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Original Article

Evaluation of Chemical Composition of *Vaccaria oxyodontha* Boiss Plant Essential Oils and Antioxidant and Antibacterial Effects on Gram-Negative and Gram-Positive Bacteria

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Abstract

Background & Aims: *Caryophyllaceae* is a large family of about 2200 herbaceous or subshrub species. *Vaccaria oxyodontha Boiss* is of the family Caryophyllaceae. This plant grows as a weed in agricultural land of sugarcane, wheat, barley, forage, and summer crops such as sugar beet, potato, cotton, and onion. All parts of the *V. oxyodontha Boiss* plant, especially its root, contain a type of saponin, which shows its medicinal value. According to traditional Iranian medicine, this plant is a blood purifier and has anti-infective, utilized for cough, anti-rheumatism, and anti-inflammatory properties. This study first aimed to investigate the chemical composition of *V. oxyodontha* Boiss essential oil (EO) and the antioxidant and antibacterial properties of the hydroalcoholic extract of this plant on gram-positive and gram-negative bacteria.

Materials and Methods: In this study, EOs were extracted from the aerial parts of *V. oxyodontha Boiss* using the combination of hydrodistillation and microwave-assisted hydrodistillation methods. Then, the antioxidant properties and antibacterial activity of the extract were evaluated, and the chemical compositions of the EOs of *V. oxyodontha Boiss* were determined using gas chromatography-mass spectroscopy (GC-MS). The extract's antioxidant activity was studied by the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) method. In addition, the plant antimicrobial effects were investigated by the agar disk diffusion method, and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined as well. Finally, the antibacterial activity of the mentioned plant was compared with the antibiotic discs of gentamicin (10 mg) and vancomycin (30 mg).

Results: The major compounds in the EOs were 2-Pentadecanone,6,10,14-trimethyl (10.52%) and 1,2-Benzenedicarboxylic acid (19.27%). Half maximal inhibitory concentration (IC_{50}) of *V. oxyodontha Boiss* was 0.49 µg/mL, which was higher than that of vitamin C (IC_{50} value of 0.56 µg/mL). Further, MIC and MBC for the *V. oxyodontha Boiss* extract against *Staphylococcus aureus* and *Escherichia coli* were 62.5 and 125 mg/mL, as well as 31.25 and 62.5 mg/mL, respectively.

Conclusion: The results of GC-MS demonstrated that the EO of the *V. oxyodontha Boiss* plant contains antioxidant and antibacterial compounds. Its antioxidant properties are higher than vitamin C. The highest diameter of the inhibition zone caused by the methanol extract of the *V. oxyodontha Boiss* plant was against *S. aureus* and *E. coli* (23.7 ± 0.29 mm and 15.65 ± 0.33 mm, respectively). Compared with the antibiotics vancomycin, the *Vaccaria oxyodontha Boiss* represented a stronger inhibitory effect in the inhibition of *S. aureus* ($P < 0.05$).

Keywords: *Vaccaria oxyodontha* Boiss, Oils, Volatile, Anti-bacterial agents, Adverse effects, Chemistry

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1. Introduction

Caryophyllaceae is a large family of about 2200 herbaceous or subshrub species and 86 genera. The family is known for its ornamental plants and saponin compounds. *Caryophyllaceae* is one of the largest plant families with 38 genera in Iran and Europe [1]. The *Vaccaria is* of the family *Caryophyllaceae* is a one-year static plant with 30-60 cm height and multiplies by seed. It has the shoot branch at the end. Bi-directional inflorescence, with numerous flowers and pink or bright red, is located at the end of branching stems. Furthermore, the flower Bowlin is a free part in compression mode and is collectively in the form of a jug with a tight span. Given the many activities of the plants belonging to the *Vaccaria* genus,

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the characterization, determination, and comparison of their essential oils (EOs) and extracts seem highly logical. *Vaccaria oxyodontha Boiss* is synonym with *Vaccaria hispanica* (Miller) Rauschert [2,3].

To the best of our knowledge, no scientific study has so far focused on the *V. oxyodontha Boiss* plant. However, extensive studies have been published on other plants in the *Brassicaceae* family. According to the Chinese pharmacopeia, the seeds of *Vaccaria segetalis* can treat urinary diseases and kidney infections [4]. Falih et al [5] showed the anticancer and anti-infective effects of Saponaria vaccaria plant, and utilized it for treating coughs, rheumatism, skin diseases, and hepatic eruptions. Likewise, Xu et al expressed that the Vaccaria n-butanol extract decreases proinflammatory cytokines and the infection risk of *Trichinella spiralis* [6]. Many studies have evaluated the antioxidant, anti-inflammatory, and blood flow activator effects on bile disorders, liver eruption, and other disorders and diseases of the body in *Vaccaria* plant family.

2. Objectives

In this study, EOs from the aerial parts of *V. oxyodontha Boiss* were extracted using a combination of hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD). This study first investigated the chemical composition of *V. oxyodontha Boiss* plant EOs by the gas chromatography-mass spectroscopy (GC-MS) method. Then, the antioxidant properties of its methanolic extract were evaluated with the 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) method. The antibacterial activity of the extract was examined against *Escherichia coli* and *Staphylococcus aureus*.

3. Materials and Methods

3.1. Chemicals

The 2,6-ditertbutyl-4-methyl phenol (butylated hydroxytoluene) standard antioxidant agent, nitrofurantoin antibiotic, nalidixic acid antibiotic, and DPPH were all purchased from Sigma-Aldrich GmbH (Munich, Germany). The Mueller-Hinton agar and Muller-Hinton broth culture media were purchased from Merck Company, Germany. Standard strains, including *S. aureus* and *E. coli*, as gram-positive (ATCC 1885) and gram-negative (ATCC 1625) bacteria, respectively, were provided by Iran Medical Sciences University, Tehran, Iran.

3.2. Plant material

The new aerial parts of *Vaccaria oxyodontha Boiss* were harvested in the middle of May 2013 from a farm belonging to the Agricultural Research Station of Damghan province, Iran (Figure 1). The aerial parts were separated with meticulous care to extract the oil and finally dried at the shadow in a clean place to avoid extra damage and minimize cross-contamination.

3.3. HD method

Conventional HD was performed using a circulatory commercially available Clevenger apparatus (Ashk Shishe Company, Tehran). The *V. oxyodontha Boiss* sample was dried at shade (one week) and weighed precisely. It was then immersed in the ratio of 1:10 of the plant (g) to water (mL), and the volatile oils were collected at sequential times. The experiments were conducted twice for each distillation time. The maximum distillation period was three since no more EOs were obtained after that time. Next, the EOs were separated and kept at - 4°C before analysis.

3.4. MAHD method

The microwave oven (Figure 2) used for MAHD was provided by (Samsung, South Korea) trademark operating at 2450 MHz. The maximum power of the oven was regulated at 1000 W. The dimensions of the interior cavity of the oven were 29 × 37 × 40 cm. The microwave oven was modified by drilling a hole at the top. A flat bottom flask with a 1000 mL capacity was placed in the oven and directly connected to the Clevenger apparatus through the

Figure 1. Image of *Vaccaria oxyodontha* Boiss Plant

Figure 2. Image of Microwave Oven Apparatus

hole. For MAHD, 50 g portions of the plant sample were soaked in 500 mL of distilled water at room temperature (25°C) for one hour to hydrate its external layers, and the excess water was drained off. Soaking time, during which the maximum amount of absorption is achievable, was determined from a preliminary experiment. Afterward, the moistened plant material was placed in a flat-bottom flask combined with a Clevenger apparatus. During the process, the vapor continuously passed through the condenser located outside the microwave cavity where it was condensed. The MAHD process was performed at different times and repeated until obtaining no more EO. For each condition, experiments were replicated twice. The EOs were collected in amber brown vials, dehydrated with anhydrous sodium sulfate, capped under nitrogen, and kept at 4°C until analysis.

3.5. Preparation of the extracts of Vaccaria oxyodontha Boiss

In the first step, the aerial plant parts (*V. oxyodontha Boiss*) were cut up and ground in a coffee mill (Moulinex Corporation, France). This step was followed by transferring the obtained powder to dark-colored flasks, mixing with 85% (v/v) methanol at a material-to-solvent ratio of 15:100 (mg/v), and heating at 50°C for 30 minutes. Next, the slurry was filtered through Whatman No. 1 filter paper. The residue was re-extracted twice more, and combined supernatants were evaporated to dryness under vacuum at 40°C using a rotary (IKA, Germany). Finally, the prepared extracts were stored at 4°C in the refrigerator until further analysis.

3.6. GC and GC-MS analyses

The quantitative and qualitative evaluations of the oils were performed using GC and GC-MS instruments. The GC analysis was performed on a Varian (CP 3800) gas chromatograph equipped (Agilent 5975 C) with a split/splitless (10:1) injector (290°C) and a flame ionization detector (250°C). N_2 was used as the carrier gas (0.8 mL/min). The capillary column used CP-Sil 5 CB (30 m × 0.25 mm × 0.25 µm film thickness). The oven temperature was held at 50°C for 5 minutes and heated to 240°C at a rate of 3°C minute-1 ramp, followed by a further rise to 300°C with a programmed 300 °C with a programmed 5 °C min-1 ramp. A final hold was allowed for a complete column clean-up for 3 minutes. Quantitative data were obtained through the system area percentage. GC-MS determinations were performed on an HP-6890 GC system coupled with a 5973 network mass selective detector equipped with an HP-5MS capillary fused silica column (30 m × 0.25 mm ID × 0.32 µm film thickness). The operating conditions were the same as those described above, but the carrier gas was He with a 0.8 mL/min flow rate. Mass spectra were taken at 70 eV and recorded over the m/z range of 20-500 Amu. All chromatographic measurements were performed in triplicate, and the mean of the retention times and percentage compositions of each component were taken into consideration. Thus, the same times were discarded if they differed by more than 1 second, and the experiments were repeated in duplicate.

3.7. Identification and quantification of EO compounds

In this work, the constituents of the oil were determined and characterized by:

i. Comparing their mass spectral fragmentation pattern regarding authentic samples and retention indices (RI) relative to C_9-C_{21} n-alkanes with those given in the literature [2] and some of our previous reports;
ii. Storing the data in an MS library (Wiley 275);
iii. Matching the fragmentation pattern with those in the National Institute of Standards and Technology Mass Spectral Library package with a resemblance percentage above 85%.

The relative percentage amounts of the components were directly calculated from the peak area using an HP-6890 GC system on the HP-5MS column considering the sum of all eluted peaks as a hundred percent without using the correction factor. Finally, the identical volumes of each essence were separately injected into GC-MS [7-10].

3.8. Determination of antioxidant activity by the DPPH method

The method proposed by Nabavi et al was used to assay the extracts' antioxidant capacity and the free radical-scavenging effect on the DPPH radical [11].

Accordingly, 100 µL of the extract, at concentrations of 200, 400, and 600 µg/mL, was mixed with 2 mL of solution DPPH (0.004 g DPPH solution within 100 mL Methanol). The same process was repeated for vitamin C to compare the extract's antioxidant power with vitamin C. Then, it was incubated in a dark room for 15 minutes, and absorbance (A) was measured at 517 nm for all samples (BioTek Epoch, USA). Methanol and DPPH were employed as blank and control samples, respectively. The antioxidant activities of the samples were determined using the ascorbic acid standard curve. The antiradical activity of the samples was expressed by IC_{50} (mg/L) according to previous research [12]. The DPPH scavenging effect (%) is expressed by Eq. (1) as follows:

$$DPPH\ scavenging\ effect\ (\%) = (A_o - A_1)/A_o \times 100 \quad (1)$$

where A_o and A_1 represent the absorbance of the control (blank) and the absorbance in the presence of samples (the *V. oxyodontha* Boiss extract and ascorbic acid), respectively. Numerical IC_{50} values denote the sample's concentration, which is required to scavenge 50% of DPPH free radicals.

3.9. Preparation of various dilutions of the extract

Different dilutions of the extract (1, 1/2, 1/4, 1/8, 1/16, 1/32, and 1/64) were prepared using sterile distilled water to ensure anti-bacterial properties.

3.10. Preparation of Microbial Strains

In this study, the bacteria were *S. aureus* RTCC 1885 and *E. coli* PTCC 1330, lyophilized and prepared from the Faculty of Pharmacy's Microbial Collection of Tehran University of Medical Sciences (Iran).

Bacterial lyophilized ampoules (*S. aureus* and *E. coli*) were first opened under sterile conditions and transferred to nutrient broth (Merck, Germany), and then incubated for 24 hours at 37°C. Then, to ensure the bacteria's purity from the nutrient medium, it was linearly cultured on the differential culture medium overnight and incubated for 48 hours at 37°C. A loop was then taken from the bacterial colony and inoculated into the broth nutrient 24 hours before each test. A new 24-hour culture was prepared for each test to evaluate the antimicrobial effects. Finally, the microbial suspension was prepared with a concentration equal to 0.5 McFarland.

3.11. Determination of microbial susceptibility

The protocols used in this study were based on the guidelines of the Clinical and Laboratory Standards Institute (CLSI) tables. The disc diffusion method was applied to determine the susceptibility of microbial strains against methanolic extracts. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were employed to determine microbial susceptibility. MIC was determined by dilution in a liquid medium (microbroth dilution). for the MBC test, concentration of extract that inhibited of bacterial growth in MIC step, were cultivated on Muller-Hinton agar medium. All the experiments were conducted in triplicate.

3.12. Statistical analysis

SPSS software (version 20) and Kruskal-Wallis and Dunn's tests were utilized for data analysis. The data are presented as mean values ± standard deviations (SD). The values of $P < 0.05$ for Kruskal-Wallis and Dunn's tests were considered significant.

4. Results

4.1. Chemical constituents of the EOs of Vaccaria oxyodontha Boiss

The chemical constituents of the EOs obtained from *V. oxyodontha* Boiss are shown in Table 1 and the chromatogram in Figure 3. The major compounds in the EOs were 2-pentadecanone, 6,10,14-trimethyl (10.52%), and 1,2-benzenedicarboxylic acid (19.27%).

Table 1. Chemical Compositions of the Essential Oils of *Vaccaria oxyodontha* Boiss Using GC-MS

Compounds	RT[a]	%
Dodecane n-dodecane adakane	12.053	1.96
Fenchyl acetate	12.443	2.32
MINT FURANONE	12.531	0.99
Naphthalene, decahydro-1,5-dimethy	12.633	1.83
Cyclohexane	15.457	3.35
Caryophyllene	15.939	0.78
(+)-Aromadendrene 1H-Cycloprop [121608 000489-39-4 59 e]azulene	16.479	1.41
3-Buten-2-one, 4-(2,6,6-trimethyl-	16.946	1.81
2H-3,9a-Methano-1-benzoxepin,	17.243	0.73
Phenol, 2,5-bis(1,1-dimethylethyl	17.312	0.85
1S,CIS-CALAMENENE	17.536	0.62
(-)-Caryophyllene oxide (-)-5-O	18.018	0.84
(-)-Spathulenol	18.397	1.63
Caryophyllene oxide	18.490	2.87
Cyclohexene, 6-ethenyl-6-methyl-	18.670	3.54
1H-Cycloprop[e]azulene, 1a,2,3,5,6	18.787	0.64
cis-Z-.alpha.-Bisabolene epoxide	18.879	1.89
Naphthalene, 1,2,4a,5,8,8a-hexahyd	19.025	4.77
1H-Cycloprop[e]azulene, 1a,2,3,5,6	19.235	1.42
(+)-.delta.-selinene naphthalene	19.366	1.67
Valencene Naphthalene, 1,2,3,5,	19.454	2.21
(+) Spathulenol	19.726	1.85
CAPNELLANE-8-ONE 2H-Cyclopenta	19.950	1.38
(3E,5E,8Z)-3,7,11-Trimethyl-1,3,5,	20.247	2.20
6-Isopropenyl-4,8a-dimethyl-1,2,3,	20.447	5.20
cis-.alpha.-Copaene-8-ol Tricyclic	21.070	0.67
Octadecane	21.338	1.48
2-Pentadecanone, 6,10,14-trimethyl	21.927	10.52
Tetratriacontane	23.436	1.27
Dotriacontane	24.172	0.60
2-Hexadecen-1-ol,3,7,11	24.288	6.62
Pyridinium, 1-hexadecyl-, chloride	24.493	0.93
Hexatriacontane	25.228	0.67
Nonadecane (CAS) n-nonadecane	25.637	0.84
4,5. alpha. -Epoxy-3-methoxy-17-meth	25.978	1.71
Pentacosane	26.026	4.10
1,2-Benzenedicarboxylic acid	26.280	19.27
Heptacosane	26.922	2.59
		100

Note. [a] Rt: Retention time; GC-MS: Gas chromatography-mass spectroscopy.

4.2. Antioxidant capacity

Antioxidant capacity was tested using a methanolic solution of the 'stable' free radical, DPPH. A freshly prepared DPPH solution displays a deep purple color. This purple color usually disappears when an antioxidant

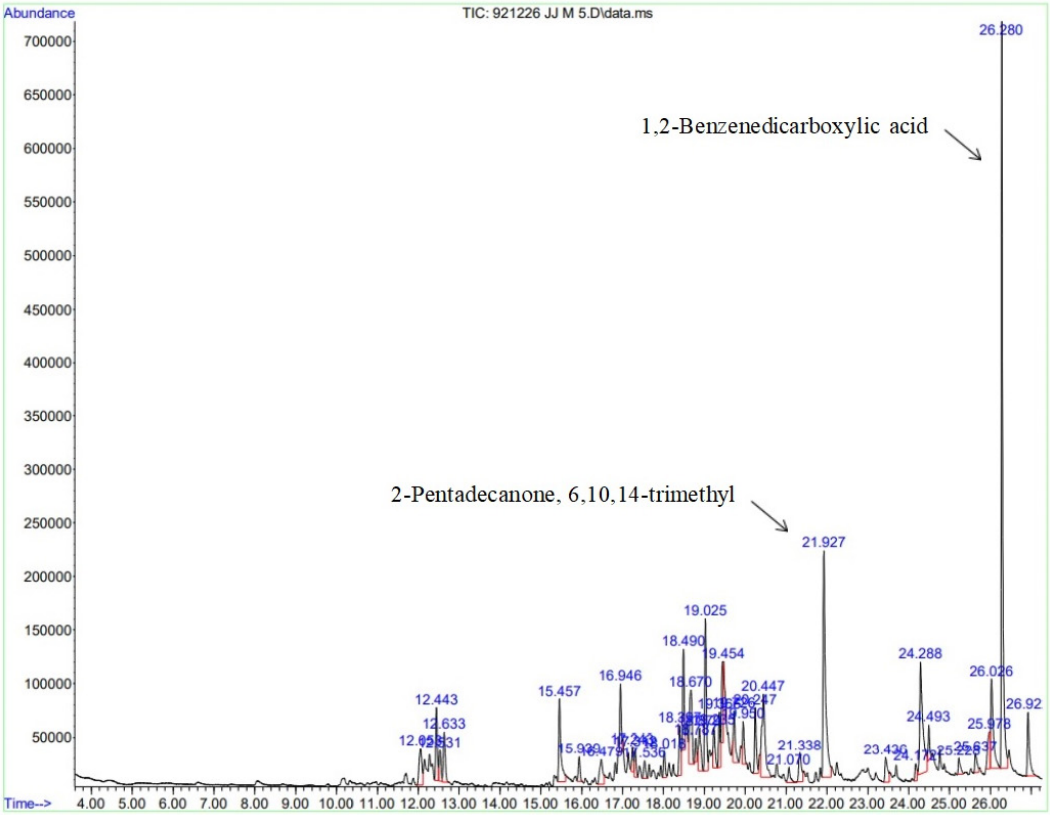

Figure 3. GC Chromatogram of the Essential Oils of *Vaccaria oxyodontha* Boiss

is present in the medium of the test. Antioxidant molecules quench DPPH free radicals, and DPPH is converted to a colorless product, resulting in a decrease in absorbance at 517 nm. The DPPH radical scavenging activities of the *V. oxyodontha* Boiss and the vitamin C extract at different concentrations are illustrated in Figure 4. The IC_{50} value of the extract for *V. oxyodontha* Boiss was 0.49 µg/mL, which was higher than that of vitamin C (an IC_{50} value of 0.56 µg/mL).

4.3. Antibacterial capacity

The diameter of the growth inhibition zone of the *V. oxyodontha* Boiss extract against the *S. aureus* strain and *E. coli* in the disk diffusion method was 23.5 and 18.65 mm, respectively.

Table 2 lists the mean and SD of the inhibition growth zone (mg/mL) related to the methanolic extract of *V. oxyodontha* Boiss at different concentrations against *S. aureus* and *E. coli*. The concentration of 500, 250, and 125 mg/mL of the extract had the highest antibacterial effect compared to vancomycin against *S. aureus*. Nevertheless, the extract effect was weaker against *E. coli* than gentamicin.

According to the results of CLSI Tables, the minimum growth inhibition zone for gentamicin antibiotic (10 µg disc) against *E. coli* strain was 19-26 mm. In the minimum growth inhibition zone for the antibiotic vancomycin (30 µg disc), the diameter of the growth inhibition zone against the *S. aureus* strain was 14-15 mm. Based on the results of Table 3, the intergroup comparison with the Kruskal-Wallis test, *V. oxyodontha* Boiss extracts had antimicrobial properties equal to gentamicin and Vancomycin antibiotics ($P<0.05$). MIC and MBC for the *V. oxyodontha* Boiss extract against *S. aureus* and *E. coli* were 62.5 and 125 mg/mL, as well as 31.25 and 62.5 mg/mL, respectively.

5. Discussion

Herbs are directly used to treat disease globally, including in Iran, China, India, and other countries in Asia and Western Europe. These plants are the *Vaccaria* family [13]. Most plants in this family are weeds on agricultural land. Flavonoids, polyphenols, kaempferol, saponins, cyclic peptides, and starch are active ingredients (synonyms) in these plants with many medical applications [14].

According to Cam et al [15], the seeds of *Vaccaria hispanica* (Miller) Rauschert are naturally distributed in all parts of Turkey. This study indicates that these *Vaccaria* family plants have potential applications in the food and pharmaceutical industries and cosmetics industries. However, this plant is not so well known. They are not yet cultivated and are not used industrially [15]. According to previous studies, the plants of this family are rich in saponins. In general, the biological role

Table 2. Mean and standard deviation of the inhibition growth zone (mg/mL) of the methanolic extract of *Vaccaria oxyodontha* Boiss in Different Concentrations Against *Staphylococcus aureus* and *Escherichia coli*

Zone of Inhibition Mean of Methanolic Extract of *Vaccaria oxyodontha* Boiss for Tested Bacteria (mm)		Different Concentrations of Extracts (mg/mL)	
S. aureus	***E. coli***		
23.7 ± 0.29	18.65 ± 0.33	500	
19.58 ± 0.42	16 ± 0.08	250	
17.95 ± 0.14	13.86 ± 0.26	125	
13.86 ± 0.26	13.48 ± 0.22	62.5	
11.58 ± 0.42	8.58 ± 0.42	31.25	
7.6 ± 0.45	5.33 ± 0.33	15.62	
2.53 ± 0.73	Indeterminate	7.8	
14.2 ± 0.08	0	Vancomycin	Positive control
0	24.1 ± 0.09	Gentamicin	
0	0	(DMSO)	Negative control

Note. DMSO: Dimethyl sulfoxide; SEM: Standard error of the mean. Values are expressed as the mean ± SEM.

Table 3. MIC and MBC of the Extracts of *Vaccaria oxyodontha* Boiss

Methanolic Extract	MIC (mg/mL)			
	Staphylococcus aureus		***Escherichia coli***	
	MBC	**MIC**	**MBC**	**MIC**
Vaccaria oxyodontha Boiss	62.5	125	31.25	62.5
Vancomycin (30 µg disc)	2 ≥	4-8	-	-
Gentamicin (10 µg disc)	-	-	2-4	4-8

Note. MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration. The group's inhibition growth zone's mean and standard deviation was 18.30, 3.3, respectively ($P < 0.05$).

of saponins is unclear in plants. However, no study has examined the effect of saponins in treating various cancers and infections. It is likely to have hypocholesterolemic properties and immune system stimulants and anti-inflammatory, antibacterials, insecticide fungicides, anti-leishmaniasis, and antioxidant properties [16]. Studies related to chemotherapy and therapeutic activities of the seeds of the plants of this family have been developed in recent years. In their study, Zhou et al demonstrated that *Vaccaria* hispanica has at least 63 different metabolites, some of which have medicinal properties [3].

According to Figure 4, a comparison between the *Vaccaria oxyodontha Boiss* extract and vitamin C, a natural antioxidant, represented that the extract had a more inhibitory effect against free radicals secreted by DPPH compared to vitamin C. Based on the antioxidant properties of this plant, they can be used in the pharmaceutical, health-cosmetics, and food industries as effective antioxidants. Biswas et al evaluated the hepatoprotective activity of the ethanolic root extract of "*Vaccaria pyramidata*" against CCl_4-induced hepatotoxicity in Wister rats [17] and reported that the *Vaccaria pyramidata* root extract had a protective and antioxidant effect on liver cells. In addition, it decreased

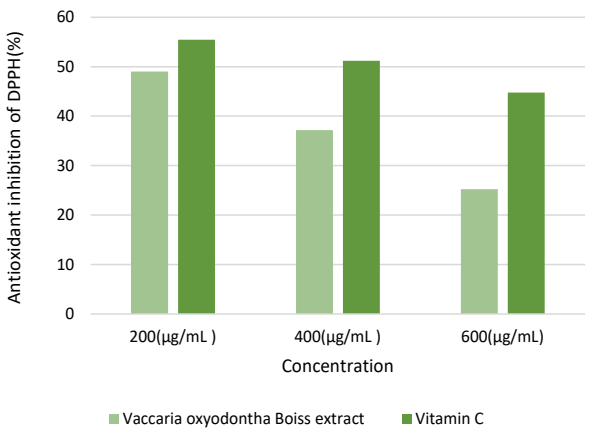

Figure 4. Antioxidant Activity of the *Vaccaria oxyodontha* Boiss Extract Compared With Vitamin C at Different Concentrations

triglycerides in rats compared to the control group.

This study investigated the antibacterial properties of the *Vaccaria oxyodontha Boiss* extract against *S. aureus* and *E. coli* using the disk diffusion method and Kerby-Bauer protocol. This test was performed in comparison with the two gentamicin and Vancomycin antibiotics. Based on the findings (Table 1), the *Vaccaria oxyodontha Boiss* extract was more potent against *S. aureus* than the Vancomycin antibiotic. Further, the diameter of the growth inhibition zone of this extract against *E. coli* was 18.65 mm. According to Mao et al [4,18], *Vaccaria segetalis* seed extracts significantly reduced bacterial load, and white and red blood cells in the urine, as well as inhibiting pathological damage to the bladder. Therefore, it can be an alternative therapeutic agent for urinary infections.

6. Conclusion

Vaccaria oxyodontha Boiss is one of the medicinal plants used in traditional medicine in Iran and some Asian countries. The results of the present study for identifying the EO constituents revealed that the highest composition of the EO was found in 2-Pentadecanone,6,10,14-trimethyl (10.52%) and 1,2-Benzenedicarboxylic acid (19.27%). The IC_{50} of the extracts of *V. oxyodontha* Boiss was equal to 0.49 µg/mL, which is a significant antioxidant property in plants. Additionally, it can be used as a strong antibacterial. Due to the unique antioxidant and antibacterial properties of this plant extract and EOs, it can find their unique place in the pharmaceutical, health, and medical industries with further research.

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Conflict of Interests

The authors declare no conflict of interests.

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