Antimicrobial, antioxidant, cytotoxic and apoptotic activities of Satureja khuzeanica

Satureja Khuzeanica’nın Antimikrobiyel, Antioksidan, Sitotoksik ve Apoptotik Aktivitesi

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ABSTRACT

Objective: Natural products with antimicrobial effect are currently investigated in order to eliminate the use of synthetic antibiotics that cause the resistance of microorganisms. This study determines the antibacterial, antioxidant activity, and also the cytotoxic and apoptotic effect of Satureja khuzeanica on various cancer cell lines.

Materials and methods: The individual constituents were identified by their identical retention indices referring to known compounds from the literature. Methanol and ethanol extracts of S. khuzeanica (25 and 50 mg/mL) were screened against four Gram-positive and four Gram-negative bacteria. The plant extracts were tested for their antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The interaction between the extracts and plasmid DNA were analyzed by agarose gel electrophoresis. In addition, the cytotoxic activity of the extracts was evaluated on MCF-7, DLD-1, osteosarcoma, and fibroblast cell line. Finally, gene expression of caspase-3, Bax and Bcl-2 were investigated by real-time PCR.

Results: Our result indicated that both extracts showed good inhibitory activity against both Gram-negative (Escherichia coli ATCC 35218) and positive (Bacillus subtilis ATCC 6633) bacteria. In addition, the methanol extract of S. khuzeanica had strong antioxidant activity (IC50 = 37.0 ± 0.3 μg/mL). The extracts showed a strong effect on plasmid DNA. The methanol extract of S. khuzeanica showed a good concentration-dependent cytotoxicity. Finally, IC50 = 47.00 ± 1.12 μg/mL, ethanol, and water extract had apoptotic effect in MCF-7 cell line.

Discussion: MCF-7 was detected as the most sensitive cell line therefore further studies should be done on this plant extract as a potential anticancer agent against breast cancer.

Key Words: Satureja khuzeanica, plasmid DNA, MCF-7, cytotoxicity

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ÖZET

Amaç: Antimikrobiyel etki gösteren doğal ürünler, mikroorganizmaların direncine neden olan sentetik antibiotiklerin kullanımını ortadan kaldırmaktadır. Bu çalışmada, Satureja khuzeanica bitkisinin antibakteriyel, antioksidan aktivitesi ve çeşitli kanser hücre hattında sitotoksik ve apoptotik etkisi araştırılmıştır.

Yöntem: Tek tek biyöleneler, literatürden bilinen bileşiklere atıfta bulundukları özdeş alıkonma endeksleri ile tanınmıştır. S. khuzeanica’nın (25 ve 50 mg / mL) metanol ve etanol özütünün düğüm Gram-pozitif ve düğüm Gram-negatif bakteri üzerindeki etkisi belirlendi. Bitki özütlerinin 2,2-difenil-1-picrilhidrazil (DPPH) radikaline karşı antioksidan aktiviteleri hesaplandı. Özütlerin plazmid DNA üzerine etkisi agar jel elektroforezi ile analiz edildi. Ayrıca, özütlerin MCF-7, DLD-1, osteosarkom ve fibroblast hücre hatları üzerinde sitotoksis aktiviteleri araştırıldı. Son olarak, gerçek zamanlı PZR ile özütlerin kaspaz-3, Bax ve Bcl-2’nin gen ifadesi üzerine etkisi araştırıldı.

Bulgular: Bizim sonuçlarımız, her iki özütünün de hem Gram-negatif (Escherichia coli ATCC 35218) hem de pozitif (Bacillus subtilis ATCC 6633) bakterilerle karşı yönlü bir inhibitory aktivite gösterdiğini ortaya çıkarmıştır. Ayrıca, S. khuzeanica’nın metanol özütünün kuvvetli bir antioksidan aktivitelerine sahip olduğu bulunmuştur (IC50 = 37.0 ± 0.3 μg/mL). Özütler plazmid DNA üzerine güçlü bir etki göstermiştir. S. khuzeanica’nın metanol özütü konsantrasyon başlığı güçlü sitotoksik aktivite göstermiştir. Son olarak, IC50, 47.00 ± 1.21 μg/mL olarak hesaplanmıştır, etanol ve su özütü MCF-7 hücre hattında apoptotik etki göstermiştir.

Sonuç: MCF-7 en hassas hücre hattı olarak tespit edildi. Meme kanserine karşı potansiyel antikanser ajan olarak ileri çalışanlar önerilmektedir.

Anahtar Sözcükler: Satureja khuzeanica, plasmid DNA, MCF-7, sitotoksite

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INTRODUCTION

Using medicinal plant to treat diseases is becoming popular in human societies (1). Aromatic plants, known for both aromatic and antiseptic properties, are used in spices and natural food preservatives, perfume industry for aromatherapy, etc. (2, 3). During the last decades, natural products with antimicrobial effect have been investigated for eliminating the use of synthetic antibiotics that cause the resistance of microorganisms and have side effects to human health (4).

Most aromatic plants belonging to the family of Lamiaceae, such as Satureja, Sideritis, Salvia, Origanum, and Thymus are annual or perennial semi-bushy aromatic plants that inhabit arid, sunny, stony, and rocky habitats (5). Several Satureja species locally known as 'Kekik' exist in Turkey (6). There are about 30 species of Satureja in the world. Satureja khuzestanica is an endemic species in Iran (7). Distribution of the genus Satureja overlaps the region of southern and southeastern Europe, Asia Minor, northern Africa, and Mediterranean area. Satureja species are commonly used for making herbal tea, flavoring agents (condiment and spice), and medicinal purposes. Infusion and decoction of aerial parts of Satureja species are used to produce a tonic, carminative, digestive, and expectorant and for the treatment of colds in Iranian traditional medicine. Due to the presence of secondary metabolites such as flavonoids, steroids, terpenoids, and tannins, they are known for their healing properties for a long time and have been used as traditional remedies to treat various ailments such as cramps, muscle pains, nausea, indigestion, diarrhea, and infectious diseases (8).

Recently, antimicrobial (9,10), antiviral (11,12), antiproliferative (13), antitumor activity (14), anti-inflammatory (15), antioxidant (16), anti-apoptotic (17), antiparasitic (18), and vasodilatory (19,20) activities have been reported from different species of Satureja. This plant is also used in ethnomedicine as an analgesic and antiseptic agent. Medicinal properties of the genus Satureja have made it one of the most popular plants throughout the world (10).

The polyphenols have the antioxidant, antiproliferative, and apoptosis-inducing properties related to the prevention of diseases such as cancer (20). All aromatic plants, as polyphenol compounds containing plants, have potential anti-inflammatory, anti-inflammatory, antimicrobial, and anticancer activities (21). S. khuzestanica is a subshrub, branched stem, 30 cm high, densely leafy, broadly ovate-lobular plant covered with white hairs. In a previous study on essential oil analysis and its antimicrobial activity, carvacrol was found as a dominant compound of S. khuzestanica. The essential oils of S. khuzestanica mainly consist of carvacrol (80.6%), p-cymene (4.8%), myrcene (1.5%), γ-terpinene (2.3%), and terpinene-4-ol (2.1%). Extract and essential oil of this plant and carvacrol (as the main constituent of its essential oil) have shown propitious antimicrobial and antioxidant activities (22,23).

Bcl-2 and Bax are two important regulator genes in the mitochondrial apoptotic pathway. Bax promotes cell death through permeabilization of the mitochondrial outer membrane in response to different cellular stresses. In contrast, Bcl-2 prevents apoptosis by inhibiting the activity of Bax. Therefore, the balance between pro- and anti-apoptotic members of this family can determine the cellular fate. Bax and Bcl-2 are the major members of a Bcl-2 family whose potential roles in tumor progression and prognosis of different human malignancies have been of interest in various studies during the last decade (24, 23).

Breast cancer is one of the most common female cancers in the world (24). Improvement in the survival and quality of life of breast cancer patients requires the design and discover new therapies and drugs that are effective and less side effect. As far as we know, there is no report on the anticancer activity of an extract of S. khuzestanica. Thus, many studies have been recently undertaken to investigate the effects of this herb extract to discover its therapeutic potentials. The aim of this research is to study the antibacterial, antifungal, and antiviral activities of the extract against some important human pathogenic bacteria. 

MATERIAL and METHODS

Preparation of the extracts

Khorramabad University of Iran. June 2013 at an altitude of 1170 m. The plant used in this study was identified as S. khuzestanica MAb of S. khuzestanica powder (30 g) was added to 450 mL ethanol, methanol, and water at ambient temperature for 24 h. The suspension was filtered through a Whatman filter paper. The solvent was evaporated with a rotary evaporator at 40°C. The dried extracts were stored in the dark at 4°C until further use. The leaves of S. khuzestanica were dried at room temperature. S. khuzestanica

The leaves of S. khuzestanica were dried at room temperature. S. khuzestanica powder (30 g) was added to 450 mL ethanol, methanol, and water at ambient temperature for 24 h. The suspension was filtered through a Whatman filter paper. The solvent was evaporated with a rotary evaporator at 40°C. The dried extracts were stored in the dark at 4°C until further use. Gas chromatography/mass spectrometry

Fid- GC was carried out using a Hewlett-Packard 6890 with HP-5 capillary column (phenyl methyl siloxane) 25 m, 0.25 mm i.d., the ratio of 1:25, and a flame ionization detector. Temperature programmer: 60°C (2 min) rising to 240°C at 4°C/min; injector temperature 250°C, detector temperature, 260°C. GC-MS was performed using a Hewlett-Packard 6859 apparatus with a quadrapole detector on an HP-5 column operating at 70 eV ionization energy, using the same temperature programmer and carrier gas. Retention indices were calculated using retention times of n-alkanes that were injected after the oil chromatography (5).

Antimicrobial activity

Microorganisms and growth conditions

For the bioassays, we used the following bacteria Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922, Escherichia coli (ATCC 35218) Bacillus subtilis ATCC 6633, Bacillus cereus NRRL B-3711, P. vulgaris (NRRL B-123), and Enterococcus faecalis ATCC 29212, and antifungal activity against Candida albicans ATCC 10231 and Candida tropicalis ATCC 13803. All the organisms tested were collected from Gazi University, Ankara, Turkey. The bacteria species were resuspended in Mueller Hinton broth at 37°C. The turbidimetry of the suspension was adjusted to the McFarland 0.5 turbidity standard and, then, spread over Mueller Hinton agar culture medium plates (3).

Disc diffusion assay

In this method, concentrations of methanolic, ethanolic, and water extracts of S. khuzestanica (25 and 50 mg/mL) were prepared and sterilized using membrane filters of 0.4 μm. Then, 50 μL of each concentration of the extracts, was added to each sterile blank disc (Padtan Tab, Iran). In the following, from the fresh culture of H. pylori, 1 McFarland solution in brucella broth (Himedia) was prepared and 100 μL of the solution was taken by sterile swab and cultured on brucella agar medium containing 5% defibrinated sheep blood 7% inactivated fetal bovine serum (FBS). The dried discs of the extracts were placed on the culture on appropriate distances. After placing the discs, the media were kept in a “CO2” incubator (with 10% “CO2”) at 37°C for 24 h. Antibacterial activity was determined by measuring the zone around the test discs. The growth inhibition diameter was an average of three different measurements. The result was expressed as the diameter of inhibition zone and compared with standard antibiotic Ampicillin (Amp, 10 μg) and Chloramphenicol (C, 30 μg).

Antioxidant activity

Determination of DPPH radical scavenging activity

Antioxidant activity of the extracts was determined based on its ability to react with the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Yamasaki et al. 1994). Briefly, 0.1 mmol/L solution of DPPH was added to S. khuzestanica extracts solutions at different concentrations and after 30 min incubation, the absorbance was measured at 517 nm. Absorbance that was measured at 517 nm. Absorbance that was measured at 517 nm. Absorbance that was measured at 517 nm. Absorbance that was measured at 517 nm. Absorbance that was measured at 517 nm. Absorbance that was measured at 517 nm. Absorbance that was measured at 517 nm. Absorbance that was measured at 517 nm. Therefore, the percentage of inhibition, IC50 (the concentration giving 50% inhibition), was determined from the graph plotting inhibition percentage against extract concentration. Extract concentration providing 50% inhibition IC 50 was calculated using the following formula:

\[
\text{IC}_{50} = \frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Sample absorbance}} \times 100
\]

Where sample absorbance is the absorbance of the test control and absorbance is absorbance of the test extract. Extract concentration providing 50% inhibition IC50 was calculated from the graph plotting inhibition percentage against extract concentration. Extracts were carried out in triplicate. BHT and ascorbic acid were used as a positive control.

Total phenolic content

Total phenolics were determined using Folin-Ciocalteu reagent (Singleton & Rossi, 1965). 100 μL of each plant extract (1 mg/mL) or gallic acid standard solutions was mixed with 200 μL of Folin-Ciocalteu reagent (1.1 diluted with distilled water) and 2 mL of aqueous “Na2CO3” 1M. After standing for 1 h at room temperature, absorbance was measured at 765 nm. Results were expressed as mg Gallic acid equivalents (GAE) /g DW.

Cytotoxicity assay

Cell lines

The human cancer cell line MCF-7 (breast cancer), DLD-1 (colorectal adenocarcinoma), osteosarcoma, and normal gingival fibroblast cells were obtained from Kirikkale University. All cancer cell lines were maintained in the recommended RPMI-1640 medium supplemented with 10% fetal bovine serum, L-glutamine (3 mM), streptomycin (100 μg/mL), and penicillin (100 IU/mL). Cells were grown in a humidified atmosphere of 95% air and 5% “CO2” at 37°C.
Treatment of cell lines
Stock solutions (100 mg/ml) of methanol extract, prepared in dimethyl sulfoxide (DMSO), were dissolved in the corresponding medium to the required working concentrations. All cancer cell lines and fibroblast cells (5000 cells per well) were seeded into 96-well microtiter plates and 24 h later after the cell adherence. The cells were treated with the extracts (ranging from 3.125 to 100 μg/ml) except for the control cells to which a nutrient medium only was added. Nutrient medium (RPMM 1640) was incubated for 48 h.

Determination of cell survival
The effects of extracts on cancer cell survival were determined by MTT test. Briefly, 20 μl of MTT solution was added to each well. Samples were incubated for further 4 h at 37°C in 5% CO2 and humidified air atmosphere. The supernatant was removed and replaced with 100 μL of DMSO. The optical density of wells was measured with a microplate spectrophotometer reader. Optical density was measured at 570nm. Each test was repeated three times (29).

Statistical analysis
SPSS 11 were used for statistical analyses. Experimental results were expressed as mean ± S.D. of three parallel measurements. P-values < 0.05 were regarded as significant.

Table 1

| NO | Compound Name             | Ret.Time | Similarity | KIstd | KIVAL | Area (%) |
|----|---------------------------|----------|------------|-------|--------|----------|
| 1  | α-Thuene                  | 9.5      | 96         | 930   | 931    | 0.61     |
| 2  | α-Pine                   | 9.8      | 98         | 939   | 940    | 0.42     |
| 3  | Camphone                | 10.4     | 97         | 954   | 959    | 0.05     |
| 4  | β-Pine                   | 11.3     | 97         | 979   | 988    | 0.12     |
| 5  | Myrcene                 | 11.5     | 94         | 991   | 995    | 1.16     |
| 6  | 6-Methyl-3,5-heptadien-2-one | 11.7   | 86         | -     | 1001   | 0.38     |
| 7  | 3-Octanol                | 12.0     | 96         | 991   | 1011   | 0.04     |
| 8  | 3-Carene                 | 12.2     | 93         | 1031  | 1017   | 0.1      |
| 9  | α-Terpine                | 12.5     | 95         | 1017  | 1026   | 0.25     |
| 10 | para Cymene             | 12.9     | 93         | 1025  | 1038   | 3.35     |
| 11 | β-Ocime                  | 13.4     | 95         | 1050  | 1053   | 0.02     |
| 12 | γ-Terpine               | 13.9     | 97         | 1060  | 1068   | 0.67     |
| 13 | cis Sabinen hydrate     | 14.5     | 92         | 1070  | 1087   | 0.44     |
| 14 | Terpinolene             | 14.8     | 95         | 1089  | 1096   | 0.07     |
| 15 | Linalool                | 15.3     | 96         | 1097  | 1112   | 1.02     |
| 16 | 4-Terpineol             | 18.1     | 97         | 1177  | 1201   | 1.94     |
| 17 | α-Terpineol             | 18.7     | 95         | 1189  | 1218   | 0.17     |
| 18 | Thymol                  | 21.6     | 93         | 1290  | 1315   | 0.28     |
| 19 | Carvacrol               | 22.7     | 89         | 1299  | 1353   | 86.29    |
| 20 | β-Caryophyllene          | 25.2     | 93         | 1419  | 1443   | 0.13     |
| 21 | Geranyl aceton           | 25.7     | 97         | 1455  | 1463   | 0.18     |
| 22 | α-Franesene             | 27.1     | 95         | 1506  | 1512   | 0.33     |
| 23 | β-Bisabolene            | 27.3     | 96         | 1506  | 1522   | 0.97     |
| 24 | α-Bisabolene            | 28.1     | 89         | 1507  | 1553   | 0.15     |
| 25 | Caryophyllene oxide      | 29.7     | 94         | 1583  | 1616   | 0.53     |
| 26 | β-Udesmol               | 30.0     | 89         | 1651  | 1630   | 0.25     |
| 27 | α-Bisabolol             | 32.0     | 89         | 1686  | 1711   | 0.08     |

RESULTS

In the present study, the extract yield percentages obtained from S. khuzestanica were 17.5, 16.8, and 22.54% methanol, ethanol, and water, respectively. Overall, water extraction gave better recovery percentages.

Identification of components
The linear retention indices for all the compounds were determined by connecting the sample with a solution containing homologous series of CB-C22 n-alkanes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature and also by comparing their mass spectra either with the known compounds or with the Wiley mass spectral database (Table 1).
Table 2. Antimicrobial activity of methanol and ethanol extract of S. khuzestanica

| Microorganism         | Ethanol extract (µM) | Methanol extract (µM) |
|-----------------------|----------------------|-----------------------|
| B. cereus NRRL B-3711 | 25±0.3               | 25±0.5                |
| S. aureus ATCC 25922  | 12.5±0.7             | 12.5±0.2              |
| E. coli ATCC 25922    | 6.25±0.2             | 6.25±0.8              |
| E. coli ATCC 35218    | 6.25±0.0             | 12.5±0.5              |
| E. fecalis ATCC 292112| 12.5±0.4             | 6.25±0.5              |
| P. vulgaris (NRRL B-123)| 25±0.7               | 12.5±0.8              |
| P. aeruginosa ATCC 27853| 6.25±0.5             | 12.5±0.0              |
| C. albicans ATCC 10231| 6.25±0.3             | 6.25±0.2              |
| C. tropicalis ATCC 13803| 12.5±0.4             | 12.5±0.0              |

About 50% DMSO (solvent of the extracts) was used as a negative control by disc diffusion method. The highest antimicrobial activities were observed against Candida albicans.

Antioxidant activity

Free radical-scavenging activity potentials of test plant extracts were evaluated for estimating their antioxidant capacities. The DPPH results of S. khuzestanica extracts in different concentrations are presented in (Fig. 1) the positive standard (BHT), methanol, ethanol, and water extracts showed significant antioxidant activities.

Figure 1. DPPH radical scavenging activities methanol, ethanol and water extracts of S. khuzestanica.

To determine antioxidant capacity, 10 concentrations (mg/mL) were tested for each extract. The lower IC50 value indicates a higher antioxidant activity. The methanol extract IC50=37.7±0.8 µg/mL exhibited a higher DPPH scavenging activity than those of methanol IC50=51.3±0.8 µg/mL and water extracts IC50=65.5±0.5 µg/mL. Also, DPPH scavenging activities of the methanol and ethanol extracts were higher than the standard BHT. DPPH radical scavenging activity of test samples decreased in the order of ethanol>methanol>BHT>water.

All extracts of the studied plants exhibited antioxidant activities. Phenolic compounds of these plants could be contributed to their antioxidant activities. Some studies reported a positive correlation between total phenolic content and antioxidant activity. These results indicated that S. khuzestanica extracts have different antioxidant capacities. Also, the broad range of activity suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activities of S. khuzestanica detected in this study demonstrate the potential application value of these extract.

Amount of total phenolic constituents

Phenolic content of gallic acid was used as a standard; the contents of phenolic in plant extracts were expressed as gallic acid equivalents to help the comparison. The standard curve equation is,

\[ y (\text{absorbance})=0.0085x (\mu\text{g gallic acid})-0.0209, R^2=0.9858. \]
The cytotoxic activity of methanol and ethanol extracts on MCF-7 cells was higher than those of other cancer cell lines. The cytotoxic activity of methanol, ethanol, and water extracts on all cancer cell lines decreased in the order of methanol>ethanol>water; also the results indicated that the cytotoxicity order of extracts was Fibroblast > MCF-7 > DLD-1 > osteosarcoma. The results indicated that the cytotoxicity of methanol and ethanol extracts was influenced by the concentration (100-3.125 μg/ml). Methanol extract exhibited a remarkable cytotoxic effect on MCF-7 and DLD-1 cells. Among all cancer cells, only MCF-7 cells were highly susceptible to methanol and ethanol extract.

**Hoechst 33342 and PI double-staining assay**

Since apoptosis is the major mechanism of most anticancer agents, we investigated the apoptosis-inducing activity of *S. khuzestanica* extracts using Hoechst 33342 and PI double-staining assay. The results showed that the nuclei of cancer cells not treated with *S. khuzestanica* extracts exhibited diffuse staining of chromatin. Also, cells not stained by PI did not present a brighter blue fluorescence (control). With the treatment of *S. khuzestanica* extracts, more nuclei exhibited brighter blue fluorescence, indicating the nuclear condensation in apoptotic cells (Fig 3, 4). Furthermore, the apoptosis-inducing activity of methanol, ethanol, and water extracts on all cancer cell lines decreased in the order of methanol>ethanol>water. These results suggested that *S. khuzestanica* extracts could induce apoptosis in cancer cell lines.

**Table 3.** The IC50 values of the methanol, ethanol and water extracts on various cancer cell lines

| Cell lines  | Extracts  | IC50 values (μg/ml) |
|-------------|-----------|---------------------|
| Fibroblast  | Methanol  | 29.86               |
| Fibroblast  | Ethanol   | 33.92               |
| Fibroblast  | water     | 62.41               |
| MCF-7       | Methanol  | 30.125              |
| MCF-7       | Ethanol   | 40.125              |
| MCF-7       | water     | 40.125              |
| Osteosarcoma| Methanol  | 47.94               |
| Osteosarcoma| Ethanol   | 63.56               |
| Osteosarcoma| water     | 65.24               |
| DLD-1       | Methanol  | 26.92               |
| DLD-1       | Ethanol   | 36.03               |
| DLD-1       | water     | 61.75               |

**Figure 3.** Analysis of apoptosis by double-staining with Hoechst 33342/PI in MCF-7 and osteosarcoma cancer cell line that treated with methanol, ethanol and water extract of *S. khuzestanica*, observed under the fluorescence microscopy. The control cells were untreated with extracts.

**Figure 4.** Analysis of apoptosis by double-staining with Hoechst 33342/PI in DLD-1 and fibroblast cancer cell line that treated with methanol, ethanol and water extract of *S. khuzestanica*, observed under the fluorescence microscopy. The control cells were untreated with extracts.

**Figure 5.** The Results displays height of the left bar indicates the target/reference ratio of the Bax and Caspase 3 value in MCF-7 cell lines and the right bar shows the bcl2 value in MCF-7 cell lines.
When the extracts interact with plasmid DNA, the DNA damage due to the conformational change can be visualized via gel electrophoresis (Fig. 6). In the electrophoretograms, the lane (p) applies untreated plasmid DNA as a control, while the lanes 1 to 7 apply to plasmid DNA interacted with decreasing concentrations of the extracts (from 10000 μM to 156 μM). The extracts have a strong effect on plasmid DNA, especially at lanes 3 and 4, where in the intensity and mobility of form I was decreased. In the case of lanes 5, 6, and 7, the extracts (except methanol at 48 h) indicate a very little effect on the mobility and intensity of plasmid DNA (Fig. 6).

Figure 6. Electrophoretograms applying to the interaction of plasmid DNA with decreasing concentrations of methanol and ethanol extracts. for 24 (A), (B), 48 (C) a (D) hours incubation respectively. Lane P applies untreated pBR322 plasmid DNA. Concentrations (in μM) are as follows: lanes 1 to 7 apply to plasmid DNA interacted with decreasing concentrations of the extracts: lane 1: 10000; lane 2: 5000; lane 3 2500; lane 4: 1250; lane 5: 625; lane 6: 312; lane 7: 156 (μM). The arrow and the bottom bands correspond to form II (single nicked open circular), form I (covalently closed circular or supercoiled) and form III (linear) plasmids, respectively.

DISCUSSION

Satureja is an analgesic and antiseptic plant found in the southern parts of Iran. The composition of the essential oils of wild and cultivated S. khuzistanica and the antioxidant, antidiabetic, anti-inflammatory, and reproduction stimulatory properties of this plant have been recently reported from Iran. Carvacrol is one of the most important components of many species including those belonging to Satureja genus. This phenolic compound shows antiseptic, antibacterial, antifungal as well as antimicrobial and anti-inflammatory properties in Satureja spp (23,26).

The results of antimicrobial activity showed that among the extracts studied in this work, methanol extract had a more powerful antimicrobial effect than ethanol and water extracts against important eight human pathogenic bacteria.

According to antimicrobial results, ethanol, methanol, and water extracts of S. khuzestanica had different antimicrobial effects against these pathogens, and these effects may result from their phytochemical properties including phenolic compounds (6).

Antimicrobial effect of the extracts was different, depending on the type of microorganisms; thus, the Gram-positive bacterium S. aureus has a higher sensitivity compared to Gram-negative bacteria E. coli. Previous works showed that the Gram-positive bacteria are more sensitive to plant extracts than the Gram-negative ones, due to differences in the cell structure of Gram-negative and Gram-positive bacteria. An explanation for this behavior is that Gram-positive bacteria have a higher mucopeptidate content in their cell wall composition while Gram-negative bacteria have only a thin layer of mucopeptidate and most of their cell structure is lipoprotein and lipo polysaccharides. Thus, Gram-negative bacteria are more resistant. These observations are consistent with the results obtained by (27) who showed the high antimicrobial effect of these extracts. It seems that the presence of thymol, carvacrol, P-cymene, and gamma-terpinene cause the strong antimicrobial effect of these extracts. Several mechanisms are discussed to explain the antimicrobial effect of carvacrol and thymol. It may be effects of hydrophobic and chemical structure of these compounds toward the cell membrane (18).

The biological precursor of carvacrol, P-cymene, is hydrophobic and causes expansion of the cytoplasmic membrane. When combined with carvacrol in vivo, P-cymene incorporates into cytoplasmic membranes, facilitating transport of carvacrol across them (Baren, et al. 2006). Thus, the antimicrobial activity of carvacrol is increased by the presence of its precursor P-cymene, owing to described synergistic effect.

The antimicrobial effect of thymol and carvacrol is due to the damage in membrane integrity induced by a change in pH hemostasis. Also, by the equilibrium of inorganic ions, although P-cymene does not have antimicrobial activity, the antimicrobial activity of thymol or carvacrol is increased (28).

The flavonoids are almost ubiquitous in plants and are powerful chain-breaking antioxidants acting as metal chelators and free radical scavengers (29). Nitrogen compounds (alkaloids), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites in plants also play important roles in antioxidant activity (1).

Several antioxidant compounds have different polarities; therefore, different solvents are frequently used for the isolation of antioxidants. Antioxidant activity of the extracts is drastically dependent on the type of solvents (30).

According to our antioxidant results, the extract dissolved in ethanol had the most powerful antioxidant activity in DPPH free radical scavenging methods and contained the highest phenolic compounds. According to Miliusas and colleagues, there is a correlation between flavonoids and antioxidant activity. Also, a significant correlation was found between the content of phenolic compounds and the DPPH free radical scavenging (31). Our antimicrobial results were also in agreement with the literature that exhibited a good correlation between antimicrobial and antioxidant activity. The methanol and ethanol extracts of S. khuzestanica were detected with strong antioxidant and antimicrobial activities. Although several studies have reported antioxidant and antimicrobial activities of some Satureja species, our results proved that S. khuzestanica has strong antioxidant activities. Among species studied, S. khuzestanica was found to be more active against studied microorganisms than the other species.

In the cytotoxic assay, ethanol, methanol, and water extracts exhibited cytotoxic effects on MCF-7, DLD-1, and osteosarcoma cells. Methanol extract had a more powerful cytotoxic effect than ethanol extract on MCF-7, DLD-1, and osteosarcoma cells. There are some reports that contribute to the relationship of cytotoxicity with antioxidant activity. Therefore, antioxidant potential of the methanol extract may contribute to its cytotoxic activity.

These phenolic compounds could be responsible for the observed cytotoxic activity of the methanol and ethanol extract. Since the cytotoxic activity of S. khuzestanica extracts has not been reported up to now, additional research is needed to investigate its cytotoxicity.

Dysregulation of the mitochondrial pathway of apoptosis is one of the most important events during carcinogenesis. Bcl-2 protein family, including antiapoptotic (Bcl-2 and Bcl-xl) and proapoptotic (Bax and Bak) members play an essential role in the regulation of this pathway. According to our results, Bax/Bcl-2 ratio was significantly (P < 0.05) correlated with MCF-7 cell line.

CONCLUSION

Our analysis on the antibacterial, antioxidant cytotoxicity and apoptotic effect of S. khuzestanica in different cancer cell lines provided a better insight into understanding the therapeutic strategies and molecular mechanisms of breast cancer in order to predict genetic risk. Thus, it would be possible to obtain a novel drug that could potentially be less toxic and more cost-effective against cancer. Our results suggest that these methanolic and ethanolic extracts might have the potential to be used in, for example, anticancer agents.

Conflict of interest

No conflict of interest was declared by the authors.

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