



Study of compounds, cytotoxicity and biological activities of essential oil of *Satureja rechingeri* Jamzad

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Abstract

Nowadays, the essential oil has received a special position for the treatment of diseases. Although *Satureja rechingeri* Jamzad is an endemic species of Iran, unfortunately few studies have been conducted on its biological properties. In this study, along with the analysis of the compounds of *Satureja rechingeri* essential oil, cytotoxic, antioxidant and antibacterial properties of the essential oil of this species were investigated. The compounds of prepared essential oil were analyzed by GC-FID and GC-MS using Clevenger. Disc diffusion and MTT methods were used to determine the antibacterial activity and cytotoxicity of the essential oil, respectively. The antioxidant activity of the essential oil was measured by two methods of reducing power assay and DPPH free radical scavenging. p-Cymene (46.5%) was the most identified compound in the essential oil. The essential oil showed higher inhibitory effect on seven bacterial strains relative to the standard antibiotics. The studied essential oil showed significant concentration-dependent inhibitory effect on four cancer cells of Vero, SW480, MCF7 and JET3 with 50% lethal effect of 15.6, 125, 15.6 and 250 µg/mL for each line, respectively. The highest adsorption (2.6 nM) was at 500 µg/mL for reducing power assay and 50% free radical inhibition at a concentration of 375 µg/mL for DPPH antioxidant assay. In general, the essential oil of *Satureja rechingeri* with high antioxidant, antibacterial and anticancer activity can be used as a cheap and affordable natural product in clinical and pharmaceutical fields.

Keywords *Satureja rechingeri* · Essential oil · Antioxidant · Antibacterial · Cell cytotoxic

Introduction

The essential oils are aromatic compounds that are found in various organs of plants and due to evaporation due to air vapor these are called “volatile essential oils” (Guenther 1991). The essential oils may be obtained directly

from changes in protoplasm and resin from the cell wall or from the hydrolysis of some glycosides that are different in the different plant families where the essential oil is formed and replaced. The essential oils are mainly involved in biosynthesis, metabolism and biological activities of the plant depending on the climatic conditions of the plant environment. Various factors such as harvest time, method of collection, drying, packing and storage in the store affect the quality and quantity of herbal essential oils (Skoćibušić and Bezic 2004). Today, due to the disadvantages of synthetic antibiotics such as side effects, high cost and the most important problem: the resistance of pathogenic microorganisms to these antibiotics, natural compounds is widely used for the treatment of diseases (Pokorny 2007). Numerous studies have been conducted on the biological properties of essential oils including anti-inflammatory (Purohit and Kapsner 1994), antibacterial (Yousefzadi et al. 2014), antioxidant and anti-cancer (Salehi et al. 2007) and properties. *Satureja* belongs to Lamiaceae family with 30 species, some of which are one year and most of them are perennial

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(Ahmadi et al. 2009). *Satureja* species are mainly native to the eastern Mediterranean and West Asian regions and generally grow in areas with moist climates and deep soil to arid and sunny areas with rocky soil (Jamzad 1996). Iran is one of the most important reservoirs of *Satureja* germplasm in the world, with eight of the 14 species endemic to Iran (Jamzad 2009). *Satureja rechingeri* species have gray leaves and high trichome on the surface of the stem, leaves and other organs (Jamzad 1996). Various studies have shown that different species of *Satureja* species are mainly native to the eastern Mediterranean and West Asian regions and generally grow in areas with moist have antimicrobial and antioxidant properties (Giweli et al. 2012). Although *Satureja rechingeri* species are native to Iran, only few studies have been conducted to investigate the biological properties of this species, including the cytotoxicity of aqueous and methanolic extracts of *Bellaria artemia salina*, antioxidant and antibacterial activity of methanol extract as well as wound healing (Alizadeh and Naturforsch 2015; Mashjoo et al. 2016; Sahraei et al. 2016). Searches in databases show despite the cytotoxic effect of many species of *Satureja* spp., *S. khuzestanica* (Yousefzadi et al. 2014), *S. sahandica* (Yousefzadi et al. 2012) and *S. hortensis* (Ramshini et al. 2016), has been studied, so far the properties of the cytotoxic effects of *Satureja rechingeri* have not been studied. The present study has attempted to elucidate the cytotoxic, antioxidant and antibacterial properties of this species as raw materials for the production of pharmaceutical products.

Materials and methods

Collecting and drying samples

After collecting the plant, the samples were placed in yarn fabrics that were prepared for this purpose, transferred to the laboratory and dried for a week in shade with appropriate air conditioning. The plant was verified by Department of Plant systematic at Agriculture and Natural Resources Researches Center of Hormozgan, Bandar Abbas, Iran.

Extraction of essential oil using Clevenger

In order to extract the essential oil by hydrodistillation, about 100 g of dried and grounded of the plant was poured into 2-liter balloons and covered with distilled water. After installing Clevenger, we let it boil in water for 2–3 h. Due to the oil nature of the essential oils, the essential oil was floated on an aqueous layer, which we readily separated, to extract the essential oil, using sodium sulfate salt without water. The essential oil was poured into small tubes and kept in the freezer – 20 °C until the test.

After preparation of the essential oil and its injection into FID-GC, suitable conditions were obtained for the best separation. Then, by using gas chromatography coupled mass spectrometry (GC–MS), the essential oil compounds were quantitatively and qualitatively identified.

Identification of essential oil compounds

For gas chromatography analysis of essential oil, we used Varian CP 3800 (5DB) gas chromatography with 25 mm length, 0.25mm inner diameter and 0.25mm thin layer thickness. The oven temperature was maintained at 60 °C for 1 min and then increased to 250 °C at a rate of 4 °C/min and maintained at this temperature for 10 min. The temperatures of the injection and detector parts were 250 and 280 °C, respectively, and we used nitrogen gas at a flow rate of 1.1 mm/min as carrier gas.

Essential oil analysis

For the essential oil analysis, we used a coupled mass Thermo-quest Finnigan Trace gas chromatography equipped with a DB5 column with 60 m length, internal diameter of 0.25 mm and thin layer thickness of 0.25 mm. The oven temperature increased 60–250 °C at a rate of 4 °C/min and was maintained at 250 °C for 10 min. we used helium carrier gas with a flow rate of 1.1 mL/min and ionization energy of 70 electron volts. The compounds were identified using various parameters such as time and inhibition index (RI), study of mass spectra, comparison of these spectra with standard compounds and information contained in GC–MS computer library (Adams 2001). The relative percentage of each of the constituents of the essential oil was obtained from subtracting the area under the curve in GC chromatogram by normalizing the surface and ignoring the response coefficients.

Introduction of bacterial strains

In this study, eight strains of Gram-positive and Gram-negative bacteria were obtained from the Pasture Institute (Tehran) were used in the anti-microbial activity assay. The characteristics of the standard bacteria were used are shown in Table 1.

Antimicrobial test (disk diffusion method)

We used disk diffusion method in order to determine the antibacterial activity of the essential oil by Muller Hinton Agar culture medium. Bacterial disk analysis

Table 1 Characteristics of the bacterial strains used

Bacteria	Gram	Standard
<i>Enterococcus faecalis</i>	+	ATCC 29,212
<i>Bacillus subtilis</i>	+	ATCC 465
<i>Bacillus pumilis</i>	+	PTCC 1274
<i>Staphylococcus aureus</i>	+	ATCC 25923
<i>Staphylococcus epidermidis</i>	+	ATCC 12228
<i>Klebsiella pneumoniae</i>	–	ATCC 10031
<i>Escherichia coli</i>	–	ATCC 25922

(Kirby-Bauer) was performed according to NCCLS method (NCCLS 1997). The culture medium was prepared in 7 g/200 mL distilled water. The culture medium was transferred to autoclave (120 °C) with other sterilized media for 15 min. The autoclaved molar Hinton agar medium was distributed in sterile plates for sterilization at later stages. The pre-prepared bacterial tubes' turbidity was compared with 0.5 McFarland's standard of 1.5×10^8 bacterium per mL. Then, the swab was immersed in a suspension of bacterial suspension (10^6 CFU/mL) in three directions over Muller Hinton agar medium to culture the bacteria on the plates. The desired concentrations of essential oil and standard antibiotic were poured onto sterile paper discs (6 mm in diameter) and then the discs were fixed on bacteria-infected agar culture medium. The diameter of the non-growing halos was determined after 24 h of incubation at 37 °C. The diameters of these halos were measured using Hi Antibiotic Zone Scale ruler and the mean results were calculated three times. The standard antibiotic ampicillin was considered as the positive control.

Determination of minimum inhibitory concentration (MIC) of the essential oil

We used micro-dilution susceptibility assay, as recommended by NCCLS (1999), in order to determine the minimum inhibitory concentration (MIC) of bacterial growth by *Satureja rechingeri* essential oil. 96 well micro plates were used for this purpose. Each concentration of essential oil was poured into the wells of each column up to column number 12. All wells with the exception of row 8 wells were pelleted from the 100 μ L of prepared stock solution of bacteria at a concentration of 1×10^6 CFU (Colony Forming Unit). The eighth row wells had essential oil and culture medium only as the control. Column 12 served as a control for bacteria only to determine the bacterial turbidity containing culture medium and bacteria. Finally, the micro-plates were incubated at 37 °C for 24 h. In order to determine the minimum inhibitory concentration, the first well that had no turbidity, in other words no bacterial growth, was used as MIC.

Cell lines

Four cancer cell lines of kidney (Vero), clone (SW480), uterine (JET3) and breast (MCF7) were used for this species essential oil cytotoxicity study. Four cell lines were prepared from the cell bank of Pasteur Institute of Iran. The cells were grown and cultured in RPMI1640 culture medium supplemented with 10% FBS 100 units per mL of streptomycin and penicillin antibiotics and 1 μ g/mL of the antifungal Fondizone.

Determination of cytotoxicity by MTT (Methyl Thiazol Tetrazolium)

MTT test is a colorimetric assay based on the reduction and breakdown of tetrazolium yellow crystals by the chemical formula of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-1-(MTT) tetrazolium bromide performed by succinate dehydrogenase enzyme and the formation of insoluble blue crystals Horiuchi et al. (1988). In order to perform the test, first, 1×10^5 cells were cultured in each of the 96-well plates and the cells were allowed to adhere to the plate and stabilize. Then, to investigate the effect of the essential oil on cell growth and proliferation, the essential oil was injected at concentrations of 1000, 500, 250, 125, 62.5, 31.2, 8.6 and 7.15 in wells with the cell and plate was incubated for 24 and 48 h in a CO2 incubator. Over time, the supernatant was discarded, 200 μ L of culture medium containing 0.5 mg/mL of MTT solution was added to each well and incubated for 2–4 h. Before colorimetric, the culture medium was first removed from the wells and then 100 μ L of DMSO solvent was added to each well for reading. Finally, the optical absorption of each well was read at 570 nm and using the standard curve the number of cells was calculated. It should be noted that for each cell line a linear relationship is found between the number of cells and the optical absorption of the final solution. So for each cell type we should draw a standard curve corresponding to the same cell line.

Antioxidant assay

Antioxidant assay of Reduction Power

This test was performed on the basis of the reductive ability of iron chloride (III) to iron chloride (II) by essential oil, according to the method of Sun et al. (2011) with slight variations. In this method, yellow to dark green or blue is the basis of measurement. For this purpose, 50 μ L of different concentrations of the essential oil (100–500 μ L) and/or

standard ascorbic acid at concentrations of 10–50 μL was mixed with 50 μL of 0.2 M phosphate buffered salt (pH 6.6) and 50 μL potassium ferricyanide (1%), then for 20 min was in a warm bath of 500 cc and dark conditions, the reaction was completed by adding 50 μL of trichloroacetic acid (10%) and the reaction solution was centrifuged for 10 min at 3000 rpm. Then, 50 μL of distilled water and 10 μL of chloroform (0.1%) were added and stored at room temperature for 10 min. The samples' absorption was read at 700 nm using Alpha Spectrophotometer (South Korea). Increasing sample absorption means increasing reducing power. Distilled water was also used as Blanc and ascorbic acid as Standard.

Antioxidant assay of DPPH radical scavenging

In order to evaluate free radical scavenging (DPPH) of diphenyl-1-picrylhydrazyl, first 50 μL of different concentrations (100–500 $\mu\text{g}/\text{mL}$) of 1:1 ratio of *Satureja rechingeri* essential oil was added to 96-well micro-plates with 50 mL of methanol DPPH 0.1 mmol. The microplates were incubated for 30 min at 37 °C and in darkness. The absorption at 518 nm was read by ELISA reader against methanol Blanc. In this method, ascorbic acid at a concentration (10–50 $\mu\text{g}/\text{mL}$) was used as standard and the percentage of free radical scavenging of DPPH was calculated by the formula $I(\%) = [(A_0 - A_t) / A_0] \times 100$. In this formula, A_0 and A_t are the control and sample adsorption, respectively. Finally, the results were expressed as IC_{50} (some of the antioxidants required to inhibit 50% DPPH free radical concentration) (Bajpai et al. 2013).

Statistics

All data are reported as mean \pm SD. Analysis of variance was performed using SPSS 19.0 (IBM, SPSS) for Windows. Significant differences between means were determined using the Duncan's multiple range test in ANOVA.

Results

Identification of essential oil compounds

Essential oil production efficiency was 1.25% by weight in *Satureja Khozestanica* (Table 2). Identification of components of essential oil of *Satureja rechingeri* using GC and GC–MS showed that among the five identified compounds, p-Cymene with 46.5% was the highest and gamma-terpinene

Table 2 Antimicrobial activity of *Satureja rechingeri* essential oil

Components	Retention index	Percentage
Thymol	1290	6.2
P-Cymene	1023	46.5
γ -Terpinene	1018	8.1
Limonene	1031	6.3
Carvacrol	1297	4.2

Table 3 Antimicrobial results of *Satureja rechingeri* essential oil with concentration (10 mg/disc)

Microorganism	<i>Satureja rechingeri</i>		Antibiotic Ampicillin ^c
	IZ ^a	MIC ^b	
<i>B. subtilis</i>	28 \pm 0.5	0.46	14 \pm 0.4
<i>B. pumilus</i>	27 \pm 0.5	0.46	15 \pm 0.3
<i>E. faecalis</i>	15 \pm 0.3	3.75	11 \pm 0.3
<i>S. aureus</i>	20 \pm 0.1	1.87	13 \pm 0.3
<i>S. epidermidis</i>	30 \pm 0.2	0.35	19 \pm 0.5
<i>E. coli</i>	17 \pm 0.3	3.75	12 \pm 0.2
<i>K. pneumoniae</i>	12 \pm 0.7	7.5	–

^aInhibitory zone also includes disk diameter (6 mm)

^bMinimum inhibitory concentration mg/mL

^cConcentration tested 10 mg/disc

Non-active (-), mean activity (7–14), high activity (14<), Results (shown as mean \pm SD) were obtained from three independent experiments, each performed in duplicate

with 8.1% was the second compound of essential oil of *Satureja rechingeri* (Table 2).

Antibacterial activity

As shown in Table 3, the essential oil of *Satureja rechingeri* showed significant inhibitory effect on seven bacterial strains compared to standard ampicillin antibiotic. Among the studied bacteria, Gram-positive bacteria *Staphylococcus epidermidis* with inhibition zone (30 \pm 0.2 mm) were the most sensitive and Gram-negative bacterium *Klebsiella pneumoniae* with inhibition zone (12 \pm 0.7 mm) were the most resistant bacteria to the essential oil of this plant. Also the essential oil of this plant had high inhibitory effect on two strains of *Bacillus subtilis* (28 \pm 0.5 mm) and *Bacillus pumilus* (27 \pm 0.5 mm) compared to ampicillin standard (14–15 \pm 0.4 mm). The minimum concentration required to inhibit the bacteria was in the range of 0.35 to 7.5 mg, the lowest concentration was related to Bacteria *Staphylococcus epidermidis* (0.35 mg/mL) and the highest concentration was related to *Klebsiella pneumoniae* (7.5 mg/mL) (Table 3).

Cytotoxic effects of essential oil

The effects of the cytotoxicity of *Satureja rechingeri* essential oil on four cell lines were studied using MTT technique. In all four cell lines, the lethality of the essential oil increased with increasing concentration of essential oil, but no difference was found in the essential oil lethality for 24 and 48 h. In uterine cancer line (JET3), the lowest and highest lethality percentages of essential oil were observed at concentrations of 7.8 and 1000 $\mu\text{g/mL}$ (13 and 78%, respectively), and 50% lethality was observed at 250 $\mu\text{g/mL}$ (Fig. 1). In Vero cancer cell line, 37% lethality was observed at the lowest concentration (7.8 $\mu\text{g/mL}$) and 87% lethality was observed at the highest concentration (1000 $\mu\text{g/mL}$). 50% lethality of Vero cancer cell was observed at concentrations of 15.6 $\mu\text{g/mL}$. In the

breast cancer cell line (MCF7), 35% lethality was observed at a concentration of 7.8 $\mu\text{g/mL}$ and 90% lethality was observed at a concentration of 1000 $\mu\text{g/mL}$. 50% lethality was observed by essential oil on MCF7 cancer line at concentration of 15.6 $\mu\text{g/mL}$ (Fig. 2). In SW480 clone cell line, *Satureja rechingeri* essential oil at the lowest (7.8 $\mu\text{g/mL}$) and highest (10 $\mu\text{g/mL}$) concentrations showed 10 and 75% lethality, respectively as well as 50% lethality was observed at 125 $\mu\text{g/mL}$ concentration (Fig. 2). In general, the most susceptible cells to the essential oil of *Satureja rechingeri* are Vero and MCF7 with 50% lethality at a concentration of 15.6 $\mu\text{g/mL}$. Next, we have SW480 cancer cell with 50% lethality at a concentration of 125 $\mu\text{g/mL}$. JET3 cancer line is also the most resistant cell to essential oil with 50% lethality at a concentration of 250 $\mu\text{g/mL}$.

Fig. 1 The results of cytotoxicity of *Satureja rechingeri* essential oil on two cancer lines (JET3) choriocarcinoma (Vero) monkey kidney. Values expressed as mean \pm SD of three independent experiments. a, b, c, d, e, f, g; show significant differences between essential oil concentrations on JET3 at 5% level ($P < 0.05$). k, l, m, n, o, p; show significant differences between essential oil concentrations on Vero at 5% level ($P < 0.05$)

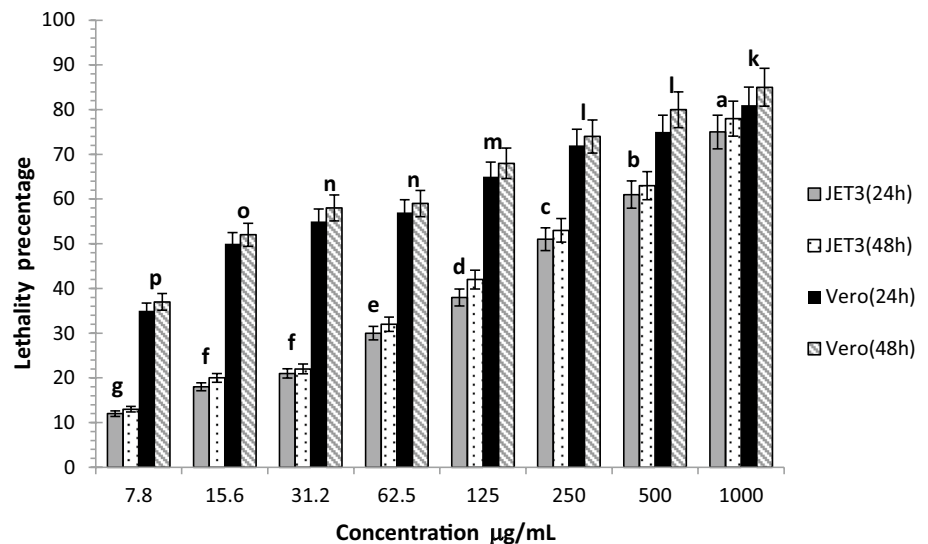
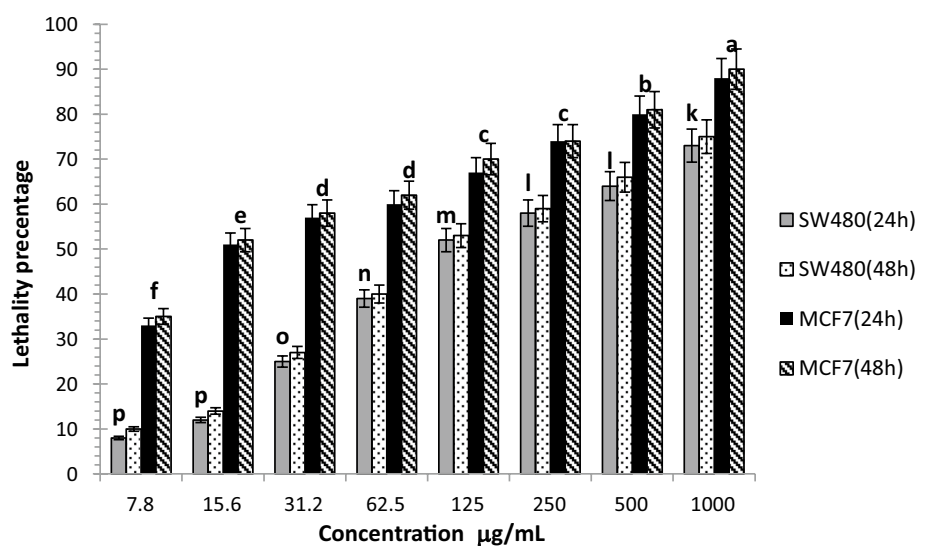


Fig. 2 The cytotoxic effects of *Satureja rechingeri* essential oil on two cancer lines; Human Breast Adenocarcinoma (MCF7) and Human colon adenocarcinoma (SW480). Values expressed as mean \pm SD of three independent experiments. a, b, c, d, e, f; show significant differences between essential oil concentrations on SW480 at 5% level ($P < 0.05$). k, l, m, n, o, p; show significant differences between essential oil concentrations on MCF7 at 5% level ($P < 0.05$)



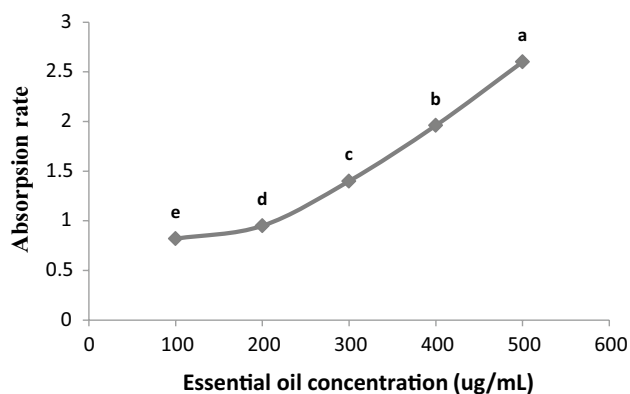


Fig. 3 Antioxidant reducing power of essential oil of *Satureja rechingeri* at various concentrations. Values expressed as mean \pm SD of three independent experiments. **a–e** Different letters show significant differences between essential oil concentrations at 5% level ($P < 0.05$)

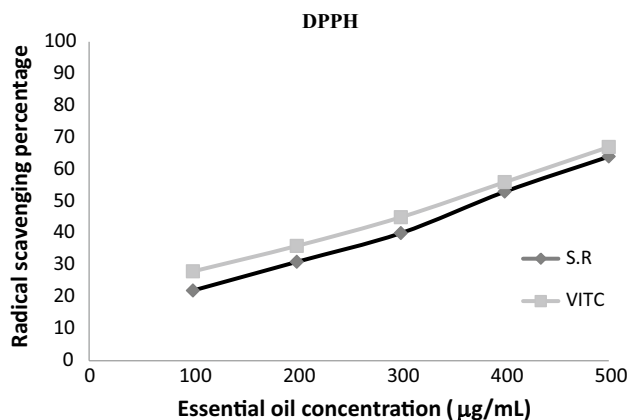


Fig. 4 DPPH free radical scavenging of *Satureja rechingeri* essential oil compared to ascorbic acid as standard at different concentrations. Values expressed as mean \pm SD of three independent experiments

Antioxidant activity

In study of the reducing power of this essential oil, the increase in reducing power was followed by a process dependent on the concentration of the essential oil, which increased with increasing the essential oil concentration (Fig. 3). In measuring reducing power, increasing the absorption of samples means increasing reducing power. The highest absorbance was 2.6 nm at a concentration of 500 $\mu\text{g/mL}$ and the lowest absorbance was 0.82 nm at a concentration of 100 $\mu\text{g/mL}$ ($P < 0.05$). In the antioxidant assay of free radical scavenging DPPH by spectrophotometry, as shown in Fig. 4, as the concentration of essential oil of *Satureja rechingeri* increased, the activity of free radical scavenging showed the highest inhibition at a concentration of 500 μL (65%) and the lowest inhibition at

a concentration of 100 μL (22%). The concentrations of essential oil and standard ascorbic acid, which resulted in 50% of the radical inhibition, were 375 and 350 $\mu\text{g/mL}$, respectively.

Discussion

The study results of the effect of *Satureja rechingeri* Jamzad essential oil in this study showed that the highest compounds were related to p. Cymene and then gamma terpinene, while the analysis of the essential oil of this species showed different results in other studies. For example, Darvishnia et al. (2015) in the analysis of the essential oil showed carvacrol and then p.Cymene compounds and Noosh-Kam et al. (2016) also identified the two carvacrol and gamma terpinene compounds as the most common compounds of *Satureja rechingeri*. In a study at three stages of *Satureja montana* species growth, at pre-flowering stage carvacrol and at flowering stage p.Cymene were the main components of the essential oil (Skočibušić and Bezic 2004). Study of diversity of eight populations of *S. sahendica* from Zanjan, Kurdistan and Azerbaijan Provinces shown that although all samples belonged to the same species, the percentage of the main compounds of the essential oil was different and formed various types (Sefidkon and Jamzad 2004). In general, studies showed that various chemical compounds of the essential oils of *Satureja* species is depended to climate, ecological condition, harvesting stage, stage of plant growth and laboratory conditions (Skočibušić and Bezic 2004).

The study results of the antimicrobial effect of *Satureja rechingeri* essential oil showed the antibacterial effects of this essential oil on seven gram positive and negative strains. In this study, Staphylococcus epidermidis was the most sensitive bacterium to the essential oil studied. Staphylococci are more medicinal resistant than other bacteria and mainly cause cutaneous infections, pneumonia, purulent arthritis, osteomyelitis, bacteremia and endocarditis and food poisoning in the human. The study results showed that both strains of *Bacillus subtilis* and *Bacillus pumilus* were highly susceptible to essential oil of *Satureja rechingeri*. In a study by Ehsani et al. (2017), essential oil of three species of *Satureja* including *Satureja rechingeri* showed high antibacterial activity. According to studies by Kalemba and Kunicka (2003), the strong antibacterial properties of the essential oils can be attributed to the presence of volatile materials and their predominant monoterpene phenols. As shown in the essential oil analysis of this species in this study, the essential oils contained the compounds p.Cymene, gamma terpinene, thymol and carvacrol. These secondary compounds, due to the hydrophobic properties, may release lipids to the bacterial cell wall and increase membrane permeability (Fitsiou et al. 2016) and will cause bacterial death

(Ulte et al. 2002). In general, in this study, gram-positive bacteria were more susceptible to the essential oil of *Satureja rechingeri* which is consistent with other studies on the high sensitivity of Gram-positive bacteria such as Ehsani et al. (2017). Studies have shown that gram-positive bacteria are more susceptible to secondary plant compounds than Gram-negative bacteria. In Gram-negative because of the cell wall lipopolysaccharide the permeability of secondary compounds is associated with more complications (Ulte et al. 2002).

Herbal medicines, despite their antioxidant and anticancer properties and minimal side effects observed, are suitable options for combination with chemical medicines that require continuous scientific laboratory and clinical investigations. Previous studies showed that the potent antibacterial extracts showed high cytotoxicity against human tumor cell lines (Gozari et al. 2019). The results of the present study showed that the combination of *Satureja rechingeri* essential oil in a dose-dependent and time-dependent manner inhibited the growth of JET3, Vero, MCF7 and SW480 cancer cells, which is similar to the study results of Yousefzadi et al. (2014) on cytotoxicity of *Satureja Khozestanica* essential oil dose-dependent effects on JET3, Vero, MCF7 and SW480 cancer cells inhibited the growth of these cells. These results are also consistent with the study results of Esmaeili-Mahani et al. (2018) on toxicity of methanolic extract of *Satureja Khozestanica* on MCF7 cancer cell lines. Given that cancer progression is very closely related to oxidative stress and inflammation, a compound that has anti-inflammatory or antioxidant properties may also be a cell anti-malignant agent (Moheghi et al. 2011). In the present study, *Satureja rechingeri* essential oil showed a significant antioxidant activity in DPPH free radical scavenging and reducing power. In a study by Fitsiou et al. (2016), the essential oil of two species of *S. thymbra* and *S. parnassica* was studied based on DPPH free radical scavenging, in which the essential oil of *S. thymbra* was characterized by high antioxidant activity of thymol and carvacrol. In many studies, two phenolic compounds, thymol and carvacrol alone or in combination with other oils showed high antioxidant activity (Guimarães et al. 2010). *Satureja* essential oil contains gamma-terpene, which in studies by Ruberto and Baratta (2010), gamma terpene has shown high antioxidant activity in of DPPH free radicals scavenging, as shown in this study. In general, *Satureja rechingeri* contains compounds such as carvacrol, thymol, gamma terpene, and p.Cymene, which have very high antioxidant properties (Ruberto and Baratta 2010). Phenolic and flavonoid compounds have numerous biological properties such as antioxidant properties, free radical scavenging and delay oxidative damage in important molecules (Kris-Etherto et al. 2002). Given that the antioxidant activity of phenolic compounds in plants is mainly due to their reducing power and chemical structure,

by donating electrons to free radicals they can neutralize complex formation and metal ions and the single and triple oxygen molecule silence through displacement or peroxide break down (Kumaran and Karunakaran 2006). Therefore, it is possible that the phenolic and flavonoid compounds present in the compound of *Satureja rechingeri* essential oil inhibit DPPH free radicals and triple iron chloride reduction.

Conclusions

According to the study results, it can be said that the essential oil of *Satureja rechingeri* showed significant antioxidant, antibacterial and anti-cancer properties, as it has significant antibacterial and antibacterial properties. The anti-bacterial and DPPH free radical removal showed activity higher than or equal to the standards used. The study results provided a basis for further studies on the purification of these compounds for clinical and scientific use of these natural and available products.

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Declarations

Ethical statement This article does not contain any studies involving animals performed by any of the authors. This article does not contain any studies involving human participants performed by any of the authors.

Conflicts of interest Mitra Arman has no conflict of interest. Kiana Pirian has no conflict of interest. Mostafa Alinaghizadeh has no conflict of interest. Fatemeh Khosheghbal has no conflict of interest. Reza Nahavandi has no conflict of interest. Saeid Tamadoni Jahromi has no conflict of interest.

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