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Original Article

Growth Inhibitory Effect of *Anthemis haussknechtii* Root Extract, as a Source of Parthenolide, on Breast Cancer Cell Line

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Abstract

Parthenolide is major Sesquiterpene lactones present in *Anthemis haussknechtii* Boiss. & Reut. (feverfew). This compound has many effects on different disease such as migrain and cancer. Parthenolide was reported from *Tanacetum parthenium* (L.) Sch.Bip. but other plants of Asteraceae family could contain parthenolide. In this study parthenolide was extracted and identified with two methods, Fourier Transform Infra-Red Spectroscopy (FTIR) and High Performance Liquid Chromatography (HPLC). Breast cancer cell line, MDA-MB-231, was exposed to different concentrations of parthenolide for 24 hours. Half maximal inhibitory concentration (IC_{50}) was evaluated using Methylthiazol Tetrazolium (MTT) test. Based on results, 1000 µg/ml concentration is the minimum lethal dose that kills approximately 50% of cells after 24 hours. The results revealed that the *A. haussknechtii* parthenolide dramatically decreased survival of cancer cell line by inducing apoptosis. This is the first report of cytotoxicity effect of A. *haussknechtii* extract on breast cancer cell line.

Keywords: Cell Viability, FT-IR; HPLC, MTT test, Parthenolide

Introduction

Medicinal herbs have been used for centuries to treat different kind of diseases. Members of Asteraceae family have been well-known as remedy for various diseases. The Asteraceae species as source of natural antioxidant used to be used in medicine and pharmaceutical [1]. In Iran, *Anthemis* L., *Matricaria* L., *Tripleurospermum* Sch.Bip. and *Tanacetum* L., four different genera from Asteraceae family, have been used as Chamomile. Antibacterial and antioxidant activity of essential oil from members of *Anthemis* genera have been reported by other researches [1,2]. Terpenoids, as plant secondary metabolites, are abundant in this family and have key roles in environmental interaction, such as plant defenses. Sesquiterpene lactones are a major group of terpenoids that formed by assembly of a 15-carbon skeleton into bisabolane, cuparane, cadinane, humulane and germacrane, as backbones, and functional groups [3]. Parthenolides are the most important sesquiterpene lactones because of their effect on various diseases. In *Tanacetum parthenium* (L.) Sch.Bip., parthenolide is the principal bioactive compound [4].

Cancer is the second leading cause of death in around the world. Breast cancer is a complex and heterogeneous disease with both genetic and environmental risk factors. Due to the mortality rates of breast cancer in many African and Asian countries, research on breast cancer seems to be vital. Breast cancer cell lines are classified based on histological type, tumor grade, lymph node status and the presence of predictive markers such as estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) [5]. The growth-inhibitory effect of *Terminalia chebula* Retz. fruit

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was confirmed on several malignant cell lines including MCF7, S115, HOS- 1, PC-3 and PNT1A cancer cell lines [6]. The anti-proliferative activity of *Cimicifuga racemosa* (L.) Nutt. extracts on estrogen receptor positive MCF7 and estrogen receptor negative MDA-MB231 breast cancer cells was studied by WST-1 assay [8, 7]. The effects of aqueous extracts of some Chinese medicinal herbs such as *Anemarrhena asphodeloides* Bunge and *Artemisia argyi* H. Lév. & Vaniot, were evaluated on cancer cell lines [9].

Alkaloids such as vinblastine, vincristine and ellipticine alkaloid frequently have been used to treat cancer [10]. This secondary metabolite via different mechanisms, such as induction of apoptosis and inhibition of topoisomerase I and II and ROS production, killed cell lines [11,13]. Parthenolide is currently being studied for their ability to induce apoptosis or differentiation in some types of cancer cells. The best known mechanism of parthenolide role is the inhibition of nuclear factor-κB (NF-κB) activity via inhibitor-κB kinase [14]. It can induce apoptosis by inhibiting the activity of the NF-kB transcription factor complex. As genes under NF-kB control can be an apoptosis inhibitor, parthenolide can down-regulate anti-apoptotic genes [15]. In this study we demonstrated the human-antitumor activity of *Anthemis haussknechtii* Boiss. & Reut. extract as a new source of parthenolide on breast cancer MDA-MB-231 cell line treatment.

Material and Methods

Parthenolide Extraction, from *Anthemis haussknechtii* Boiss. & Reut. root, and loading on nanoparticle

A. haussknechtii was collected from Malayer, Hamadan province, Iran (Fig. 1). 5 g of Plant materials (flower, leave, stem and root) were ground to a fine powder using a mortar and pestle in liquid nitrogen. Three biological replicates were used for each sample. Extraction was performed with 15 ml methanol:formic acid (1000:1, v/v). The extracts were briefly vortex for 5 min and then sonicated for 15 min. Subsequently, the extracts were centrifuged and filtered through a 0.2 µm inorganic membrane filter [3].

Root extract was loaded on nano-particle MS-APTES (Amino Peropil Theaxy Saline) for increasing solubility and improvement drug delivery.

Cell Line

MDA-MB-231 Cell line was donated by Dr. Mosa Gardaneh (NIGEB). MDA-MB-231 cell line is negative for ER, PR and HER2 and positive for EGFR. Cancer cell lines were cultured in DMEM medium supplemented with 100 U mL^{-1} of penicillin, 100 µg mL^{-1} of streptomycin, and 10% fetal bovine serum (FBS). The cells were grown at 37 ˚C in a humidified 5% CO_2 incubator.

Cell Viability by MTT Assay and SEM Analysis

The viability of cultured cell lines was determined by the Methylthiazol Tetrazolium (MTT) colorimetric assay, based on the reduction of a tetrazolium salt by mitochondrial dehydrogenase of viable cells. Cell lines were seeded (8000 cells/well) in a 96 well plate and incubated for 24 hours at 37°C in 5% CO_2 incubator and then different concentrations of *A. haussknechtii* flower, stem, leaves and root extract (31, 62, 125, 250, 500 and 1000 µg mL^{-1}) were added to each well and plate was incubated for 24 hours. After incubation, 20 µL MTT (5 mg mL^{-1}) and 180 µL of DMEM media was added to each well. Plate was incubated in the incubator for 5 hours at 37 °C. Then, the medium was omitted and formazan crystals, produced from reduction of MTT by mitochondrial dehydrogenase of viable cells, were dissolved in 200 µL of dimethylsulfoxide. Absorbances of treated and controls were read by ELISA plate reader (Multiskan MS) at 570-630 nm. The percentage of viable cells was determined based on this absorbance. For treated cells, viability was calculated compared with control cell. The morphological changes occurred due to root extract treatment on cell line MDA-MB-231 at 1000 $\mu g.mL^{-1}$ for 24 h has investigated through scanning electron microscopy (SEM).

Statistical Analysis

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences) software. Analysis of Variance (ANOVA) and Duncan's test were carried out to detect changes among the groups. Results represent groups that do not share a letter or letters significantly different for the MTT test. Differences with P values < 0.001 were considered significant.

HPLC Analysis

HPLC analysis was performed for effectively extract using Cecil 1100 series (Cecil Inst., Ltd.,

Cambridge, United Kingdom) equipped with a 1100 series pump and UV absorbance detector and a column oven C_{18}(CTS-30 Younglin, Korea). The mobile phase consisted of ultra-pure water and acetonitrile with a flow rate of 1.5 $mLmin^{-1}$ at room temperature (25 ˚C) [3]. Column was washed and equilibrated for 15 min before the injection. An authentic standard of parthenolide (Sigma, USA) were prepared at four concentrations of 50, 100, 200 and 400 ppm in methanol and was used to make a calibration curve. Crude extract of root, stem, leaves and flower that confirm by HPLC analysis have parthenolide pick, use to determining cell viability by MTT assay.

Fourier Transformation Infrared Spectroscopy (FT-IR)

Fourier Transformation Infrared Spectroscopy spectra were obtained on FT-IR spectrophotom-eter (BRUKER, Germany) using KBr discs. The *A. haussknechtii* flower, stem, leaves and root extracts (2 µL) were coated on the KBr discs for infrared spectrometry analysis. The discs were approxi-mately 5 mm in diameter and 1 mm in thickness. The instrument was operated under dry air purge, and the scans were collected at scanning speed of 2 mm s^{-1} with resolution of 4 cm^{-1} over the region of 4000– 400 cm^{-1}.

The *Anthemis haussknechtii* Boiss. & Reut. specimens were collected from Malayer, Hamadan province, Iran and the roots underwent to get parthenolide based on parthenolide extraction protocol. Albeit To see which organs of *A .haussknechtii* contain parthenolide, several different tissues was selected including flower, leaves, stem and root analyzed by HPLC.

The inhibitory effect of four extracts from *A. haussknechtii* on breast cancer cell line was examined by MTT assay. This method measures cytotoxicity, cell proliferation and cell activation. Cancer cell line MDA-MB-231 was exposed to six concentrations of *A. haussknechtii* flower, stem, leaves and root extract for 24h to assay the inhibitory effect of the extracts on cancer cell growth. As root extract showed the highest effect on cancer cell line, our experiment has been followed with this fragment. The half maximal inhibitory concentration (IC_{50}) was used to measure the suppression of cancer cell growth. For root extract, this context was calculated as 1000 μgmL^{-1} in MDA-MB-231 cell line (Figure 2). Analysis of proliferation inhibitory rate (Figure 2) confirmed that the more concentrated extract, the more potential in cell growth inhibition. Analysis to follow morphological changes of MDA-MB-231 cell lines with 1000 µg.mL-1 root extract of *A. haussknechtii* visualized by SEM (Fig. 5).

Result

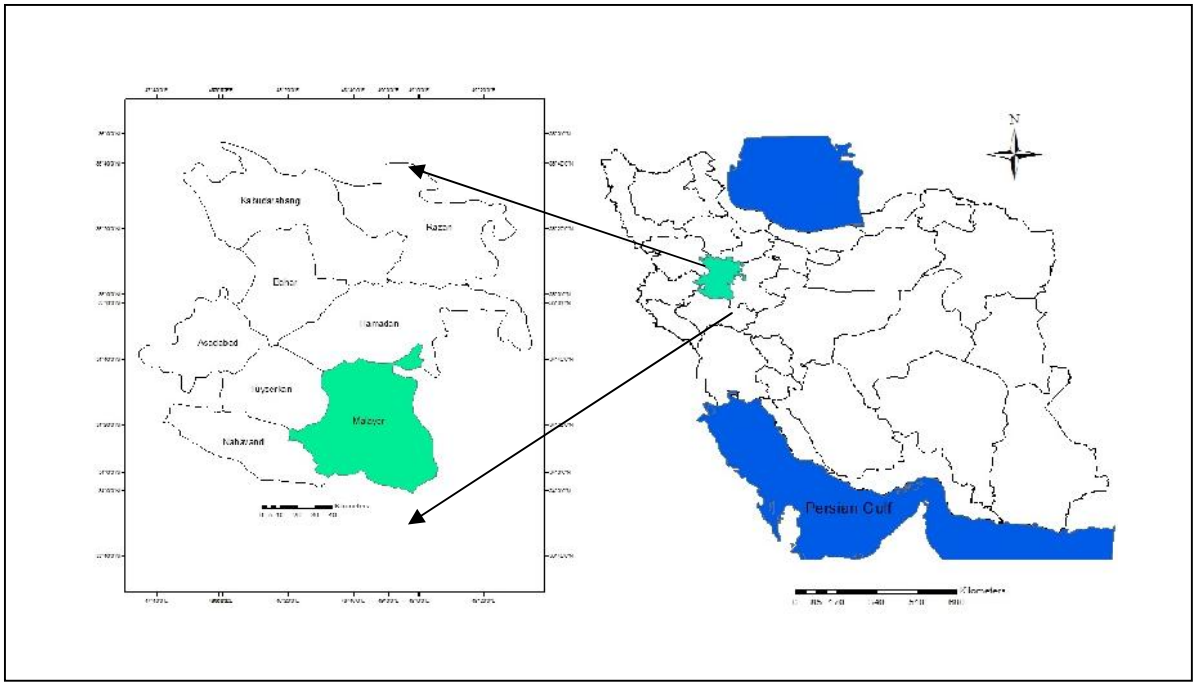


Fig. 1 The geographical origin of *Anthemis haussknechtii* Boiss. & Reut.gene pool used in this study. Malayer, Hamadan province, Iran

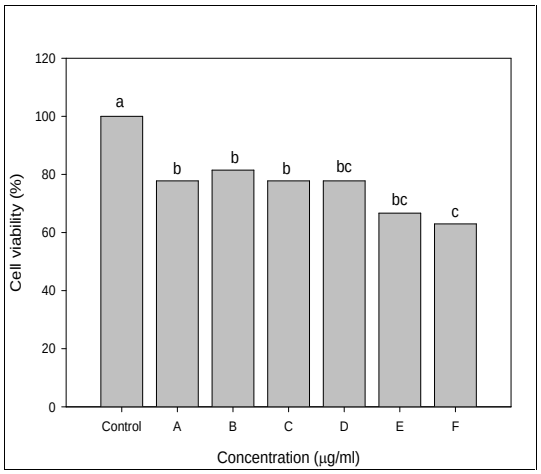
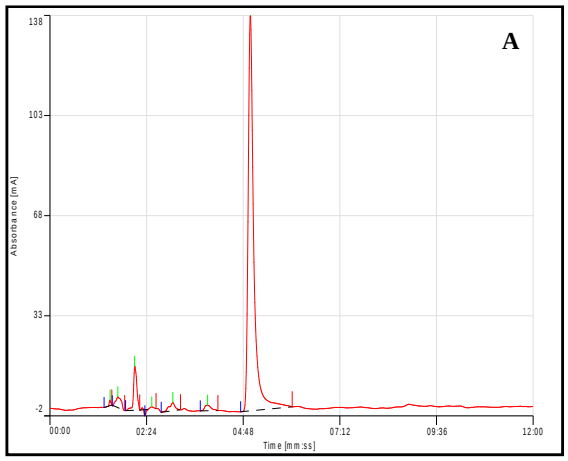
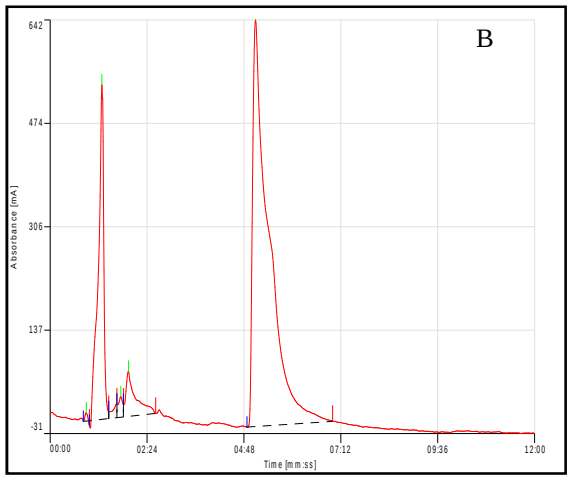


Fig. 2 Inhibition of different concentrations, (31, 62, 125, 250, 500 and 1000 µg mL^{-1}) and control of *Anthemis haussknechtii* Boiss. & Reut. root extract on proliferation of MDA-MB-231 cancer cells by MTT Assay.

To detect the concentration of parthenolide in of *A. haussknechtii* extract, the calibration curves was drown as shown in Figure 3. Figure 3 B demonstrates the absorbance of the *A. haussknechtii* extract compared with parthenolide standard (Fig. 3 A).





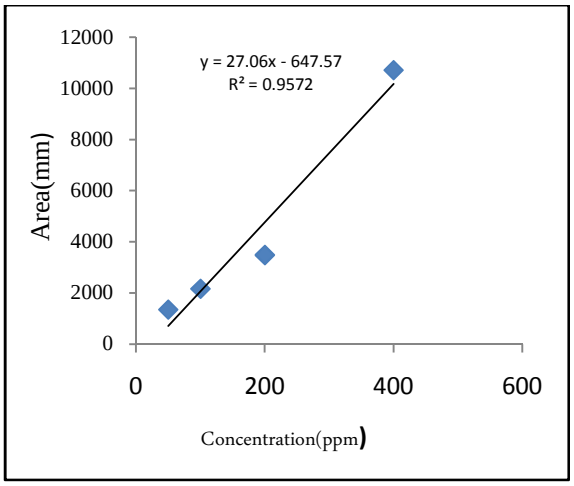


Fig. 3 HPLC chromatogram of parthenolide standard (A), *Anthemis haussknechtii* Boiss. & Reut. root extract (B) and calibration curve for parthenolide(C).

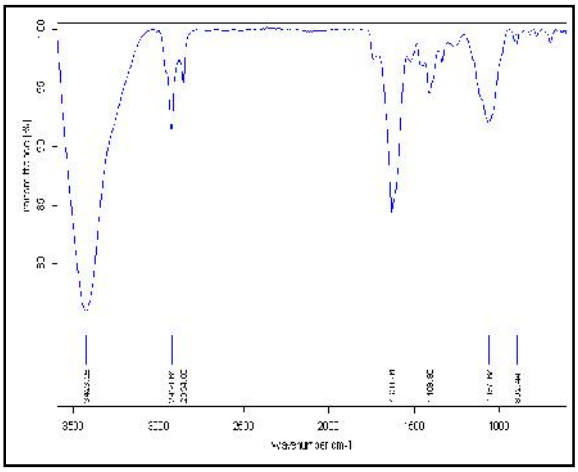


Fig. 4 FT-IR spectra for *Anthemis haussknechtii* Boiss. & Reut. root extract. C-O functional group in 1100 nm and C=O in 1730 nm resembling parthenolide molecular formula ($C_{15}H_{20}O_{16}$)

HPLC analysis was used to detect the *A. haussknechtii* root extract components, compared with parthenolide standard solutions. The chromatograms approved the presence of parthenolide as their retention time at 4.8 minutes, was in accordance with the standard solution (Figures 3 A and B). FT-IR spectra of *A. haussknechtii* root extract represented C-O functional group in 1100 nm and C=O in 1730 nm resembling parthenolide molecular formula ($C_{15}H_{20}O_{16}$) (Figure 4).

Loading *A. haussknechtii* root extract on MS-APTES nanoparticle could improve solubility of extract but it has no effect on its anticancer properties (data not shown).

A B

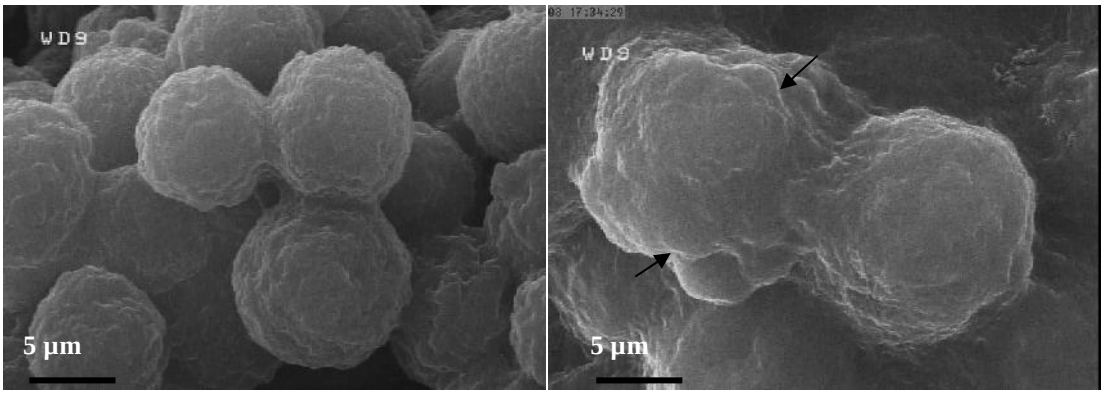


Fig. 5 Morphological changes of MDA-MB-231 treated with 1000 µg.ml^{-1} root extract visualized by scanning electron microscopy (SEM). A) Control, B) treated cell

Discussion

The central Zagros of Iran (including Ilam, Lorestan, Kermanshah and Hamadan province) is so important because of vital role for protection of ecosystem, species and genetic biodiversity [16]. Among vascular plants of western of Iran, Asteraceae is the largest family (29 species) [16]. The seventh largest tribe of Asteraceae with about 109 genera worldwide is the Anthemideae [17]. For decades, Feverfew has been used by Europeans as a treatment for migraine, asthma, rheumatism, and gynecological problems. Researches have showed responsible for medicine property of Feverfew is parthenolide. Other members of Asteraceae are such as *A. haussknechtii* that reported antioxidant and antibacterial activity of it essential oil [2]. Demand for herbal plant for treating cancer is an increasing trend because synthetic drugs have side effects which they may elicit [2]. Recent studies have shown that parthenolide (PTL) is an anticancer compound with anti-proliferation and pro-apoptotic potential against a variety of cancer such as prostate, melanoma ,breast and pancreatic carcinoma [18, 19 and 20]. In this study we explore a member of Asteraceae family, *A. haussknechtii* for detecting parthenolide. Our study showed that the parthenolide mainly present in *A. haussknechtii* roots. We isolated the parthenolide with partial extraction from *A. haussknechtii* roots, confirmed by two analytical methods, FTIR and HPLC. Although, different secondary metabolite have been reported to be present in *Anthemis* genera extract, such as essential oils [2], parthenolide was detected as the major components of A. *haussknechtii* root extract by HPLC. As regard to the spectrum of *A. haussknechtii* root extract consisted of 1100 cm^{-1} band which addresses of C-O presence, 1700 cm^{-1} that is sign of C=O existence and 3400 cm^{-1} which means the presence of OH band. Following the verification of parthenolide presence in root extract through wave number in the range 1100- 1700 cm^{-1}.

In conclusion, the results of the current research showed that root extract of *A. haussknechtii* contain parthenolide. In this work, we investigated anticancer effect of *A. haussknechtii* root extract against cancer cell line, MDA-MB-231. We conclude that root extract could inhibit the growth of MDA-MB231 cells via induction of apoptosis.

Although some members of Asteraceae family were studied for some potential, other species such as *A. haussknechtii* are rarely studied. Other exploration of their bioactive compounds develops useful knowledge on the use of them for medicine and pharmaceutical industries. Further studies are suggested to elucidate the mechanism of action of parthenolide from *A. haussknechtii* on more human cancer cell lines.

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