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Published at http://www.ijate.net http://dergipark.gov.tr/ijsm **Research Article**

Antioxidant and Antibacterial Effects of Some Medicinal Plants of Iran

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Abstract: Medicinal plants used in the treatment of diseases earlier times are potential sources of new drugs. The present study was undertaken to study the chemical composition, antioxidant and antibacterial activity of certain medicinal plants of Iran by gas chromatography and mass spectrometry (GC/MS), DPPH and disk diffusion method. According to the results of GC/MS, there are 46 kinds of chemical compounds including mucilage, fatty acids, flavonoid and diterpenes in flower of *Echium khuzistanicum*. There are aldehydes (7.9%), phenols (7.5%), fatty acids (5.8%) and furfural (5.4%) in the methanol extract of *Echinops cephalotes*. Furfural, steroids, vitamin B and flavonoids are the main compounds of *Marrubium anisodon*. Results of the antibacterial test showed that *Staphylococcus aureus* and *Bacillus subtilis* were more sensitive to methanol extract of *Echium khuzistanicum* root. *Pseudomonas auruginosa* was more sensitive to DMSO extract of *Marrubium anisodon* at 600 mg/ml concentration. Maximum flavonoid and phenol contents were belonging to *Echinops cephalotes*. *Marrubium anisodon* showed the best DPPH free radical scavenging activity.

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1. INTRODUCTION

Plants can produce a variety of chemicals so that new compounds always are discovered and extracted from plants. Each of these compounds may have therapeutic effects like antibacterial and antioxidant activities [1].

The antioxidant system in plants and animals comprise both-low molecular mass and high molecular mass antioxidants. Low molecular mass antioxidants described to date include water-soluble compounds such as reduced glutathione, ascorbic acid, and lipid-soluble ones such as carotenoids (including β-carotene), retinol, α-tocopherol. They usually operate as free radical scavengers. Various compounds of a plant such as fibres, carotenoids, phenols, flavonoids, isoflavones ,and ascorbic acid eliminate free radicals and have antimutagenic and antioxidant activity[2]. The ability of elimination of active oxygen makes these compounds converted to factors which protect human from ailments like cancer[3]. In recent years, there are considerable attention towards the identification of plants with the antioxidant ability [4].

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Several drug resistances have been observed in human pathogenic microorganisms due to the excess usage of commercial antimicrobial drugs in the treatment of infection [5]. Also in all countries including developed countries, the prevalence of foodborne diseases are still considered a serious issue. So, a permanent search is needed to discover effective methods and materials to treat the food infection caused by microorganisms [6]. We need to identify and introduce new medicinal and aromatic plants with effective natural antibiotics, high biological value and low side effects [7].

Echium khuzistanicum Mozaff is a biennial plant of Boraginaceae family which grows in the southwest of Iran [8]. In about 150 species of Boraginaceae, naphthoquinone pigments such as alkannin and shikonin derivatives exist in roots. Alkannin (S enantiomer) and shikonin (R enantiomer) and their derivatives have a lot of medicinal properties like anti-allergic, antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory and wound healing [9-15]. Shikonin plays a significant role in the treatment of the obesity, intestinal ulcers, skin diseases, cancers ,and AIDS [16].

Marrubium anisodon K. Koch is a plant of the Lamiaceae. There is the various activity such as antioxidant and anti-inflammatory effect in this genus [17]. *Echinops cephalotes* DC is a plant of the Asteraceae. This genus is remarkable regarding chemical composition, tens of alkaloids extracted from various parts of them which used in industry, agriculture, and medicine. Alkaloids, saponins, polyphenols, carotenoids, and phytosterols were detected in this genus [18]. The aim of this study is the assessment of the chemical composition, antioxidant and antibacterial activity of these plants of Iran for the first time.

2. MATERIAL AND METHODS

2.1. Plant Material

M. anisodon and *E. cephalotes* were collected from the medicinal plant garden of Hamadan (Natural Resources Department, Hamedan Natural Resources and Agriculture, Education and Research Center, Medicinal Plant Garden, Hamedan, Iran) and *E. khuzistanicum* was planted in a greenhouse. The plant's seeds were collected from Alhaii region around Ahwaz (the southwest of Iran). The plants were identified by the botanist, Dr. Dinarvand (Faculty member, Natural Resources Department, Khuzistan Natural Resources and Agriculture, Education and Research Center, Ahvaz, Iran).

2.2. Extraction of Plant Material

The samples were dried at the room temperature in the dark and further was ground in a mortar. About 10 grams of each plant powder extracted in 100 ml of methanol by soxhlet till the solvent in siphon tube of an extractor become colorless. The extract was concentrated at temperature below 40°C and was used for determination of flavonoids, phenols, free radical scavenging, antibacterial activity and GC/MS analysis [19].

2.3. Determination of Total Flavonoid

Flavonoids were determined using Aluminum chloride[20]. 0.5 ml of extract solution (1mg/ml) with 1.5 ml methanol, 0.1 ml Aluminum chloride (10%), 0.1 ml Potassium acetate (1M) and 2.8 ml distilled water was mixed. After 30 min, sample absorption was read at 415nm by a double beam Lambda 45 UV–visible spectrophotometer. The total flavonoid content was determined using a standard curve of quercetin (R^2= 0.981). Total flavonoid content is expressed as µg of quercetin equivalents/ 100 mg of sample. Total flavonoid was calculated by using the following equation:

$$\text{Absorbance} = 0.0077 \text{ quercetin } (\mu g) - 0.0532$$

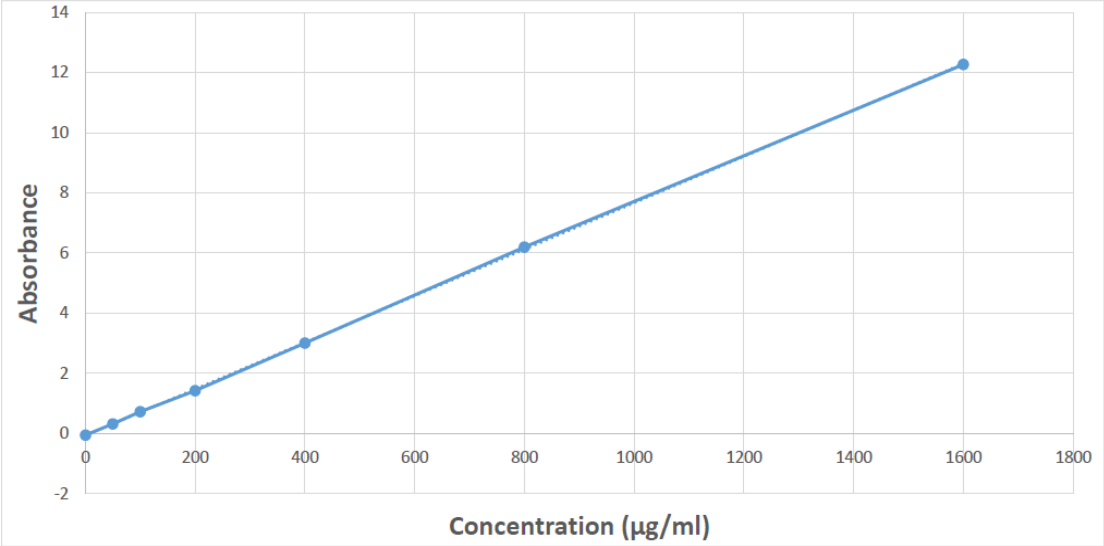

Figure 1. Quercetin standard calibration curve

2.4. Determination of Total Phenols

Total phenolic content in each extract was determined by using Folin-Ciocalteu reagent [21]. 200 microliter of extract (1mg/ml) was mixed with 1ml (1N) Folin-Ciocalteu reagent (Sigma-Aldrich, Germany) and 5.8 ml of distilled water, followed by 3ml 20% Sodium Carbonate (Na_2Co_3) 3min later. The mixture was shaken for two h at room temperature in the dark, and absorbance was measured at 165nm. All tests were performed in triplicate. Gallic acid (Sigma-Aldrich, Germany) used as a standard. The concentration of total phenolic compounds (TPC) was determined as µg gallic acid equivalents (GAE)/mg extract by using the following equation obtained from a standard gallic acid graph ($R^2 = 0.9877$):

$$\text{Absorbance} = 0.0012 \text{ gallic acid } (\mu g) - 0.0034$$

2.5. Free Radical Scavenging Activity

Free radical scavenging activity was determined by using the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The Ascorbic acid was used as a standard control. To study inhibition percent of DPPH, 50µl of the extract with different concentration (0.2, 0.4, 0.6, 0.8, 1mg/ml) mixed with 5ml of DPPH (0.0004%) and after 30 min, the absorption was measured at 517nm [22]. The percentage of inhibition (I) was calculated as:

$$I = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}]$$

IC_{50} values denote the concentration of the sample, which is required to scavenge 50% of DPPH free radical.

2.6. Assessment of Antibacterial Effects

Six human pathogenic bacteria were used including gram-positive bacteria of *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Streptococcus pyogenes* ATCC 19615 and gram-negative bacteria of *Escherichia coli* ATCC 8739, *Salmonella typhimurium* ATCC 14028 and *Pseudomonas aeruginosa* ATCC 9027 prepared from Reference Center of Bu-Ali Sina Hospital (Hamedan, Iran). The antibacterial effect of the extracts was examined by the disk diffusion method. Small paper disks (prepared from Padtan Teb Co. with a diameter of 6.4 mm) soaked in different concentration of the plant extract with 100, 200 and 600 mg/ml concentration. In this method, a suspension with the dilution equal to 0.5 McFarland standard was prepared by 24-hour culture of bacteria. At the next stage, 0.2 ml of the bacterial suspension

was added to each plate and surface-cultured by a sterile swab [23]. Then the disc containing 20 µl of the extract was placed on the medium using sterile forceps tip. The plates were incubated for 24 hours at 37 ° C [24]. The solvent was only used as the negative control and ten µg antibiotic gentamicin disc (Padtan Teb Co.) as the positive control. After incubation, the diameter of the inhibition halo was measured using a ruler and recorded. The antibacterial test of the extracts was done in triplicate for each concentration, and completely randomized design and ANOVA test were employed at 5% level. The results were expressed in means ± SEs.

2.7. Gas Chromatography and Mass Spectrometry

The chemical composition of the extracts was identified by GC/MS (Agilent 6890N gas chromatography coiled with Agilent 5973N mass detector). 1µl of each extract was injected. The separation of extract was performed using an HP-5 column of 30m in length and 0.25 mm in diameter and 0.25 µm in stationary phase thickness. The analysis conditions were shown in Table 1.

Table 1. Temperature program of analysis

Rate(˚C/min)	Temperature ˚C	Hold (min)
-	60.00	0.00
5.00	150.00	0.00
10.00	250.00	0.00

The solvent delay was 5 min, and the identification of the compound was based on comparing their mass spectra with those recorded in the Wiley 7n mass spectra database and with literature reports.

2.8. Statistical Analysis

All the experiments did with three replicates for each sample of plants. A completely randomized design was employed at 1% level.

3. RESULTS

3.1. Chemical Composition of Plant Extracts

To analyze results accurately and given that the chemical composition of these plants is not detected, after extraction, chemical composition of plants were checked by GC/MS. The amount and type of chemical compounds were achieved by comparing the data from GC/MS with information of libraries.

3.2. Chemical Composition of *Marrubium anisodon*

In methanol extract of this plant's aerial parts, 86 compounds were identified by GC/MS (Figure 2). The compounds present in this plant (with more than one percent), their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area %) are presented in Table 2. Furfural, steroids, vitamin B and flavonoids are the main compounds of *M. anisodon*. According to the results, furfural is the most abundant compound (20.43%). Furfural is the natural product of lignocellulose degradation. Also, furfural is obtained from dehydration of pentose sugars during cellulose depolymerization under acidic conditions [25]. Furfural and its derivatives are the main flavors of foods. Furfural at low concentrations (1-12 mM) inhibits microorganisms [26]. About 13.26% of this plant extract is cyclopentane which is in steroid structure. The extract consists of lactose (9.53%) and inositol vitamin (8.55%). This plant extract has flavonoids such as 4H-pyran-4-one (5.42%). Fatty acids such as the dodecanoic acid (1.036%) and pentadecanoic acid (1.55%), alkaloids such as alpha-pyrrolidone (2.21%) and cyclic isoprenoids such as cyclotetradecan (2.32%) were detected by GC/MS.

Previous studies have reported that there are some compounds such as diterpenes, sterol, derivatives of caffeic acid and flavonoids in this genus [27]. One acylated flavonoid glycoside and two tetrasaccharides phenylethanoid glycosides, velutinosides I-II, have been isolated from *Marrubium velutinum* shoot [28]. Marrusidins A and B are two labdane-type diterpenes isolated from the chloroform extract of *Marrubium anisodon* along with polyodonine [29]. The methanol extract of the plant showed a 27.7% inhibitory activity of acetylcholine esterase used for the treatment of the disease Alzheimer. This inhibitory effect was attributed to the components that are functionally or structurally similar to tacrine [30]. According to the results of GC / MS, it is possible that this effect of the plant is related to alkaloids such as alpha-pyrrolidine, which need to be tested, and confirmed in the laboratory. This family plants have been used to treat dandruff and hair regrowth [31]. According to the presence of vitamin B7 in this plant, it can be concluded that this plant is a good candidate for the treatment of hair loss and alopecia.

3.3. Chemical Composition of *Echium Khuzistanicum* (Flowers)

According to GC/MS results, 46 compounds were found in the methanol extract of *E. khuzistanicum* flower. Each of these compounds made a peak on chromatogram (Figure 3). The plant compounds with more than 1% are shown in Table 3 including the mucilage, fatty acids, flavonoids and diterpenes. According to the results, glucose is the highest compound in the flower extract of this plant (22.32%). Mucilage in *Borago officinalis* is hydrolyzed to glucose, galactose, arabinose and allantoin [32-36] so the glucose present in the extract of this plant can be obtained by hydrolysis of mucilage [37]. Mucilages are carbohydrates with very complex chemical structures and high molecular weights. One of the most important medicinal properties of the mucilage is their anti-inflammatory property. It is used to treat gastrointestinal ulcers (stomach and intestines) and infections of the throat mucous [38]. In flower extract of this plant, 11.23% of 9, 12, 15-octadecatrien-1-ol was found. This compound is also present in the *spartium junceam* extract. Fatty acids such as a capric acid (12.6%), octadecanoic acid

Table 2. The compounds present in *M. anisodon* (more than 1%)

No.	Name of the compounds	RT[a]	MF[b]	MW[c] g/mol	Peak area%
1	Furancarboxaldehyde	13.63	$C_5H_4O_2$	96.09	20.43
2	Cyclopentane	26.41	C_5H_{10}	70.1	13.26
3	Lactose	26.64	$C_{12}H_{22}O_{11}$	342.3	9.53
4	Neo-inositole	24.86	$C_6H_{12}O_6$	180.16	8.55
5	4H-pyran-4-one	11.08	$C_5H_4O_2$	96.085	5.42
6	12-methyl-E,E-2,13-octadecadien-1-ol	20.09	$C_{19}H_{36}O$	280.496	3.22
7	Cyclotetradecan	16.17	C14H28	196.37	2.32
8	Alpha-pyrrolidone	9.21	C_4H_7NO	85.106	2.21
9	Propanoic acid	8.05	$C_3H_6O_2$	74.07854	1.88
10	Pentadecanoic acid	25.11	$C_{15}H_{30}O_2$	242.3975	1.55
11	Dodecanoic acid	24.47	$C_{12}H_{24}O_2$	200.32	1.03

a: Retention Time, b: Molecular Formula, c: Molecular Weight

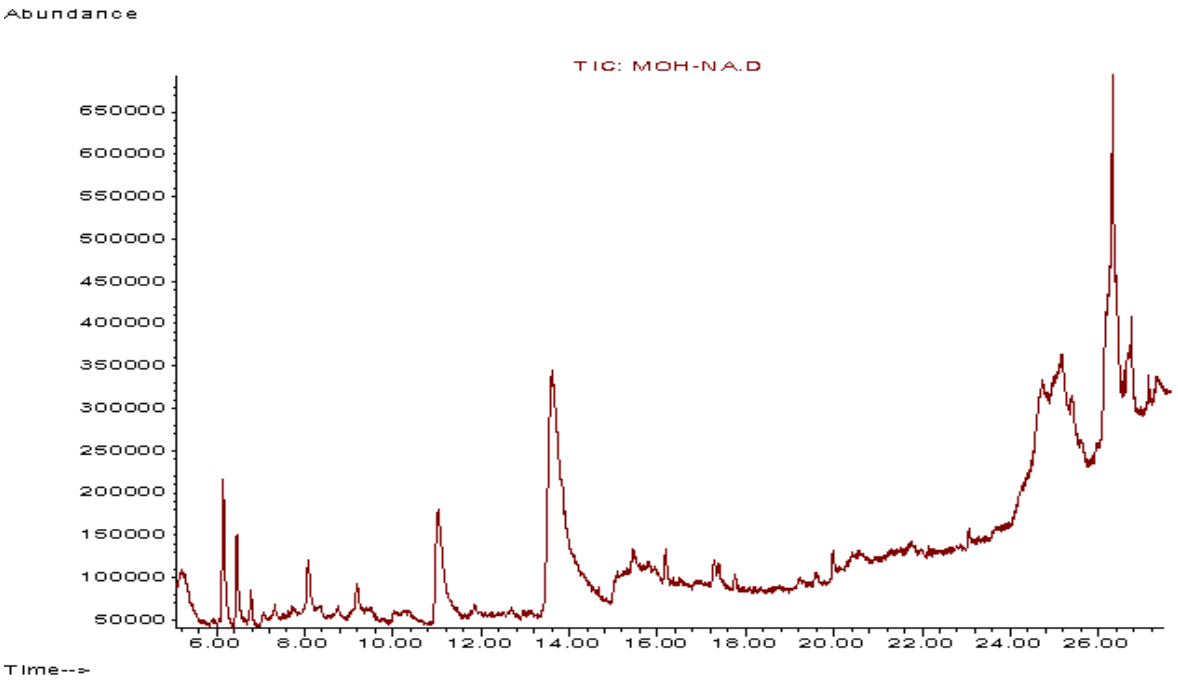

Figure 2. The chromatogram of *M. anisodon*

(3.75%) and butanoic acid (1.62%), alkaloids such as indole (2.57%), phenolic compounds such as 4-vinyl-2-methoxy-phenol (4.4%), diterpenes such as phytol (5.7%) and flavonoids such as 4H-Pyran-4-one (1.91%) and 3-Hepten-2-one (1.54%) are found in the extract. Capric acid is a 10-carbon fatty acid found in palm and coconut oil and less in animal fats and milk. This oil reduces insulin resistance and balances insulin level in humans. 36.7% of furfural and 1.99% of the sulfur compound such as dimethyl sulphone compound are also found in this plant. It is used as a food additive to maintain the quality and taste of food and treat parasitic infections and carriers of drugs (Jacob et al., 1999). Researchers try to increase the level of this fatty acid in transgenic plants [39]. Diterpene available in this plant is an alcoholic non-cyclic diterpene with antibacterial, anticancer, anti-inflammatory and diuretic effects (Furumoto, 2002). Delorme et al. (1977) reported that *Echium amoenum* has anthocyanins (13%), flavonoids (0.15%) and a small amount of alkaloids [40]. Javadzade (1995) reported that *Borago officinalis* have mucilage, tannins, Na, Ca and K. Due to the presence of different materials such as mucilage, flavonoids, phenolic compounds, diterpenes and useful fatty acids in this plant, it could be a good candidate for the treatment of many diseases and it is necessary to examine the effects of secondary metabolites of this plant.

3.4. Chemical composition of *Echinops cephalotes*

According to the results, 76 kinds of the chemical compounds found in the methanol extract of *E. cephalotes* (Figure 4). The compounds present in this plant (with more than one percent), their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area %) are presented in Table 4. Aldehydes (7.9%), coniferol (4.8%), fatty acids (5.8%) and furfural (5.4%) are found in *E. cephalotes*. According to the results, tridecanedinal is the most abundant compound in this plant (7.9%). The presence of alkaloids, saponins, plant sterols, polyphenols, and carotenoids has been detected in different parts of the echinops genus [18]. Diisodecyl ether compound derived from streptomyces had the anti-bacterial effect and was found in this plant [41]. Benzenemethanol is a type of benzyl alcohol present in many plants and an aglycone with antioxidant effect [42]. There are toxic compounds such as DDMP and benzyl alcohol in this plant so it is necessary to examine its toxicity.

3.5. Total phenol and flavonoids

The total phenol content in the samples varied from 21.24 to 177.19 µg gallic acid /mg dr.wt. Maximum amount phenol was found in *E. cephalotes* while the lowest amount was observed in the shoot of *E. khuzistanicum*.

Flavonoids are regarded as one of the most widespread group of natural constituents found in plants. The value of flavonoid content varied from 323.59 to 1305.61 µg QC/100 mg dr.wt. Maximum flavonoid content was determined in *E. cephalotes* and leaves of *E. khuzistanicum* (Table 5).

3.6. DPPH

The antioxidants are known to mediate their effect by directly reacting with ROS quenching them and chelating the catalytic metal ions. The radical scavenging activity was found to be high in *M. anisodon* followed by the flower of *E. khuzistanicum* (Table 6). IC_{50} values in *M. anisodon* and the flower of *E. khuzistanicum* are lower than ascorbic acid.

3.7. Antibacterial Effects

Data statistical analysis showed a significant difference at the level of 1%. In Tables 7-10, the diameter of the halo preventing the growth in the presence of the extracts is shown. Gentamycin was a positive control (Table 11).

3.8. Antibacterial Effects of *Echium Khuzistanicum*

The methanol extract of the root had maximum inhibition of gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The growth inhibition was increased by increasing the extracts' concentration. *Bacillus subtilis* halo diameter in the presence of methanol extract of the root was 24.33±0.57, and in the bacteria, *Staphylococcus aureus* was equal to 24.16±1.6. These halos' diameter were also bigger than with the positive control (Gentamicin). The extracts had the lowest effect on *Pseudomonas aeruginosa*.

Table 3. The compounds present in *E. khuzistanicum* (more than 1%)

No.	Name of the compounds	RT[a]	MF[b]	MW[c] g/mol	Peak area%
1	alpha-D-Glucopyranoside	20.43	$C_7H_{14}O_6$	194.1825	22.32
2	Decanoic acid	25.57	$C_{10}H_{20}O_2$	172.268	12.60
3	9,12,15-Octadecatrien-1-ol	25.81	$C_{18}H_{32}O$	264.453	11.23
4	2-Furancarboxaldehyde	13.83	$C_5H_4O_2$	96.09	7.36
5	Phytol	18.57	$C_{20}H_{40}O$	296.539	5.7
6	4-vinyl-2-methoxy-phenol	11.26	$C_9H_{10}O_2$	150.18	4.40
7	d-Talonic acid lactone	12.31	$C_6H_{10}O_6$	178.14	4
8	Octadecanoic acid	26.28	$C_{18}H_{36}O_2$	284.48	3.75
9	Indole	9.07	C_8H_7N	117.15	2.57
10	Dimethyl sulfone	13.62	$C_2H_6O_2S$	94.13	1.99
11	4H-Pyran-4-one	13.79	$C_5H_4O_2$	96.085	1.91
12	Butanoic acid	11.06	$C_4H_8O_2$	88.11	1.62
13	3-Hepten-2-one	21.61	$C_7H_{12}O$	112.172	1.54

a: Retention Time, b: Molecular Formula, c: Molecular Weight

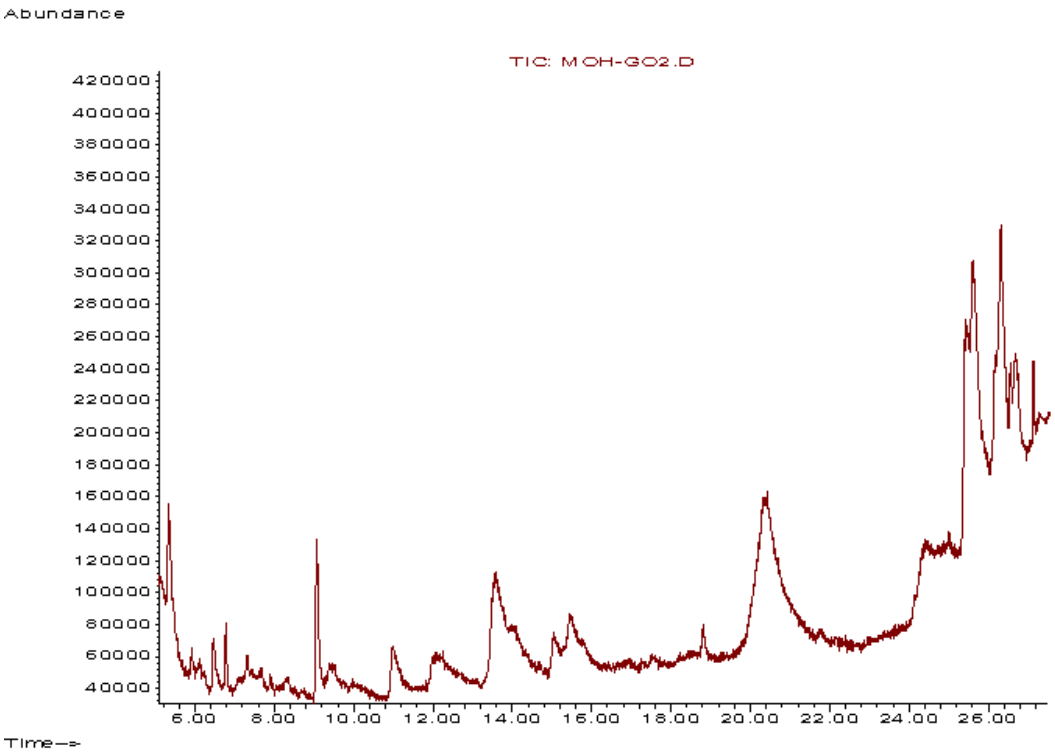

Figure 3. The chromatogram of *E. khuzistanicum*

Table 4. The compounds present in *E. cephalotes* (more than 1%)

No.	Name of the compounds	RT[a]	MF[b]	MW[c] g/mol	Peak area%
1	Tridecanedial	26.47	$C_{13}H_{24}O_2$	212.333	7.9
2	*Capronic acid*	25.14	$C_6H_{12}O_2$	116.16	5.82
3	11,13-Dimethyl-12-tetradecen-1-ol acetate	27.13	$C_{18}H_{34}O_2$	282.4614	5.76
4	2-Furancarboxaldehyde	13.83	$C_5H_4O_2$	96.09	5.46
5	Octadecanoic acid	26.47	$C_{18}H_{36}O_2$	284.48	4.91
6	Coniferol	25.61	$C_{10}H_{12}$	180.201	4.87
7	Phytol	18.57	$C_{20}H_{40}O$	296.539	4.15
8	2,2,3-Trimethyloxirane	11.21	$C_5H_{10}O$	86.132	4.08
9	2-Ethyl-2-hexen-1-ol	25.43	$C_8H_{16}O$	128.212	3.47
10	Benzenemethanol	24.38	$C_7H_{10}O$	110.156	2.94
11	2-Methyl-2-pentenal	13.90	$C_6H_{10}O$	98.145	2.59
12	Hexanoic acid	24.52	$C_6H_{12}O_2$	116.16	2.22
13	1-Methoxy-3-hydroxy methyl octane	24.81	$C_{10}H_{22}O_2$	174.281	2.13

a: Retention Time, b: Molecular Formula, c: Molecular Weight

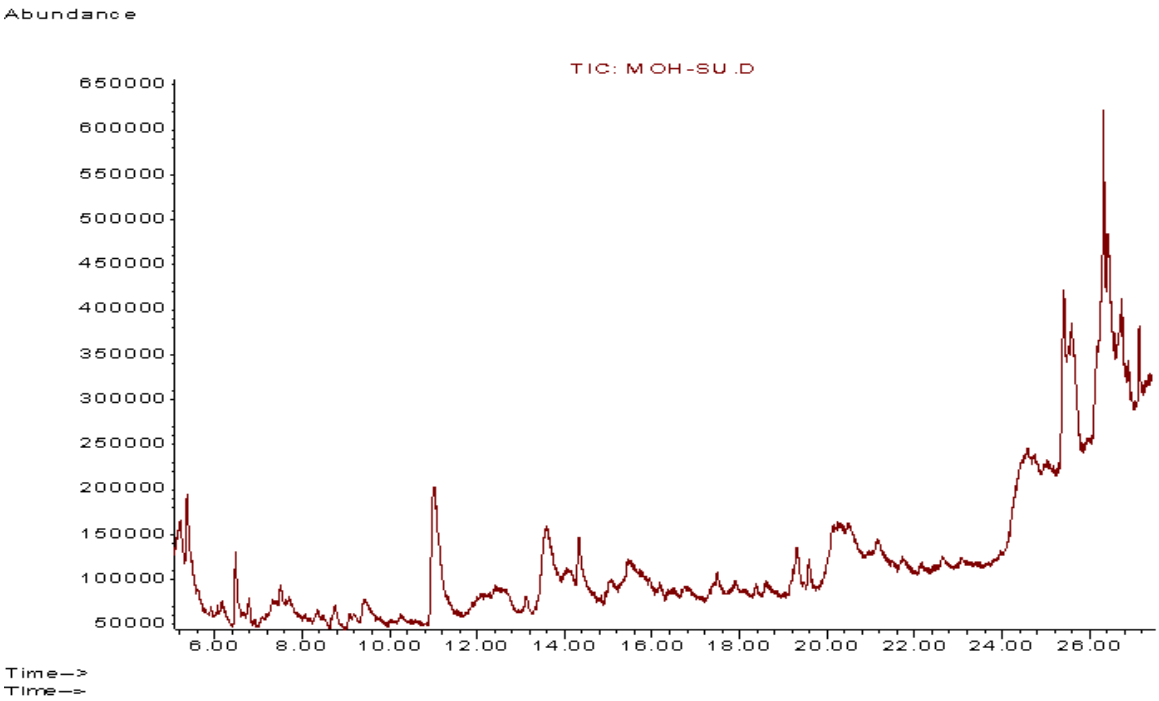

Figure 4. The chromatogram of *E. cephalotes*

Table 5. The total phenol content (µg gallic acid/mg dr.wt) and the total flavonoid content (µg QC/100 mg dr.wt)

Plant sample	*E. cephalotes*	*E. khuzistanicum* Leaf	*E. khuzistanicum* Root	*E. khuzistanicum* Shoot	*E. khuzistanicum* flower	*M. anisodon*
Phenol content	177/19±7.49[a]	60.96±1.16 [c]	28.13±0.82[d]	21.24±.083[d]	73.71± 2.44[c]	109.8±6.5[b]
Flavonoid content	1305.61[a]	1305.61[a]	323.59±4.52[b]	336.91±4.52[b]	543.69±20.61[b]	369.18±6.75[b]

Table 6. The inhibition percent (I) and IC_{50}

Variables	Ascorbic acid	*E. cephalotes*	*E. khuzistanicum* Leaf	*E. khuzistanicum* Root	*E. khuzistanicum* Shoot	*E. khuzistanicum* flower	*M. anisodon*
I	95.92±0.72[a]	7/26±0.72[e]	13.6±0.46 [e]	69.36±0.30[d]	69.08±0.08[d]	71.23± 0.28[c]	72.48±0.24[b]
IC_{50} (mg/ml)	0.39	6.94	3.67	0.72	0.72	0.14	0.14

Table 7. The diameters of clear zone (mm) in the presence of *E. khuzistanicum* shoot extracts

Bacterium	Methanol			Ethanol			DMSO		
	Concentration (mg)								
	100	200	400	100	200	400	100	200	400
S. aureus	0	10.5±0.96	20.33±1.02	0	0	11.83±0.76	8.83±1.04	11.5±0.5	13.5±0.5
B. subtilis	0	8.5±1	20.83±0.28	0	0	0	8.5±0.5	10.5±0.5	12.83±0.76
S. pyogenes	0	8.5±0.5	14±1	0	0	0	0	0	0
E. coli	8.23±0.25	16.83±1.06	23.5±0.78	0	0	12.83±0.28	0	0	0
P. aeruginosa	0	0	10±0.5	0	0	11.83±0.76	0	0	0
S. typhimurium	7.96±1.37	15±1	20.5±1.32	0	0	12.83±0.28	0	0	12.83±0.28

Table 8. The diameters of clear zone (mm) in the presence of *E. khuzistanicum* leaf extracts

Bacterium	Methanol			Ethanol			DMSO		
	Concentration (mg)								
	100	200	400	100	200	400	100	200	400
S. aureus	0	0	11.5±1.32	0	8.5±0.5	11.83±0.28	0	9.5±1.8	14.5±0.5
B. subtilis	0	6.66±0.57	11.16±0.76	7.16±0.28	7.83±0.28	12.16±0.28	0	17.83±1.2	20±1
S. pyogenes	0	0	0	0	0	0	0	0	0
E. coli	0	0	11.16±1.75	±0.58	±0.59	13.5±0.5	8.16±0.28	10.33±1.1	16.16±0.3
P. aeruginosa	0	0	8±0.5	0	0	0	0	0	8.16±0.28
S. typhimurium	0	0	17±1.32	0	9.66±0.28	16.66±0.76	8.66±0.28	13±0.5	15.16±1.6

Table 9. The diameters of clear zone (mm) in the presence of *E.khuzistanicum* flower extracts

	Methanol			Ethanol			DMSO		
Bacterium	Concentration (mg)								
	100	200	400	100	200	400	100	200	400
S. aureus	0	8.5±0.5	9.83±0.28	0	7.5±0.5	9.33±0.28	0	12.16±1.75	16.33±0.76
B. subtilis	0	0	10.83±0.76	0	0	0	0	18.33±0.28	11.5±0.5
S. pyogenes	0	8.83±0.76	9.5±0.5	0	0	0	0	0	0
E. coli	0	0	0	0	0	0	0	8.66±0.28	10.83±0.76
P. aeruginosa	0	0	0	0	0	0	0	0	0
S. typhimurium	0	0	0	0	0	0	9.66±0.76	14±1	15.66±0.57

Table 10. The diameters of clear zone (mm) in the presence of *E. khuzistanicum* root extracts

Bacterium	Methanol			DMSO			Ethanol		
	Concentration (mg)								
	100	200	400	100	200	400	100	200	400
S. aureus	12.16±1.6	16.16±1.89	24.16±1.6	10.83±0.76	14.5±0.5	20.83±0.76	0	0	0
B. subtilis	0	13.16±0.76	24.33±0.57	±110	14.33±2.08	21.66±1.15	0	0	0
S. pyogenes	9.66±0.28	13.66±0.57	21.83±0.76	0	10±0.5	13.16±0.76	0	0	0
E. coli	0	11.83±0.76	18.66±0.78	9.66±0.57	15.16±0.28	19.33±1.52	0	0	0
P. aeruginosa	0	0	12.66±1.o6	0	0	8.16±0.28	0	0	0
S. typhimurium	10.83±0.76	17.5±0.5	21±1	13.5±0.5	18.83±0.76	22±0.5	0	0	0

Table 11. The diameters (mm) in the presence of the gentamicin antibiotic as a positive control.

Bacterium	Diameter (mm)
S. aureus	17.5±0.76
B. subtilis	20.83±0.28
S. pyogenes	19±0.18
E. coli	16.25±0.52
P. aeruginosa	15±0.5
S. typhimurium	17±0.57

3.9. Antibacterial Effects of *M. anisodon*

The halo diameter of standed-growth in the presence of extracts was put in 10 groups based on mean comparison with Duncan test. The highest inhibition was seen in *Pseudomonas aeruginosa* at the concentration of 400 mg/ml of DMSO extract.

4. DISCUSSION

E. cephalotes had by far the highest amount of phenol among the plants in this study which may be due to the presence of coniferol (4.87%). Plant organs in *E. khuzistanicum* had different total phenolic content, however, the flower and leaf contain the higher phenol concentration than the other organs. This could be due to the presence of 4-vinyl, 2-methoxy phenol (4.4%) in flower of *E. khuzistanicum*. Maximum flavonoid content was determined in *E. cephalotes* and leaves of *E. khuzistanicum*. Given the importance of flavonoid compounds in the treatment of human diseases and the prevention of lipid oxidation in foods, high amounts of flavonoid in *E. cephalotes* and leaves of *E. khuzistanicum* is significant. *M. anisodon* and flower of *E. khuzistanicum* have high antioxidant activity in consensus with previous reports on the antioxidant activity in the genus of Marrubium and Echium [43-45]. There was no correlation between total phenolic content and antioxidant activity.

This activity occurs because of some compounds like phenols, flavonoids, and alkaloids in these plants. In general, the inhibitory effect on free radical DPPH depends on the type of solvent extraction, its polarity, separation method, purification of active components and method of measurement [46]. Molecular structure and position of the hydroxyl group on molecule determined antioxidant activity in flavonoid compounds [47]. Antioxidant activity in the plant was often evaluated by considering phenolic compound content. However, the antioxidant potential of the extracts does not solely depend on it. Terpenes are another major group of chemicals showed the antioxidant potential against DPPH radical scavenging activity

which could be an additional contributory factor for antioxidant activity of extracts [48]. In flower of *E. khuzistanicum* and *M. anisodon* terpenes were detected (Tables 2 and 3). *E. cephalotes* have low percent inhibition effect on free radical DPPH despite the highest amount of phenol. It is possible to conclude that the antioxidant capacity observed doesn't only come from the phenolic contents but can occur because of some other phytochemicals such as ascorbic acid, tocopherol, terpenes and the synergistic effects of them, which also affect the total antioxidant capacity. On the other hand, various kinds of phenolic compounds depending on their structure show the different antioxidant activities. The extract of *E. cephalotes* possibly has different type of phenolic compounds with different antioxidant capacities [49].

The beneficial medicinal effect of a plant is due to the secondary metabolites in the plant [50-53]. There are alkaloids, flavonoids and phenolic compounds in the methanol extract of *E. khuzistanicum* flower according to GC/MS analysis that similar results have been described by Tiwari et al. (2011) [50]. The *E. khuzistanicum* root has an excellent antibacterial effect which can be attributed to the pigment (shikonin or alkannin) in the root of this family for which antibacterial properties was reported [54]. The antibacterial properties were reported in some genus of this family, for example, Tabata et al. (1982) showed that quinone derivatives of callus culture of *Echium lycopsis* have antimicrobial properties [54] and aqueous extract of *Echium amoenum* flower has anti-viral and anti-bacterial properties [55-56].

The type of solvent that is used in the extraction determines to a large extent the active compounds that are extracted from the plant [57]. The traditional physicians used the aqueous solvent for extraction, but the results of the research showed that the organic solvent in comparison with the aqueous solvent contains more anti-microbial compounds. Most of the active antimicrobial compounds that have been identified so far are not soluble in water, so organic solvents have a higher potential for having active antibacterial materials [58]. Water-soluble compounds such as polysaccharides and poly peptides, like all types of lectins, play a more effective role in preventing the absorption of pathogens and have no real effect like antimicrobial agents [59]. Water-soluble flavonoids, which are mostly anthocyanins, and water-soluble phenol compounds are only important as antioxidant compounds and do not have a specific antibacterial effect [60]. In this study, the methanol extract of the root as an organic solvent has the highest effect on the bacteria so it can be concluded that active polar compounds in methanol extract act as antibacterial agents.

M. anisodon did not show proper antibacterial properties in consensus with previous reports. Khalil et al. (2009) reported that *Marrubium vulgare* did not have antibacterial properties on *Staphylococcus aureus* and *Pseudomonas aeruginosa* [61]. Masoodi et al. (2008) have noted the antibacterial properties of the methanol extract of *M. vulgare* only in highly concentration of extract (600 mg/ml) [62]. Aerial parts of *M. anisodon* showed 27.7% inhibitory effect on the acetylcholinesterase activity which used for the treatment of Alzheimer's disease. This inhibitory effect was known related to some components that are functionally or structurally similar to the tacrine which can be alpha-pyrrolidone alkaloids in the chemical composition of this plant [30]. This family of the plant used to treat dandruff and hair regrowth [31]. According to the presence of vitamin B7 in this plant, it can be concluded that this is a good candidate for the treatment of hair loss and alopecia.

The extract of *E. khuzistanicum* can be used in food industries as a protective agent due to high antioxidant activity. This plant is widely used in traditional medicine and is a potential source of valuable compounds such as shikonin and unsaturated fatty acids [63]. This plant is a good candidate to replace the synthetic antibiotics due to good antibacterial properties are seen in this study. Screening, identification, and isolation of the active compounds in the plants and examining the toxicity of these compounds are considered as a way for the commercialization of these compounds.

According to the results, the previous reports on the medicinal properties of the examined plants was confirmed by identification of compounds in the extract of these plants. The synthetic pathways of many secondary metabolites and associated genes in medicinal plants have not yet been completely identified. The amount of a particular secondary metabolite can be increased or decreased by identifying these synthetic pathways and genetic engineering of them. In these medicinal plants, there are valuable secondary metabolites such as alkaloids, flavonoids, diterpenes, unsaturated fatty acids, vitamin B and phenolic compounds which can be used in pharmaceutical and cosmetics industries.

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