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A screening of anti-breast cancer effects and antioxidant activity of twenty medicinal plants gathered from Chaharmahal va Bakhtyari province, Iran

[Tamizaje antitumoral contra cáncer de mama y actividad antioxidante de veinte plantas medicinales recolectadas en la provincia de Chaharmahal va Bakhtyari, Irán]

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Abstract

Context: Breast cancer is one of the most prevalent cancers that causes substantial numbers of deaths among women, worldwide. Medicinal plants can be used for discovering new anti-breast cancer drugs.

Aims: To investigate the antioxidant effects of twenty Iranian herbs and their anticancer effects against breast cancer cell lines (i.e. MDA-MB231 and MCF-7) compared with a non-cancerous human cell line (HDF).

Methods: In this study, the plant samples were collected from different regions of Chaharmahal va Bakhtyari province in Iran and their hydroalcoholic (ethanol: water; 70:30) extracts prepared by maceration method. Antioxidant activity was investigated by DPPH with reference to butylated hydroxytoluene (BHT). Anticancer effects were investigated by MTT colorimetric assay.

Results: Most of the plants examined in this study had higher antioxidant activity than that of BHT. *Satureja bachtiarica* (37.27 ± 1.56 µg/mL), *Plantago lanceolata* (43.19 ± 4.67 µg/mL), *Parietaria judaica* (45.34 ± 8.08 µg/mL), *Stachys inflata* (53.70 ± 1.80 µg/mL), and *Euphorbia szovitsii* (55.78 ± 1.37 µg/mL) inhibited the DPPH free radicals in the lower concentration compared to BHT (120.48 ± 1.42 µg/mL) and other plants. MTT assay showed that the lowest IC_{50}s values were observed for *E. microsciadia* and *E. szovitsii* among the examined plants on both breast cancer cell lines. There was no significant relationship between anticancer effects and antioxidant activity of the plants ($p>0.05$).

Conclusions: The examined plants might be considered as valuable resources of natural compounds that may possess anti-breast cancer properties.

Keywords: antioxidant activity; cytotoxicity; drug discovery; natural compounds; traditional medicine.

Resumen

Contexto: El cáncer de mama es uno de los cánceres más prevalentes que causa un gran número de muertes entre las mujeres en todo el mundo. Las plantas medicinales se pueden usar para descubrir nuevos medicamentos contra el cáncer de mama.

Objetivos: Investigar los efectos antioxidantes de veinte hierbas iraníes y sus efectos anticancerígenos contra líneas celulares de cáncer de mama (es decir, MDA-MB231 y MCF-7) en comparación con una línea celular humana no cancerosa (HDF).

Métodos: En este estudio, se recolectaron muestras de plantas de diferentes regiones de la provincia de Chaharmahal va Bakhtyari en Irán y sus extractos hidroalcohólicos (etanol: agua; 70:30) preparados por el método de maceración. La actividad antioxidante se investigó mediante DPPH con referencia al hidroxitolueno butilado (BHT). Los efectos anticancerígenos se investigaron mediante el ensayo colorimétrico MTT.

Resultados: La mayoría de las plantas examinadas en este estudio tenían mayor actividad antioxidante que la de BHT. *Satureja bachtiarica* (37.27 ± 1.56 µg/mL), *Plantago lanceolata* (43.19 ± 4.67 µg/mL), *Parietaria judaica* (45.34 ± 8.08 µg/mL), *Stachys inflata* (53.70 ± 1.80 µg / mL) y *Euphorbia szovitsii* (55.78 ± 1.37 µg/mL) inhibieron el radical libre DPPH en la concentración más baja en comparación con BHT (120.48 ± 1.42 µg/mL) y otras plantas. El ensayo MTT mostró que se observaron los valores de CI_{50} más bajas para *E. microsciadia* y *E. szovitsii* entre las plantas examinadas en ambas líneas celulares de cáncer de mama. No hubo una relación significativa entre los efectos anticancerígenos y la actividad antioxidante de las plantas ($p> 0.05$).

Conclusiones: Las plantas examinadas podrían considerarse como recursos valiosos de compuestos naturales que pueden poseer propiedades contra el cáncer de mama.

Palabras Clave: actividad antioxidante; citotoxicidad; descubrimiento de medicamento; compuestos naturales; medicina tradicional.

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INTRODUCTION

As one of the most common and main diseases of the community, cancer has imposed tremendous social, economic, and mental costs on humans. Globally, breast cancer has been one of the most leading cancers and the most common cancer in women. Many women every year die due to breast cancer (The International Agency for Research on Cancer, 2014).

Current drugs and therapies for cancer especially breast cancer cause certain side effects and have limitations causing the incidence rate of cancer to remain high despite recent advances in screening (Karimaian et al., 2017). It is therefore essential to seek out more efficient and novel therapies to reduce severity of cancer in the light of the complications of the current treatments, the resistance of different cancers to the current drugs, the stupendous costs of these cures, and the high prevalence of cancers (Ghanbari et al., 2014; Montazami et al., 2015; Tehrani et al., 2018).

The use of nature-based products such as medicinal plants is one of the approaches that have recently attracted the population attention (Yousefi et al., 2015; Asadi-Samani et al., 2016). In this regard, it seems necessary to seek out plants to fight cancer cells particularly in breast cancer, with fewer side effects, as alternatives to chemotherapy and overwhelming therapies, and treat cases who have acquired resistance to treatment. Indeed, medicinal plants can be used to prevent and treat diseases due to natural active compounds and, if appropriately used, cause no considerable side effects in the consumers (Kooti et al., 2017; Abbaszadeh et al., 2018). However, medicinal plants and their active compounds to treat specific disease(s) should be identified; in addition, the active doses of these plants and their compounds should be determined so that more effective drugs can be produced to treat various diseases such as cancers.

Taken together, this study designed to evaluate antioxidant activity of twenty medicinal plants from Chaharmahal va Bakhtiari province (Iran) and to determine their anticancer effects on breast cancer cell lines.

MATERIAL AND METHODS

Plant material

Between May to Sep 2015, the herbal samples gathered from various regions of Chaharmahal va Bakhtiari province (southwestern Iran) and identified botanically by Miss S. Khademian in Shiraz University of Medical Sciences and Dr. Shirmardi in Research Center for Agricultural & Natural Resources (Shahrekord, Iran). Medicinal plants in this study have been shown in the Table 1.

Preparation of extracts

The aerial parts of medicinal plants were cleaned and dried in the shade. The samples powdered using a mechanical grinder. For preparation each extract, 100 g of plants' powder was added to 400 mL of ethanol 70% at room temperature (RT) for 72 hr. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator (LabTech, Ev 311, MA, US). The concentrated hydro alcoholic extracts were dissolved in dimethyl sulfoxide 0.1% (DMSO; Sigma-Aldrich, St. Louis, MO, USA) (Jadhav et al., 2012; Foo et al., 2014) and then diluted in RPMI 1640 medium at a concentration of 5 mg/mL.

Preparation of cell lines

MCF7 and MDA-MB231 as human breast adenocarcinoma and HDF (Human Dermal Fibroblasts) as non-cancerous human cell line were used. Cells were obtained from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran).

Cell lines were cultured in Roswell Park Memorial Institute medium 1640 (RPMI 1640; Gibco) with 10% FBS and 1% penicillin-streptomycin (Sigma-Aldrich) in a humidified atmosphere with 5% CO_2 at 37°C.

Table 1. Iranian medicinal plants investigated in this study.

No.	Scientific names	Farsi name	Region	Family name	Yield (%)	Herbarium code
1	*Acanthophyllum glandulosum* Bunge ex Boiss.	Chobak nekaei	Saman	*Caryophyllaceae*	12.58	896
2	*Achillea wilhelmsii* K.Koch	Bomadaran	Saman	*Compositae*	10.76	207
3	*Echinophora platyloba* DC.	Khosharozeh	Saman	*Apiaceae*	19.01	249
4	*Euphorbia szovitsii* Fisch. & C.A.Mey.	Farfion	Saman	*Euphorbiaceae*	16.97	935
5	*Euphorbia microsciadia* Boiss.	Farfion	Saman	*Euphorbiaceae*	14.56	659
6	*Haplophyllum perforatum* (M. Bieb.) Vved.	Morde kazeb	Saman	*Rutaceae*	4.70	150
7	*Hertia angustifolia* (DC.) Kuntze	Karghich	Saman	*Compositae*	19.32	701
8	*Medicago sativa* L.	Yonjeh	Saman	*Leguminosae*	9.73	742
9	*Moriera spinosa* Boiss.	Kharmarjan	Shahrekord	*Brassicaceae*	19.16	623
10	*Onosma sericeum* Willd.	Gavzaban	Saman	*Boraginaceae*	12.74	841
11	*Parietaria judaica* L.	Goshmosh	Saman	*Urticaceae*	10.86	617
12	*Phlomis persica* Boiss.	Goshbareh Irani	Saman	*Lamiaceae*	12.28	700
13	*Plantago lanceolate* L.	Kardi (Barhang sarneyzei)	Saman	*Plantaginaceae*	14.58	252
14	*Salvia multicaulis* Vahl	Gol arvaneh	Sartishniz	*Lamiaceae*	21.87	301
15	*Satureja bachtiarica* Bunge	Marzeh Bakhtiyari	Saman	*Lamiaceae*	10.6	208
16	*Sophora alopecuroides* L.	Talkhbayan	Saman	*Leguminosae*	14.83	258
17	*Stachys inflata* Benth.	Sonbole badkonaki	Shahrekord	*Lamiaceae*	13.06	260
18	*Teucrium orientale* L. subsp. taylori. (Boiss.) Rech.f.	Maryam nokhodi sharghi Shirazi	Shahrekord	*Lamiaceae*	16.73	522
19	*Urtica dioica* L.	Gazaneh	Tange nikan, Kohrang	*Urticaceae*	9.91	303
20	*Ziziphora clinopodioides* Lam.	Kakoti kohi	Saman	*Lamiaceae*	9.24	253

Herbal samples gathered from various regions of Chaharmahal va Bakhtiari province (southwestern Iran) and identified botanically by Miss S. Khademian in Shiraz University of Medical Sciences and Dr. Shirmardi in Research Center for Agricultural & Natural Resources (Shahrekord, Iran). The aerial part of the plants macerated in ethanol (70%). The voucher specimens were prepared and deposited in Herbarium unit of Shahrekord University of Medical Sciences. In addition, the scientific names of plants were confirmed using The Plant List (http://www.theplantlist.org).

MTT colorimetric assay (3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay)

Cell viability was quantified by MTT colorimetric assay (Asadi-Samani et al., 2018; 2019). The cells were seeded in 96-well plates at 7.5 × 10^3 for MCF7, and 8 × 10^3 for HDF cells and incubated at 37°C. Twenty four hour after incubation, the medium was discarded and the cells were exposed with different concentrations of extracts (1 - 10 µg/mL). After 48 hr, the supernatants were removed and fresh medium containing 0.5 mg/mL

MTT was added to each well. After four hours of incubation, the supplement was removed, and the formazan crystals were dissolved in DMSO. The wells were read at 570 nanometer using ELISA reader (Stat-Fax 2100 microplate reader, Awareness Technology, Inc., FL, USA) with a reference wavelength of 630 nanometers.

Percentage of inhibition was determined as 1-(sample OD value/negative control OD value)] × 100. The IC_{50} value (the concentration with 50% cell inhibition) was calculated via the graph of inhibition percentage *versus* different extract concentrations through regression analysis and related models with a probit regression model.

DPPH scavenging assay (antioxidant activity)

Antioxidant property was determined by DPPH (2,2-diphenylpicrylhydrazyl) using Stat Fax® 2100 (Awareness Technology, Inc., FL, USA) at 517 nm wavelength with reference to a synthetic antioxidant, butylated hydroxytoluene (BHT). The results were indicated as the percentage of inhibiting free radicals using the graph plotted against the extracts concentration, and IC_{50} (the concentration with 50% DPPH free radical inhibition) was calculated (Moein et al., 2012).

Percentage of inhibition was determined as 1-(sample OD value/negative control OD value)] × 100. The IC_{50} value was calculated through regression analysis and related models with a probit regression model.

Statistical analysis

The dose–response curves of plants extracts were plotted using GraphPad Prism Software 3.0 (USA). IC_{50} values were calculated through regression analysis and related models with a probit regression model. All *in-vitro* experiments were carried out on three microplates with at least three wells. Also, the Spearman's correlation test was used for evaluation of relationship between antioxidant and anticancer activities. A value of $p<0.05$ considered statistically significant.

RESULTS

Cell viability results

In the Table 2, anticancer effects (expressed as IC_{50}s) of herbal samples on MCF-7 and MDA-MB231 has been shown. Medicinal plants with IC_{50} values more than 300 µg/mL were determined as inactive (Fadeyi et al., 2013).

E. microsciadia and *E. szovitsii* indicated the highest anti-breast cancer activities on cancer cell lines compared to other investigated medicinal plants. The lowest IC_{50} was 59.52 µg/mL which was found for *E. szovitsii* against MCF-7 cell line.

For determining the cytotoxicity activity of herbal samples on non-cancerous human cell line as a normal cell, the activity of effective extracts on HDF cell line was evaluated (Table 3). At IC_{50} concentration, *E. szovitsii* and *E. microsciadia* did not have any activity on HDF cells.

Antioxidant activity results

In the Fig. 1, inhibition rate of DPPH free radicals following utilization of selected medicinal plants against BHT (120.48 ± 1.42 µg/mL) was compared. Most of the plants had higher antioxidant activities compared with BHT. *Satureja bachtiarica* indicated the highest antioxidant activity. Also *E. szovitsii* and *E. microsciadia* had about 1.5 times and 3 times higher antioxidant activity, respectively compared to BHT.

Based on Spearman's correlation test, there was no significant relationship between anticancer effects and antioxidant activity of the plants ($p>0.05$).

Table 2. Anti-breast cancer activity of selected Iranian medicinal plants from Chaharmahal and Bakhtiari province.

Scientific names	MCF-7			MDA-MB-231		
	IC_{50} (µg/mL)	95% CI	R^2	IC_{50} (µg/mL)	95% CI	R^2
Acanthophyllum glandulosum	300<	-	-	283.30	256.6-312.8	0.9894
Achillea wilhelmsii	300<	-	-	300<	-	-
Echinophora platyloba	300<	-	-	300<	-	-
Euphorbia microsciadia	148.60	92.87-237.8	0.8235	235.40	202.0-274.2	0.9789
Euphorbia szovitsii	168.30	116.7-242.6	0.8843	59.52	47.51-74.58	0.9535
Haplophyllum perforatum	300<	-	-	300<	-	-
Hertia angustifolia	300<	-	-	300<	-	-
Medicago sativa	300<	-	-	300<	-	-
Moriera spinosa	300<	-	-	300<	-	-
Onosma sericeum	300<	-	-	300<	-	-
Parietaria judaica	300<	-	-	300<	-	-
Phlomis persica	300<	-	-	300<	-	-
Plantago lanceolata	300<	-	-	300<	-	-
Salvia multicaulis	300<	-	-	300<	-	-
Satureja bachtiarica	300<	-	-	300<	-	-
Sophora alopecuroides	300<	-	-	300<	-	-
Stachys inflata	300<	-	-	300<	-	-
Teucrium orientale L. *subsp.* taylori	300<	-	-	300<	-	-
Urtica dioica	300<	-	-	300<	-	-
Ziziphora clinopodioides	300>	-	-	300>	-	-

IC_{50} determined by MTT colorimetric assay. The percentage of inhibition was measured as [1- (optical density of test/ optical density of negative control)] × 100. The IC50 value (the concentration with 50% cell inhibition) was calculated via the graph of inhibition percentage versus different extract concentrations. IC_{50} values were calculated through regression analysis and related models with a probit regression model.

Table 3. Cytotoxicity of effective plant samples on breast cancer cell lines against HDF cell line.

Scientific names	IC50 (µg/mL)	95% Confidence intervals	R^2
Acanthophyllum glandulosum	74	70-78	0.9964
Euphorbia szovitsii	412	313-542	0.9870
Euphorbia microsciadia	451	336-605	0.9463

IC_{50} was determined by MTT colorimetric assay. The percentage of inhibition was measured as [1- (optical density of test/ optical density of negative control)] × 100. The IC_{50} value (the concentration with 50% cell inhibition) was calculated via the graph of inhibition percentage *versus* different extract concentrations. IC_{50} values were calculated through regression analysis and related models with a probit regression model.

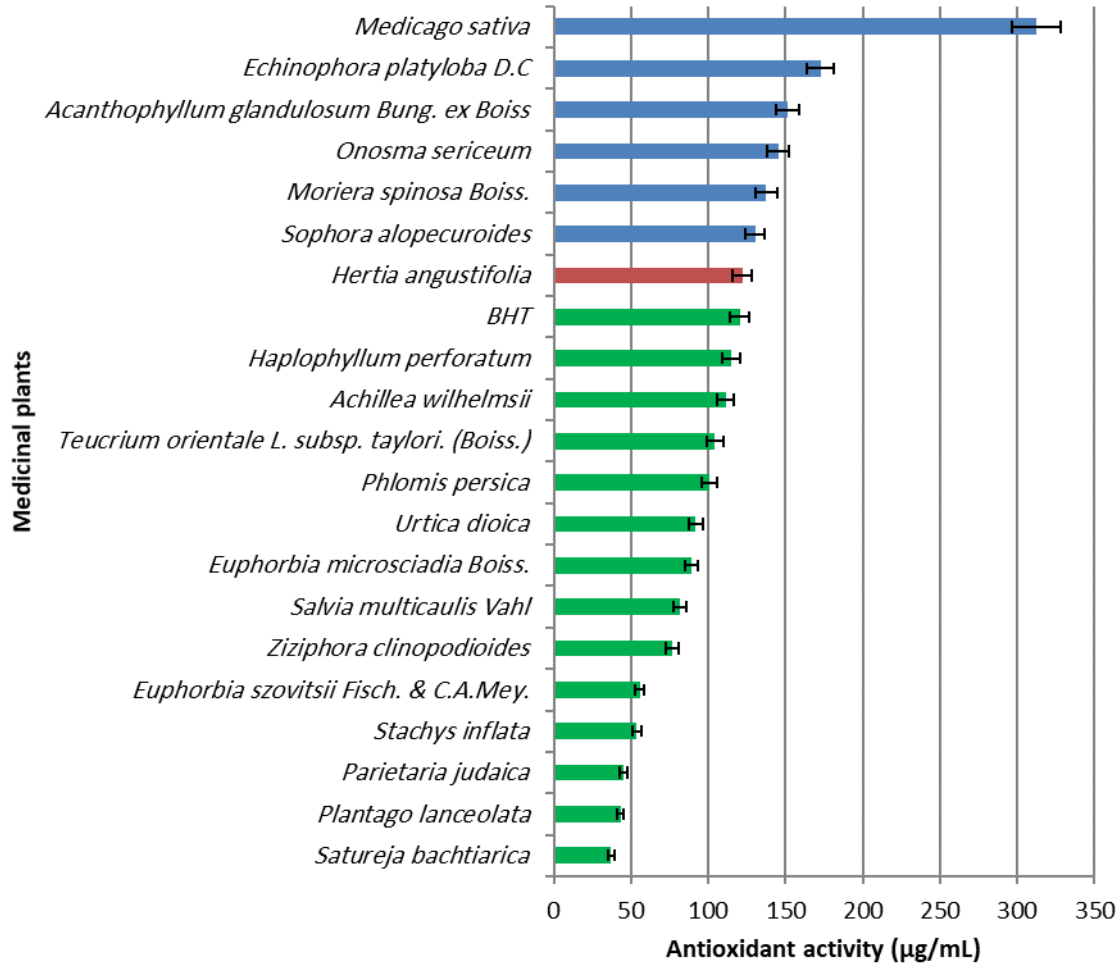

Figure 1. Antioxidant activity of medicinal plants gathered from Chaharmahal va Bakhtyari province compared with BHT.

Antioxidant activity of the plants was determined by DPPH free radical inhibition. The DPPH radical is a stable free radical, which is widely used in the evaluation of antioxidant capacity. Percentage of inhibition was calculated as 1-(sample OD value/negative control OD value)] × 100. The IC_{50} value was calculated through regression analysis and related models with a probit regression model. A low IC_{50} value indicates high antioxidant activity. Data has been indicated as mean ± SEM. Butylated hydroxytoluene (BHT) was used as a synthetic standard antioxidant.

DISCUSSION

Since the early 1950s, the plants have been extensively screened to discover new compounds to prevent cancer and be used in cancer chemotherapy and produce their derivatives. Two drugs used for blood cancer namely, vincristine and vinblastine, which were derived from the alkaloids of *Vinca* are two of the first anticancer drugs. In addition, Taxol is a product of such research (Voss et al., 2006).

The aim of the current study was to evaluate anti-breast cancer effects and antioxidant activity of twenty medicinal plants from Chaharmahal va Bakhtiari province. The findings demonstrated that out of the 20 studied plants, two which were

from genus *Euphorbia* (i.e. *E. szovitsii* and *E. microsciadia*) had the highest inhibitory effects on the growth of the studied cell lines. To the best of our knowledge, no study has yet been conducted on the anticancer effects of *E. microsciadia*, and only few chemical compounds of this plant were isolated. *E. microsciadia* is from the family *Euphorbiaceae* and is native to Iran (Karimi, 2002). Plants from the family *Euphorbiaceae* are traditionally used to treat various inflammations and tumors (Amirghofran et al., 2011). In addition, the cytotoxic and immunomodulatory effects of other species from the family *Euphorbiaceae* have been demonstrated (Jassbi, 2006). The anticancer activity of other species of genus *Euphorbia* and their derivatives on cancer cell lines have been reported. For example, the growth-inhibitory and cancer cell-killing effects of *E. tirucalli* were demonstrated on MDA-MB 231 and MCF-7 cell lines (Choene and Motadi, 2016). In addition, the inhibitory effects of *E. humifusa* Willd were investigated on metastasis and invasion; the findings demonstrated that *E. humifusa* extract could prevent the invasion and metastasis of breast cancer cells at early stages (Shin et al., 2016). Moreover, a study on the anticancer effects of *E. fischeriana*-derived diterpenoids on MDA-MB 231 cell line, showed that jolkinolide B, as a newly identified compound of this plant, could decrease adhesion and invasion of cancer cells, and therefore, might be considered a compound with metastasis-inhibitory effects (Sun et al., 2015). In addition, the inhibitory effects of *E. sogdiana* steroids were demonstrated on the growth and apoptosis of NCF-7 and MDA-MB 231 cell lines (Aghaei et al., 2016). A study indicated that paratocarpin E, a compound isolated from *E. humifusa*, could simultaneously induce apoptosis and autophagy in human breast cancer cell lines (Gao et al., 2016). In other studies, the anti-cancer activity of *Hertia angustifolia, Achillea wilhelmsii, Urtica dioica, Plantago lanceolate*, and *Echinophora platyloba* collected from other regions have been investigated. For example, study of anti-cancer activity of *Achillea wilhelmsii* C. Koch extract on MDA-Mb-468 and MCF-7 cell lines indicated that *A. wilhelmsii* extract has significant inhibitory effects on MCF-7 (IC_{50}= 51.67 ± 1.527 µg/mL) and MDA-Mb-468 (53.67 ± 1.577 µg/mL) after 48 hr of treatment (Galavi et al., 2016). In another study, anti-proliferative effects of the *Urtica dioica* extract were observed only on MCF-7 cells after 72 hr with an IC_{50} value of 2 mg/mL (Fattahi et al., 2013). Moreover, Galvez et al. (2003) in an anticancer study of different species of *Plantago* genus on MCF-7 cells, reported IC_{50} values of 32 to 114 µg/mL for the plant. These differences in the inhibitory concentration rates and observed results may be due to differences in extracts types, regions from where the plants have been collected and also plants' phytochemical profiles.

The studies conducted on medicinal plants collected from different regions have reported inconsistent findings. It is obvious that plants from different regions may have different therapeutic effects. This study demonstrated that *Satureja bachtiarica*, *Plantago lanceolata*, *Parietaria judaica*, *Stachys inflata*, and *E. szovitsii* had comparatively greater antioxidant capacities than other studied plants. In addition, most plants exhibited more potent antioxidant effects compared to that of the standard BHT. *E. microsciadia* and *E. szovitsii* with comparatively greater anticancer effects compared to other plants, had respectively 5.1- and 23.3-times higher antioxidant capacity than BHT, indicating optimal antioxidant effects of more effective extracts. Studies showed that flavonoid compounds are one of the main compounds of plants from genus *Euphorbia*. Flavonoids are the most well-known phenolic compounds with potent antioxidant properties. The protective effects of flavonoids in biological systems are due to their antioxidant capacity, scavenging free radicals, activating antioxidant enzymes, and decreasing alpha-tocopherol radicals (Ghatreh-Samani et al., 2016). Flavonoids can prevent platelets accumulation and have anti-inflammatory, antibacterial, and antitumor properties. They also inhibit protein-tyrosine kinase and protein kinase C during lymphocytes activation (Cushman et al., 1991; Guardia et al., 2001; Yang et al., 2008; Abdallah and Esmat, 2017; Bonesi et al., 2017; Pandey et al., 2017). Actually, antioxidant compounds can prevent free radical reactions and decrease cell damage or death, cardiovascular diseases, and cancers. However, for some plants, an-

tioxidant activities were not proportionate to the level of these compounds, indicating that other factors may affect the antioxidant properties of these plants.

In the present study, there was no significant relationship between anticancer effects and antioxidant activity of the plants collected from Chaharmahal va Bakhtyari province, Iran. Terpenoids are other main compounds of the *Euphoria* genus. They may be responsible for anticancer activity of the plants from *Euphoria* genus. Some previous studies reported anticancer activity of terpenoids (Mu et al., 2013; Ragasa and Cornea, 2013; Sun et al., 2015). Diterpenoids such as jolkinolide B, 17-acetoxyjolkinolide B, and 17-hydroxy-jolkinolide B could to induce apoptosis in tumors via signal transducer, activator of transcription, phosphoinositide 3-kinase/Akt pathways, and modulating the IκB kinase (Yan et al., 2008; Wang et al., 2009; 2011). Also, jolkinolide B, as a diterpenoid isolated from *E. fischeriana* Steud had anti-metastatic effect on MDA-MB-231 cells (Sun et al., 2015). Moreover, triterpenes from *E. hirta* have shown cytotoxic activity against HCT116, a human colon carcinoma cell line (Ragasa and Cornea, 2013).

This study proposes to determine the active phytochemicals of the effective plants and carry out complementary animal studies for plants introduced in the present study so that new anticancer compounds be identified for production effective anti-breast cancer drugs.

CONCLUSIONS

Based on the results gained of screening anti-breast cancer effects and antioxidant activity of twenty medicinal plants gathered from Chaharmahal va Bakhtyari provinc, *E. microsciadia* and *E. szovitsii* identified as effective sources to develop drugs for breast cancer due to comparatively more potent growth-inhibitory effects than other plants, presence of phenolic compounds, and acceptable antioxidant capacities. It is therefore recommended to determine the active compounds of these plants and their mechanisms of anticancer actions and to determine nontoxic doses in production of anticancer drugs; also, their effects should be assessed in animals and humans.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Asadi-Samani M	Rafieian-Kopaei M	Lorigooini Z	Shirzad H
Concepts or ideas		x		x
Design				x
Definition of intellectual content		x		
Literature search	x		x	
Experimental studies	x		x	
Data acquisition	x		x	
Data analysis	x	x		
Statistical analysis		x	x	
Manuscript preparation	x			
Manuscript editing		x		x
Manuscript review	x	x	x	x

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