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Investigation the Antioxidant, Antibacterial and Insecticidal Activities of *Cuscuta epithymum* and *Pyrethrum roseum* Plants using Polydimethylsiloxane (CAR/PDMS)

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Abstract

In this study, the antioxidant, anti-fungal and also anti-bacterial content in Cuscuta epithymum and Pyrethrum roseum plants were investigated. The extraction of essences is sensitive to operational conditions. Therefore, the effect of different extraction techniques by using HS -SPME fiber assembly Carboxen/Polydimethylsiloxane (CAR/PDMS), on the quality of essence oil composition was inspected and the composition of the final product was recognized using gas chromatography and mass spectroscopy.Essential Cuscuta epithymum and Pyrethrum roseum is widely used in pharmaceutical, sanitary, cosmetic, agriculture and food industries for their bactericidal, virucidal, fungicidal, antiparasitical and insecticidal properties. Their anticancer activity is well documented. The chemical composition of the essential oil from Cuscuta epithymum and Pyrethrum roseum was analyzed by GC-MS.

Keyword: antioxidants, antimicrobial, insecticidal activity, Cuscuta epithymum, Pyrethrum roseum

1 Introduction

The long term use of herbs introduced in traditional medicines confirms their value in drug discovery (1-3). Based on historical evidence, herbal therapies were used to treat convulsive seizures for centuries (4). Medicine has always played a significant role in Iranian culture and civilization. Thousands of years of history and hundreds of books have placed Iranian traditional medicine among the oldest and richest alternative medicines (5, 6). This herb is also used as a drying, salting food for tympanites; a strong substance to help digestion process produce waste of stomach, as an anti-blowing agent which makes nerve pains less troubling, and as an anti-inflammation antivirus, anti-parasite anti-fungus and antibacterial. Lately, some observations were held to see the effect of different species of this herb as an antivirus against HIV. The Prangos ferulacea is a full of money starting point of anti-oxidant and some observations claimed that a large amount of vitamin E was added to this property (7-9). There are 15 different species of Prangos ferulacea in Iran which five of them are in a low level of development. This herb needs humidity for its growth in addition to cold weather. Putting a thin coating layer of ice in a complete living wheeled machine.as an outcome, it can be discovered in snowy places with much measure end to end of cold weather (10). The herb copy of is better in clayey soils and land feeling of a material and structure has a great effect on the level of Major and not important, small part expansion of the root. The herb grows in last March and gradually till the end of April. After, it moves into a copy of phase and the fruits are ready in mid-May (11, 12). The seeds can be gathered at the time of withering by July. Pachymerus acacia is the chief trouble of

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Prangos ferulacea which is from bruchidae family and causes to come out on the leaves of the herb. The plant is a chief starting point of anti-oxidants (13).

2 Materials and methods

2.1 Materials

- Usual laboratory instruments and Clevenger using British Pharmacy standard.
- Circulation bath for constant temperature and as a condenser; type 8mLw made by mLwUH.
- Chemical analysis materials from Merck and Fluka with desired anhydrite sodium sulfate with 97% sincerity and normal hexane with 95% sincerity and standard oleic acid.

2.2 Instruments

- Gas chromatography and mass spectroscopy (GC, GC-MS): GC model HP-6890 made by HEWLETT PACKARD (USA).
- Mass spectroscopy Model HP-5973 made by HEWLETT PACKARD USA
- Gas chromatography and simultaneous gas chromatography and mass spectroscopy device (GC-MS)
- SPME fiber assembly excluding solid phase and SPME fiber holder made by SUPELCO (USA).
- Electric mill for herbaceous parts grinding, if necessary; this mill is needed for SPME method and the model is Ikawerke M20 (Germany).

Table 1: Characterisation of the used fiber in the SPME method

Characterisation of the used fiber in the SPME method
Type of fiber: (carboxen / poly dimethyl siloxane , CAR / PDMS)
Adsorbent thickness: 75 micrometer
Type of adsorbent connections: strongly network
Color: Black

2.3 Experimental procedure

2.3.1 Chromatography tests

For Cuscuta epithymum and Pyrethrum roseum plant essence oil using HS-SPME for (essential oil) methods. Analysis of the essential oil was performed using a Hewlett Packard 6890 GC equipped with a HP-5MS capillary column (30 m×0.22mm i.d., 0.25 µm film thickness) and a mass spectrophotometer 5973 from the samecompany for GC/MS detection with an electron ionization system energy (10 eV)was used. Helium was the carrier gas, at a flow rate of 1 ml/min., injector and detector MS transfer line temperature were set at 250 and 290 °C, respectively. Column temperature was initially kept at 60 °C for 5 min., and then gradually increased to 220 °C at the rate of 6 °C/min.

2.3.2 HS-SPME method

The method carried out using minimum amount of herb powder (1 g) without using any solvent. The circulating bath model mLw8 made by mLwHU was used in this experiment which had the ability of temperature control during extraction process. The fiber assembly was kept in a 10 ml glass container and the fiber holder attached to the container. The glass container was inside the circulating bath to reach the bath temperature. For heat desorption of pulled components on the fiber, the injection performed immediately into the GC-MS instrument. in This study tests the suitability of the Carboxen–polydi- methylsiloxane (CAR–PDMS) fiber.The SPME device and CAR–PDMS (75 mm) were used as fibers used in this study were purchased from internal standards (I.S.s). (results are shown as in table 1).The fibers ereMeSEt and Et S were supplied by Aldrich conditioned by inserting them into the GC system injector at 280 c for 30 min and they were immediately used to prevent contamination. Before the extraction with the fiber, the sample vials were equilibrated for 30 min at 25C. Afterwards, the stainless steel needle in which the fiber is housed was pushed through the vial septum, allowing the fiber to be exposed to the headspace of the sample for 30 min. Then, the fiber was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption at 300 c for 1min.

2.3.3 Separation and identification of components in essence oils

As the components present in essence oils are known as volatile and semi-volatile oils, therefore GC-MS method was applied for separation and identification of the components. The result spectrums were compared with standard mass spectrum of Adams (Adams, R.P., 2004). In order to confirm the identified components of standard mass spectrum, Quatz deterrence index was applied. Firstly, the Alkanes of C_8-C_{25} were injected into GC-MS and deterrence time for each Alkane was measured using KI=100n when 'n' is the number of carbons in related Alkane. Quatz deterrence index of essence oils were calculated using the following equation:

$$KI = 100\mathrm{n} + 1KI\left(\frac{t_x - t_n}{t_{n+1} - t_n}\right) \quad (1)$$

After dewatering the produced oil, the oil was diluted using normal hexane (Merck) with the proportion of 1 to 10 and then injected into GC-MS.The most widely used technique of sampling with solid phase microextraction onsists of exposing a small amount of extracting phase (coating) associated with a fibre to the sample, for a predetermined amount of time.V_f = volume of fibre coating; K_{fs} = fibre/sample distribution coefficient; V_s = volume of sample; C_0 = initial concentration of analyte in the sample.Typically, the microextraction process is considered complete when the analyte concentration has reached distribution equilibrium between the sample matrix and the fibre coating. The equilibrium conditions can be described by equation (1), according to the law of mass conservation, if only two phases are considered (for example, the sample matrix and the fibre coating):

$$C_0 . V_s = C_S^{\infty} . V_S + C_f^{\infty} . V_f \quad (2)$$

e distribution coefficient K_{fs} of the analyte between the fibre coating and sample matrix is defined as equation 3.

$$K_{fs} = \frac{C_f^{\infty}}{C_s^{\infty}} \quad (3)$$

$$C_f^{\infty} = C_0 \cdot \frac{k_{fs}.V_s}{k_{fs}.V_f + V_s} \quad (4)$$

Equations (1) and (2) can be combined and rearranged into equation (3). Finally, the number of moles of analyte *n* extracted by the coating can be calculated from equation:

$$n = C_f^{\infty}.V_f = C_o.\frac{K_{fs}.V_s.V_f}{K_{fs}.V_f + V_s} \quad (5)$$

Equation (5) indicates that the amount of analyte extracted onto the coating (*n*) is linearly proportional to the analyte concentration in the sample (C_0), which is the analytical basis for quantification using SPME.Equation (5), which assumes that the sample matrix can be represented as a single homogeneous phase and that no headspace is present in the system, can be modified to account for the existence of other compartments in the matrix, by considering the volumes of the individual phases and the appropriate distribution constants. In addition, when the sample volume is very large, i.e. $V_s >> K_{fs} \cdot V_f$, equation (4) can be simplified to:

$$n = K_{fs}.V_f.C_0 \quad (6)$$

Which points to the usefulness of the technique when the volume of the sample is unknown. In practice, the fibre can be exposed directly to the flowing blood, ambient air, water, etc. The amount of extracted analyte will correspond directly to its concentration in the matrix, without depending on the sample volume. The amount of analyte extracted onto the fibre coating is at a maximum when the equilibrium is reached, thus achieving highest sensitivity. If sensitivity is not a major concern of analysis, shortening the extraction time is desirable. In addition, the equilibrium extraction approach is not practical for solid porous coatings, due to the displacement effect at high concentrations. For these circumstances, the extraction is stopped and the fibre is analyzed before the equilibrium is reached. The kinetics of absorption of analytes onto a liquid fibre coating can be described as:

$$n = (1 - e^{-at}).C_o.\frac{K_{fs}.V_s.V_f}{K_{fs}.V_f + V_s} \quad (7)$$

where *t* is the extraction time, and *a* is a time constant, representing how fast an equilibrium can be reached. When the extraction time is long, equation (6) becomes equation (4), characterizing equilibrium extraction. If the extraction equilibrium is not reached, equation (6) indicates that there is still a linear relationship between the amount (*n*) of analyte extracted onto the fibre and the analyte concentration (C_0) in the sample matrix, provided that the agitation, the extraction time, and the extraction temperature remain constant. As equation (5) indicates, the extraction process is dependent on the distribution constant K_{fs}. This is a characteristic parameter that describes the properties of a coating and its selectivity toward the analyte versus other matrix components. Because of its solvent-free nature and the small size of the fibre coating, SPME can be interfaced conveniently to analytical instruments of various types. Only extracted analytes are introduced into the instrument, since the extracting phase is non-volatile and insoluble in most organic solvents. Thus, there is no need for complex injectors designed to deal with large amounts of solvents, and these components can be simplified for use with SPME. Depending on the method of subsequent analysis, the sensitivity of determinations using the SPME technique is very high, facilitating trace analysis. Although in most cases the analytes are only partly extracted from the sample, all extracted material is transferred to the analytical instrument, resulting in good performance. Carryover should be checked for each analyte and the desorption conditions should be chosen so that the analyte remaining on the fibre is less than 0.1% of the initial amount. The solvent free process results in narrow bands reaching the instrument, giving taller, narrower peaks and better quantification. (See Fig. 1).

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2.3.4 Data analysis

Study of the essential oil extraction by Clevenger method from Cuscuta epithymum and Pyrethrum roseum plant Clevenger method was used for the isolation of essential oil as a traditional method of extracting essential oils from medicinal plants. The effects of different extracting solvents such as n-hexane (non-polar) and methanol were studied on the extraction of the essential oil from Cuscuta epithymum and Pyrethrum roseum. Each time, the obtained essential oil was analyzed using Gas chromatography (GC). According to the obtained results of the previous studies and the beneficial effects of medicinal herbs, the aims of this study were extraction and investigation of the essential oil from Cuscuta epithymum and Pyrethrum roseum and evaluation of its effects to Inhibit blood cancer cells Growth. The results related to the essential oil are presented in the chromatograms. One of the active ingredients of essential oil is 2-methoxy-6-pentyl-1,4-dihydroxybenzene which is identified as having antimicrobial and anti-tumor properties.

3 Results and discussions

3.1 Major components of the essential oil from Cuscuta epithymum and Pyrethrum roseum

The chemical composition of the essential oil from Cuscuta epithymum and Pyrethrum roseum was determined by gas chromatography connected to a mass spectrometer (GC-MS). There were identified different compounds of the essential oil from this plant. The most important extracted compounds of the essential oil from chemical analysis by GC-MS are shown in table 2 and the obtained Figure 2. The maximum percentage of these compounds were related to bornyl acetate, Sabinene hydrate acetate, Ocimene, Thymol, Methyl eugenol, Methyl isoeugenol, Asarone, Neophytadiene, isoelemicin.

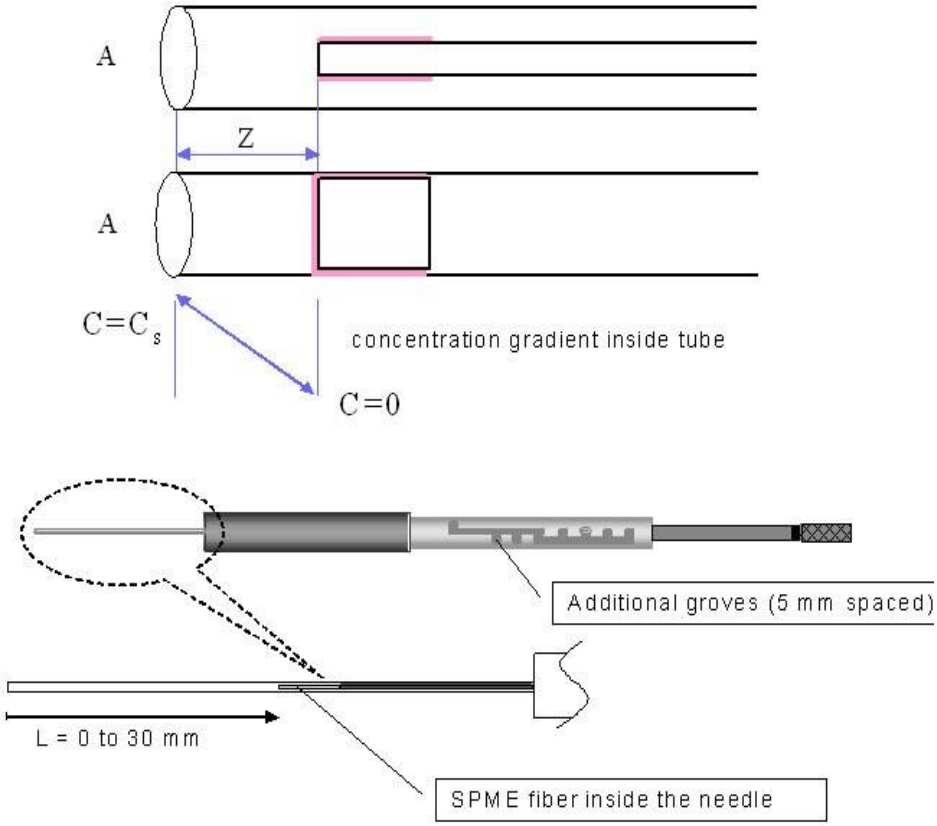

Figure 1: V_f = volume of fibre coating; K_{fs} = fibre/sample distribution coefficient; V_s = volume of sample; C_0 = initial concentration of analyte in the sample

3.2 Evaluation of the extracted essential oil from Cuscuta epithymum and Pyrethrum roseum

In order to measurement the percentage of the essential Oil components, hexane (non-polar solvent) and methanol a polar solvent were used for extraction of different compounds. The obtained extracts were analyzed by liquid chromatography. According to the chromatograms, there are limited compounds in the extract. Figure 4 and 5 show merely the obtained results and evaluation of these compounds was not provided due to lack of the required standard. The results of cytotoxic effect investigation are presented in Table 3.

Table 2: The most important constituents of the essential oil from Cuscuta epithymum and Pyrethrum roseum

Name	MF	FW	KI	Rt	%	%
Ocimene	$C_{10}H_{16}$	136	1050	5.4	9.64	4.165
Sabinene hydrate acetate<trans->	$C_{12}H_{20}O_2$	196	1256	12.38	10.01	4.234
Bornyl acetate	$C_{12}H_{20}O_2$	196	1288	13.37	8.77	3.789
Thymol	$C_{10}H_{14}O$	150	1290	13.79	8.02	3.463
Methyl eugenol	$C_{11}H_{14}O_2$	178	1403	18.22	10.77	4.646
Methyl isoeugenol	$C_{11}H_{14}O_2$	178	1492	21.79	8.09	3.494
isoelemicin<Z>, <E>	$C_{12}H_{16}O_3$	208	1570	24.74	8.25	3.564
Asarone<E>	$C_{12}H_{16}O_3$	208	1676	2893	100	43.182
Neophytadiene	$C_{20}H_{38}$	278	1841	34.08	13.58	5.866

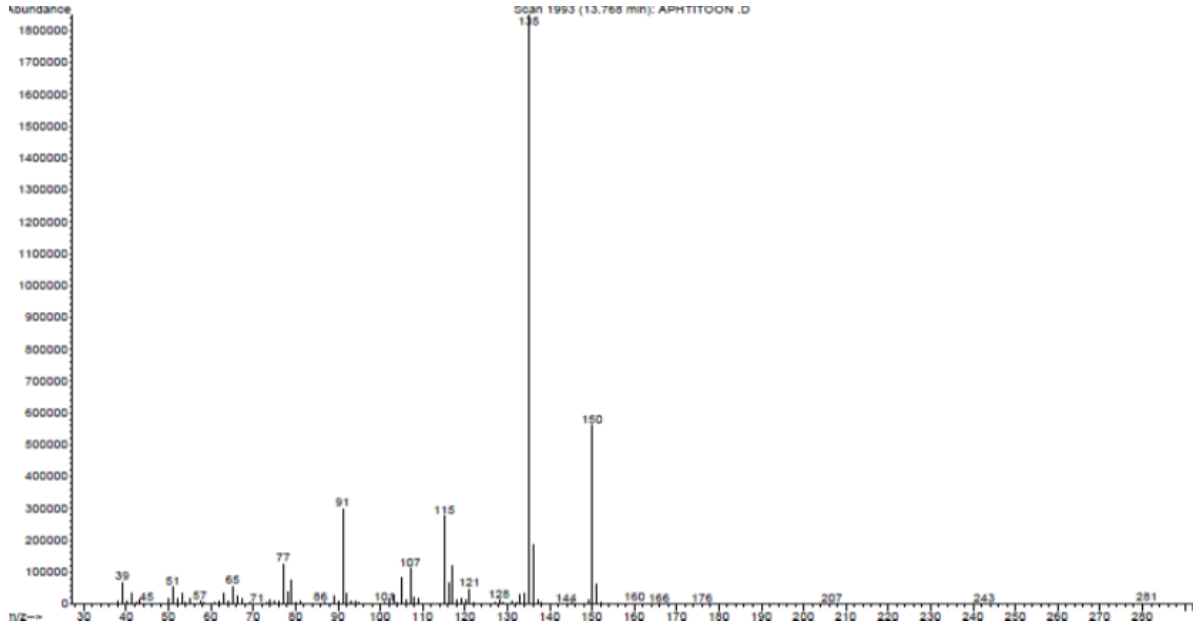

Figure 2: The mass spectra of compounds of the essential oil from Cuscuta epithymum and Pyrethrum roseum

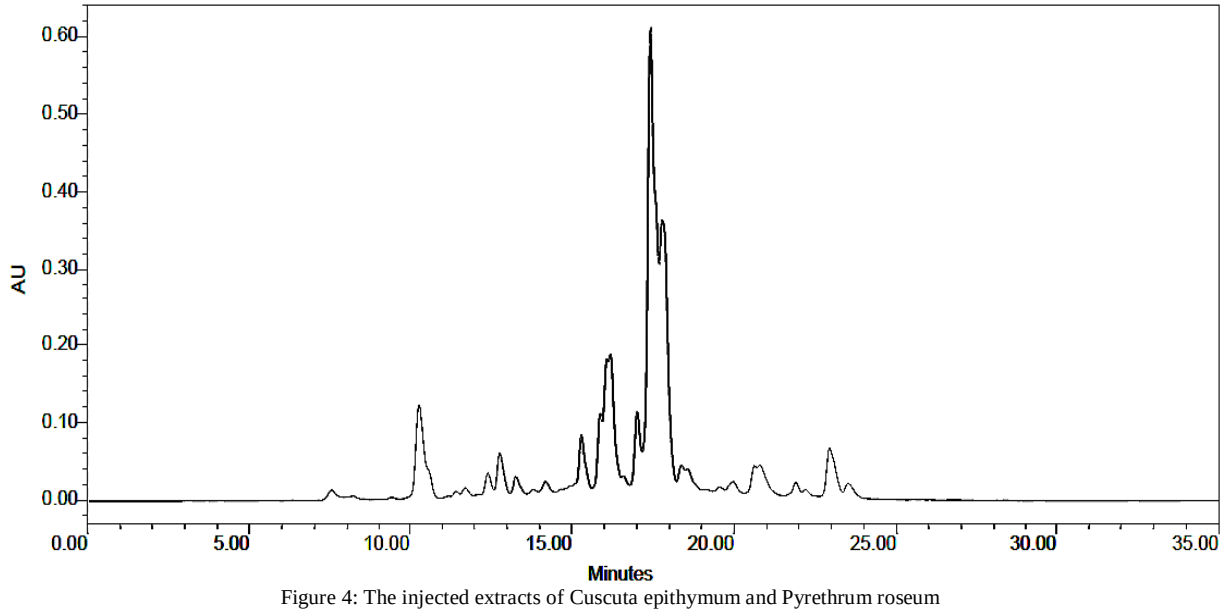

Figure 4: The injected extracts of Cuscuta epithymum and Pyrethrum roseum

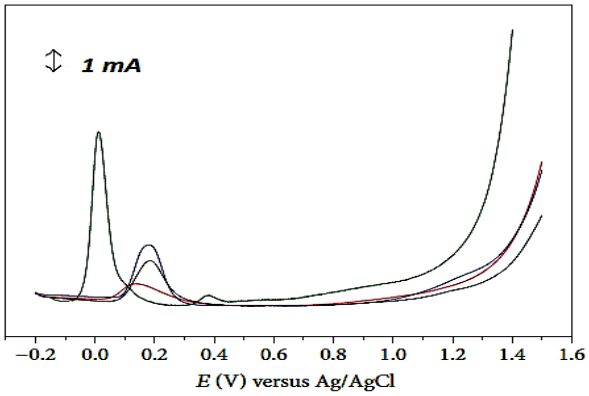

Figure 5: The injected essential oil from Cuscuta epithymum and Pyrethrum roseum

Table 3: Cytotoxic effects of the extract from Cucuta epithymum and Pyrethrum roseum

Extract	IC50(µ g/ml)
Aftimoon(hex+MeOH)	37.0±3.6
Mix (hex+MeOH)	25.48±3.3
Aftimoon (MeOH+H2O)	67.4±0.7
Cisplatin	7.0±2.4
Doxorubicin	5.4±0.3

3.3 Electrochemical study of antioxidant properties of essential oil from Cuscuta epithymum and Pyrethrum roseum:

In this study, the electrochemical method was performed to evaluate the antioxidant properties of plant essential oil. Antioxidants act as reducing agents and oxidize on the surface of inert electrodes. Therefore, the relationship between the electrochemical behavior of molecules and electrochemical capacity is considered as the basis for the study. The antioxidant power will be increased with lower oxidation potential. The carbon electrode is commonly used as the working electrode to study the anti-oxidant behavior of medicinal essential oils. Cyclic voltammetry (cv) and differential pulse voltammetry (dpv) techniques were used for examining the electrochemical behavior of essential oil. In cyclic voltammetry, potential is applied within the specified limits the working electrode and the anode current resulting from the oxidation of chemical species is determined. Antioxidant properties of the samples were investigated by comparing with the properties of salicylic acid a standard reference. Other compounds were also used such as polyphenols chlorogenic acid, cordigol, cordigone, danthrone, 1,5-dihydroxy-3- methoxyxanthone, eriosematin, flemichin D, frutinone A, mangiferin, quercetin, 1,3,6,7- tetrahydroxyxanthone.The Oxidation potential of these compounds relative to the working electrodes is in the range from 0.4 V to 0.9 V. Considering that the redox potential of most compounds with antioxidant properties is in the potential range, Cuscuta epithymum and Pyrethrum roseum essential oil can be introduced as a natural antioxidant. Voltammogram of the essential oil from Cuscuta epithymum and Pyrethrum roseum in methanol has been shown in the figure below.The essential oil from Cuscuta epithymum and Pyrethrum roseum has desirable antioxidant properties with regard to the obtained potential range, the intensity of the peaks and comparing it with common antioxidants.(see Fig. 6 and 7).

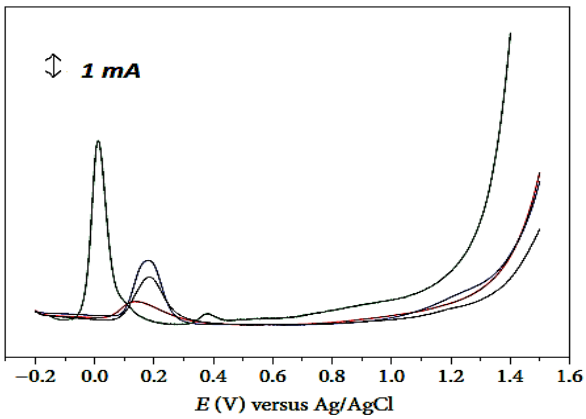

Figure 6: Differential pulse voltammetry for 0.1 mM: black (Cuscuta epithymum and Pyrethrum roseum), Blue (benzoic acid), green (tri hydroxamic acids), red (hydrocaffeic acid)

Electrochemical methods have been often used for investigation of antioxidant activity of compounds**,** evaluation of antioxidant capacity and determination of electrochemical index. Various types of electrodes can be used for this assay purposes. Cyclic or differential pulse voltammetry are often used for these electrochemical measurements.The antioxidant capacity is one of the most important antioxidant parameters. The capacity is recognized as the ability of compound to prevent the oxidative degradation of other molecules. These methods are generally based on the direct reaction of study compounds with free radicals or on the reaction with transition metals. Spectrometric methods are often employed in the investigation of antioxidant properties. However, these methods are dependent on several parameters, such as temperature and time of the analysis.Electrochemical methods are rapid, simple and sensitive in the analysis of bioactive compounds and measurement of antioxidant capacity. Antioxidants can act directly as reduction agents and they tend to be quickly oxidized in aqueous solutions with inert electrodes. Therefore, the relationship between the electrochemical behavior of compounds with antioxidant activity and accordingly with their antioxidant capacity is very considered, because compounds with low oxidation potentials have higher antioxidant power. This fact is known that imbalance between the concentration of prooxidants and antioxidants can lead to oxidative stress and these changes are very effective in the pathophysiology of patients.

3.4 The antimicrobial property of the Cuscuta epithymum and Pyrethrum roseum on the microorganisms tested

Essential oil and extracts of methanol and ethanol Cuscuta epithymum were studied in different concentrations of 5 human fungal species and on 5 human pathogenic bacteria. In the above experiments, the inhibitory effect of these agents has been increased by increasing the concentration of active ingredients. The following tables

were adjusted according to the mean diameter of the non-growth halo of the ten species tested (see Fig. 8 to 11).

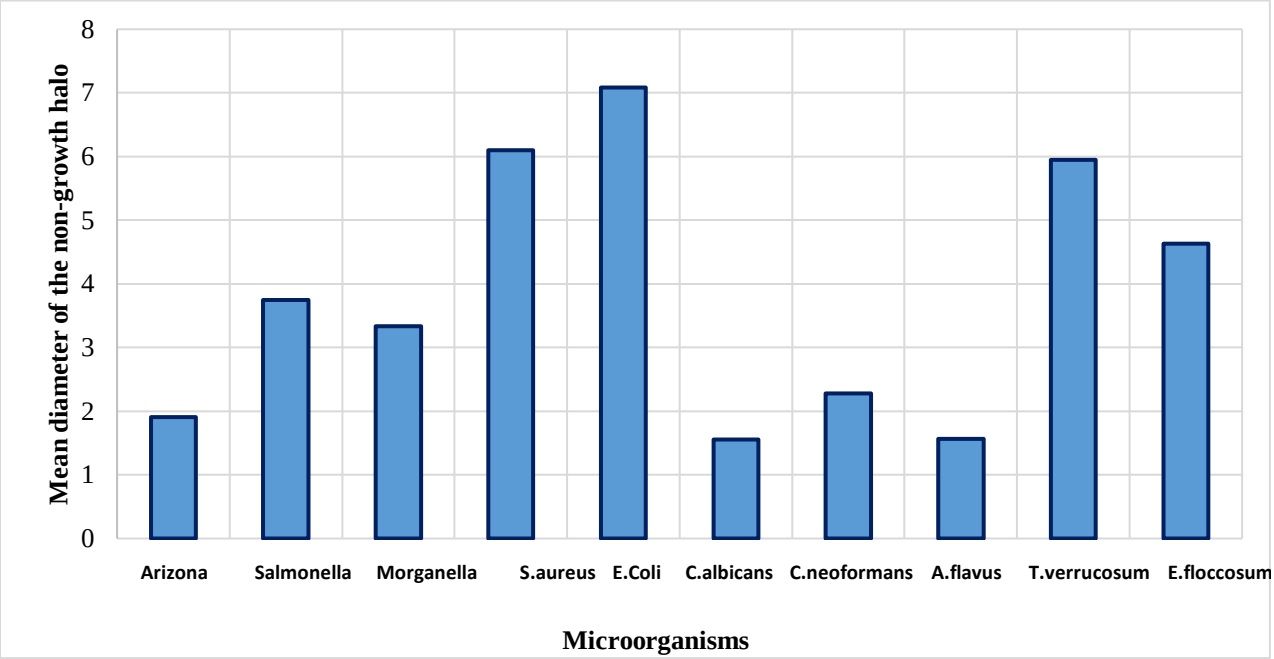

Figure 7: Minimum bactericidal concentration (MBC) Ethanol Extract

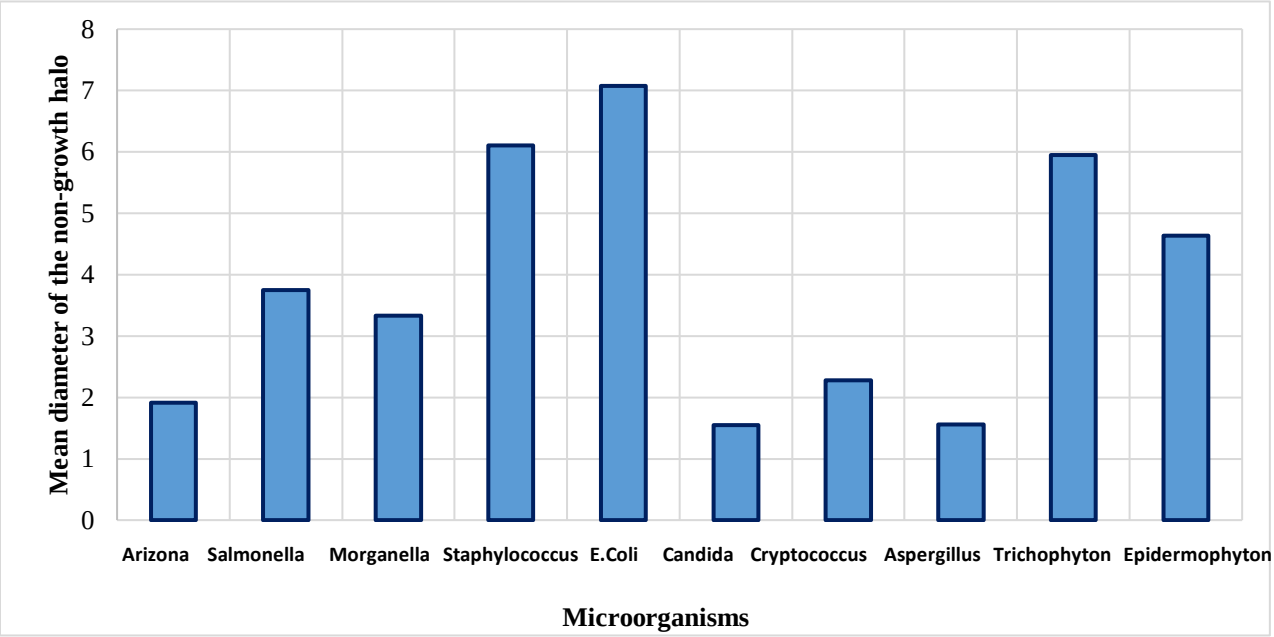

Figure 8: Minimum bactericidal concentration (MBC) Ethanol Extract

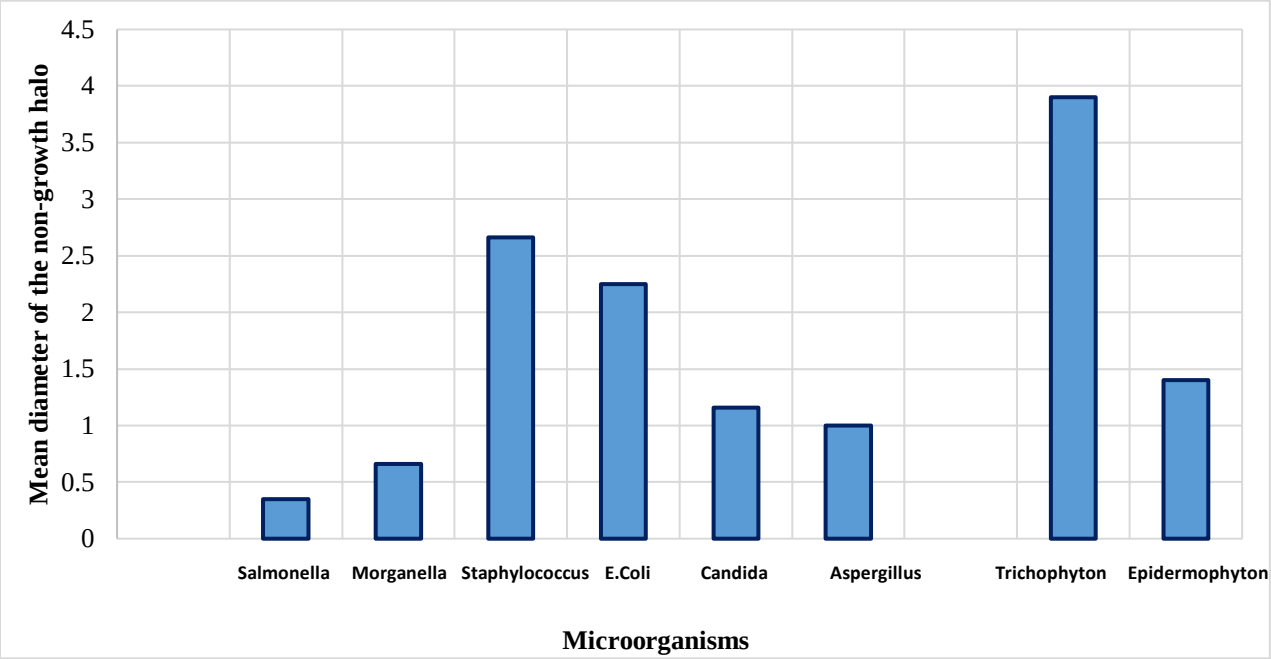

Figure 9: Minimum bactericidal concentration (MBC) Ethanol Extract

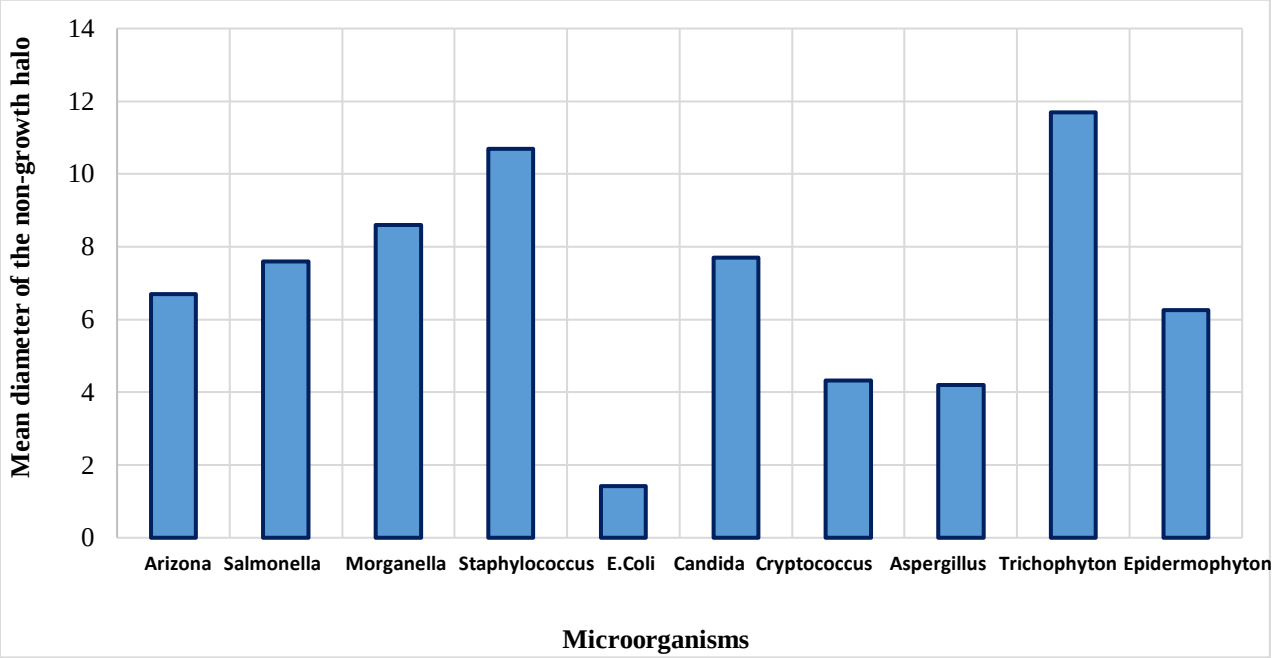

Figure 10: Minimum bactericidal concentration (MBC) Methanol Extract

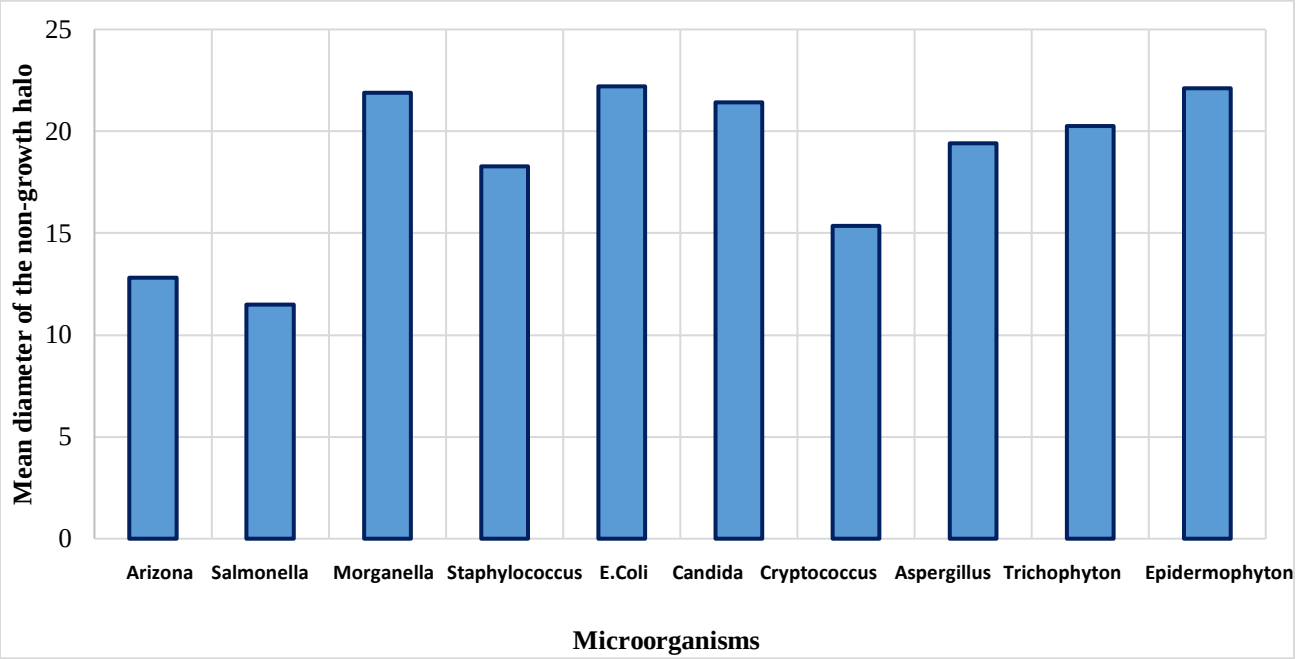

Figure 11: Minimum bactericidal concentration (MBC) Methanol Extract

3.5 Ethanol Extract

Analysis of the variance of antimicrobial property of ethanolic extract Cuscuta epithymum and Pyrethrum roseum on microorganisms was carried out (tables 4 - 8):

3.5.1 Mean type of material differentiation on the diameter of the non-growth halo of microorganisms

Cuscuta epithymum and Pyrethrum roseum has a different effect on the tested microorganisms. The results showed that this nanoparticle is effective on gram-positive and gram-negative bacteria and fungi, but depending on the type of microorganisms, its effectiveness varies. In Table 3, the average diameter of the inhibition zones of Cuscuta epithymum and Pyrethrum roseum versus various microorganisms is expressed in millimeters. In this table, according to the results of the disc diffusion method, fungi with an average diameter of the inhibition zones of the Cryptococcus neoformans are more sensitive to this nanoparticle compared to bacteria with an average diameter of the inhibition zones. Among the bacteria, Pseudomonas aeruginosa contains the largest average diameter of the inhibition zones and has a greater sensitivity to the nanoparticle than other microorganisms, while Salmonella Arizona contains the smallest average diameter of the inhibition zones. Analysis table of the variance of antimicrobial property of ethanolic extract Cuscuta epithymum and tansy on microorganisms were examined:

3.5.2 Mean diameter of the non-growth halo of ethanol extract of Cuscuta epithymum and Pyrethrum roseum against different microorganisms in millimeters

In all three types of substance, there is a significant difference in the type of sample and growth inhibitory effect, so that the essential oil of Cuscuta epithymum and Pyrethrum roseum has the most inhibitory, anti-fungal and bacterial effects, and the ethanol extract of the plant after the essential oil has a balanced property. The methanol extract has been shown to be weak in its properties.

Table 4: Analysis of variance of antimicrobial property of ethanolic extract

Meaningful level	F	Average of squares	df	Sum of squares	Source of changes	Statical indicators
0.247	1.134	16.911	9	152.197	Outgroup	The diameter of non-growth halo
		12.606	40	504.248	Intergroup	
			49	10862.743	total	

Table 5: Analysis of variance of antimicrobial property of ethanolic extract Essence

Meaningful level	F	Average of squares	df	Sum of squares	Source of changes	Statical indicators
0.128	1.673	8.863	9	79.765	Outgroup	The diameter of non-growth halo
		5.259	40	211.920	Intergroup	
			49	291.658	total	

Table 6: Analysis of variance of antimicrobial property of essence

Meaningful level	F	Average of squares	df	Sum of squares	Source of changes	Statical indicators
0.146	1.608	30.550	9	247.947	Outgroup	The diameter of non-growth halo
		19.002	40	760.078	Intergroup	
			49	1035.026	total	

Table 7: The average of material type differentiation on the diameter of the non-growth halo

classification	Number of samples	The average of material type differentiation	Type of material
B	150	1.49000±0.517626	ethanol
C	150	3.91000±0.345044	methanol
A	150	7.37800±0.649969	essence

Table 8: Analysis of variance of antimicrobial property of effective matter

F	Average of squares	df	Sum of squares	Source of changes	Statical indicators
32.461	437.933	2	875.886	Outgroup	The diameter of non-growth halo
	13.941	447	1983.156	Intergroup	
		449	2859.022	total	

4 Conclusion

Following on upon the our previous works (10, 14-24) [16-28] in this study the extraction compounds from native plants is very important due to the importance of medicinal plants in the last century and the attention of researchers to them as a safe natural resource. In Iran With various species of medicinal plants, an appropriate situation has been provided for these studies. According to the obtained results from this study of Cuscuta epithymum and Pyrethrum roseum plant and the results of cytotoxicity tests, this plant has the ability and potential for medical or therapeutic uses especially for cancer cells. The results of analysis of the essential oil by gas chromatography and mass spectrometry showed presence of a variety of compounds in essential oils. It seems the extracted essential oils have positive effects on cancer cells. Study the characteristics of each of these compounds could be a step towards developing the use of medicinal herbs. The extracted essential oils have a positive effect on cancer cells.

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