2011); rutin (Iriti et al., 2017); and quercetin (Wu et al., 2018) have been potent agents in induced apoptosis or MTT assay against the breast cancer cells. In one of the most recent studies, the researchers found that the ethanolic extracts of C. tiglium (CTL) and E. royleana (ERA) exhibit effective cytotoxic properties against human breast cancer cell lines. These extracts convinced cell cycle arrest followed by the stimulation of apoptosis through initiation of expression of caspases genes (Gull et al., 2022). In our current study, we also screened the anti-breast cancer potential of EGE. Therefore, these outcomes concluded that medicinal plants can be considered as a suitable source of biochemical agents with talented anti-cancer activity against breast cancer cells.

In a similar study, the induction of apoptosis and possible anticancer effects of two native Iranian medicinal herbs, Linum persicum and Euphorbia cheiradenia on leukemia cell lines were investigated. Accordingly, the impact of methanolic extracts of the herbs on prevention of cell proliferation and the induction of apoptosis were evaluated by MTT assay and flow cytometry. DNA fragmentation examination was also performed. The Results showed that various concentrations of L. persicum and E. cheiradenia have inhibitory possessions on different cell lines. Both cancer cell lines were highly sensitive for L. persicum with IC_{50} of 0.1 and 10 μg/ml, respectively. The outcomes also revealed a dose-dependent accumulation of cells in the sub-G1 phase. Besides, analyses of internucleosomal DNA fragmentation exhibited a classic DNA laddering in agarose gels for both extracts. Based on this, the cytotoxic properties of L. persicum and E. cheiradenia extracts on human tumor cell lines can be concluded and suggest that this potency may be partly due to the stimulation of apoptosis in leukemic cells (Amirghofran et al., 2006).

In conclusion, *Euphorbia granulata* extract (EGE) can be considered as a potent agent in free radical scavenging. HPLC analysis revealed that rutin and gallic acid were the main flavonoid and phenolic compounds in the plant extract respectively. In MTT assay, EGE inhibited cell proliferation of MCF-7 cells with a lower concentration compared to the extract of the other genus plants, which have reported previously. EGE also induced apoptosis on MCF-7 cells with a value of 28.04%. The caspase-3 activity was increased after the treatment of MCF-7 by EGE. These abilities of EGE has correlated to the plant secondary metabolites such as phenolic or flavonoid compounds that dominate in the plant extract. On the other hand, further researches are required to find the molecular mechanisms of the induced apoptotic of EGE on cancer cells.

Availability of supporting data

The authors stated also supporting data will be made available to others on request.

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CRediT authorship contribution statement

Behnam Mahdavi : Visualization, Writing – original draft. **Habibeh Zare :** Formal analysis. **Maryam Qorbani :** Investigation. **Hadi Atabati :** Investigation. **M. Reza Vaezi-Kakhki :** Formal analysis. **Amir Raoofi :** Visualization. **Vahid Ebrahimi :** Writing – review & editing.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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