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Optimizing the extraction conditions (solvent concentration and extraction time) of aqueous, alcoholic and hydroalcoholic extracts of Echinophora platyloba: antioxidant properties and its lethal effect on hydatid cyst Protoscolex in laboratory condition

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ABSTRACT

Cystic echinocossosis (hydatidosis) caused by the larval stage of cestodes of the genus Echinococcus is an important and prevalent zoonotic disease. In human patients, surgical removal of the cysts is the treatment of choice in many cases however, leakage of the larva (protoscoleces) during the surgery and formation of new cysts is a concern. Presently, different solutions such as hypertonic saline and silver nitrate are used to inactivate the cyst-dwelling protoscoleces but each have some side-effects hence searching for novel protoscolicide formulations with high efficacy and low side-effects has been always in the focus. The aim of this study was to optimize the extraction conditions of *Echinophora platyloba*, to evaluate antioxidant properties of the extracts and to assess their protoscolicical effects *in vitro*. For this purpose, hydroalcoholic extracts of *Echinophora platyloba* were extracted in ethanol concentrations of 0, 50 and 100% and during 24, 48 and 72 hours. Response surface methodology (RSM) was perform to estimate optimal extract conditions. Finally, 200, 400 and 600 ppm concentrations of optimized extracts were tested on live protscoleces from sheep and goat livers collected from Hamedan abbatoir. Results showed that *Echinophora platyloba* extract prepared with absolute ethanol in 24 hours could inactivate 100% of protoscolicess after 5, 15 and 30 minutes of exposure. This *in vitro* study suggest that ethanolic extract of *Echinophora platyloba* can potentially be applied *in vivo*.

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1. Introduction

Hydatidosis is a zoonotic disease with worldwide distribution caused by the larval stage of tapeworms of the Echinococcus granulosus complex [1]. The disease is hyperendemic in Iran and it causes a lot of health and economic damage to the affected communities [2]. It is estimated that the economic damage caused by this disease in the country is about 232 million dollars annually [3]. Dogs are the final host of the parasite and As an accidental host, humans become infected by eating water, vegetables and food contaminated with parasite eggs [4].treatmentHydatid cyst in humans with There are many complications. Although surgery is one of the most efficient methods to remove parasite cysts from the patient's body, due to the presence of cysts that are far from the reach of surgery, failure to completely remove the cyst germ layer during surgery, and the risk of disease recurrence, researchers are looking for scoliosis compounds. They are more effective and have fewer side effects. usingHypertonic salt, as a protovasculogenic agent, has caused complications such as sclerosing cholangitis and bile duct stricture [5]. The side effects of formalin are more common than other chemical compounds because the contact of formalin with the bile ducts causes the destruction of epithelial cells and ultimately liver necrosis [6]. However, the need to find herbal protoscolexicide with more effect and less side effects as an alternative has always been considered.

Plants have been used for many years all over the world to treat and prevent some diseases. In particular, several studies have been conducted in the field of protoscolexicidal effects of different plants, such as carob extract [7], essential oil of tertizak [8], mixture of ginger and eucalyptus extracts [9], and essential oil of the herb [10].In aIn a systematic review study, 52 plant species belonging to 22 families were introduced as scoliosis agents of Echinococcus granulosus, the most used of which belonged to the Lamiaceae family (25%) and Apiaceae (11.3%) and the herbal compounds berberine, thymol and thymoquinone [11]]. In the mentioned study, the results of the scoliosis effects of the essential oils of different plants showed that their effectiveness in the laboratory environment occurs after 10 to 20 minutes and with damage to the germ layer of the hydatid cyst. Some essential oils, such as Shirazi thyme essential oil, reduce the weight and size of the cyst, but do not completely eliminate the cyst [11] in the study of Malki Fard et al. (2017). Carob extract at a concentration of 50 mg/ml caused the destruction of all protoscolexes after 30 minutes [7].. In the study by Bahrami et al., 2014, the essential oil of Tretisek at a concentration of 15 mg after 60 minutes, destroyed all protovascolexes [8]. In the study of Faizi et al. (2016),A mixture of ginger and eucalyptus extractsAfter 15 and 30 minutesExposure destroyed 97.24 and 100% of protoscolexes, respectively [9]. In Hosseini et al.'s study (2016), exposure to protoscolexes with the protoscolexes after 120 minutes, 99.3% of protoscolexes were reported [10] as seen in previous studies.During exposure of 30 minutes of extract or essential oil to the cyst,Protoscolexes Not completely destroyed or at longer exposure times (60 to 120 minutes), 100%Protoscolexes they are removed.

A pleasant plant with a scientific name*Echinophora platyloba* It is from the Chetrian family and is an exclusive plant of Iran and is used as a food seasoning and flavoring food. This plant is known by the local names Khosharooz, Khoshroze, Tigh Torag and Kashander [11]. Khosharizeh from the Amblyfera family And it is a permanent plant in the Mediterranean regions as well as in the central and western provinces of Iran [12]. The ends of the leaves are prickly and they bloom from June to September. In Iran, the aerial and dried parts of some species are added to cheese and yogurt as a seasoning. Also, species related to Echinophora are used in traditional medicine to heal wounds and treat stomach ulcers due to their anti-fungal and anti-flatulent properties [12]. Previous studies showed that this plant has saponin, flavonoid and alkaloid compounds, and a pleasant methanolic extract has an inhibitory effect on the growth of bacteria and fungi [11].

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In another study, the analgesic effects of the hydroalcoholic extract of Khoshariz plant in male rats have been attributed to flavonoid and alkaloid compounds [13]. Also, anticancer activity, antimicrobial effects and liver protection against acetaminophen toxicity have been shown by this plant [12]. On the other hand, due to their phenolic structure, these compounds can be an effective substitute for synthetic antioxidants [14] because natural antioxidants, in addition to having antioxidant properties, are effective in reducing cancers and heart diseases and other diseases related to aging [15]. [. Due to the antimicrobial and antioxidant effects [17, 16] of the plant, it is necessary to achieve optimal conditions for extracting its extract, which has the maximum effective compounds present in the plant. Therefore, this study was carried out by examining the conditions affecting the extraction of plant extracts such as the type and concentration of the solvent used and the extraction time of the extract in order to optimize the extract extraction conditions in order to obtain the extracts with the highest effective compounds and the most economical method of interception. In the second part of this research, since there has been no research on the antioxidant properties of Khosharizah extract and determination of the optimal extract for scolexicidal effect, the effect of the optimal extract on Echinococcus granulosus protoscolexes was evaluated.

2- Materials and methods

2-1- Materials and equipment

In this research, Khoshareze plant was purchased from the medicinal plant market of Hamedan city. All chemicals were obtained from Merck and solvents from domestic companies with the highest purity. Also, the devices and equipment used include a household mixer (Sani, Iran), an oven (Fan Azma Gostar, Iran), a laboratory scale with an accuracy of 0.0001 (Sartorius, Germany), a two-beam ultraviolet-visible spectrophotometer (PG Instrument, England), laboratory centrifuge (Sigma, Germany), rotary evaporator (IKAVR 10, Germany), pH meter (Denver, Germany), Ben-Marie device (Fan Azma Gostar, Iran), tube shaker (Labinco Pars Khazar, Iran)) Was .

2-2- The method of preparation of Khusharize extract

After cleaning the Khoshariza plant, the leaves and stems of the plant were separated and ground. In order to prepare the extract, solvents of pure water, pure ethanol 100% and a mixture of water and ethanol (50%) were used in a ratio of 5:1. The mixtures of solvent and herbal plant were placed on a stirring heater at a temperature of 40°C for different times (24, 48 and 72 hours) and during the experiment, the level of the solvent was adjusted by adding fresh solvent at a temperature of 40°C. After different times of extraction, the extracts were smoothed with Whatman No. 40 paper and concentrated with a rotary operator device and stored in sterile containers at refrigerator temperature until the time of testing (up to 14 days).

2-3- Experiments performed on the extracts

2-3-1- Amount of total phenolic compounds

The amount of total phenolic compounds was evaluated using Folin Ciocalto method [18]. For this purpose, first 0.5 ml of each extract was diluted with 2.25 ml of distilled water and 250 microliters of Folin reagent was added to it, and after 5 minutes in the dark environment, 2 ml of 7.5% sodium carbonate solution was added to the solution. added. Then the tubes were kept for 30 minutes in a Bain-Marie at 40°C. The optical absorption of the samples was read by a spectrophotometer at a wavelength of 760 nm. The amount of total phenolic compounds of the extracts was calculated using a standard curve based on mg of gallic acid per gram of sample.

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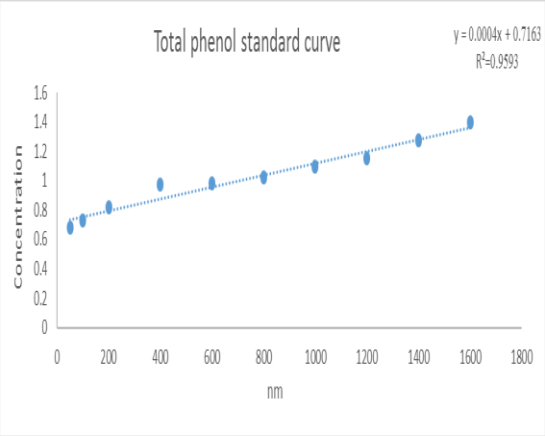

Fig 1 Standard gallic acid curve in total phenolice compounds test

2-3-2- iron reducing power test (FRAP)

Antioxidant activity was measured by the method of reducing power of trivalent iron using the method of Yıldırım et al [18]. In this method, one milliliter of extract was mixed with 2.5 milliliters of phosphate buffer and 2.5 milliliters of potassium ferricyanide. The above solution was kept at 50°C for 30 minutes. Then 2.5 ml of trichloroacetic acid was added to the mixture and the samples were centrifuged for 10 minutes. Then, from The supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of iron 3 chloride and its optical absorption was read by a spectrophotometer. Gallic acid standard was used to draw the standard graph.

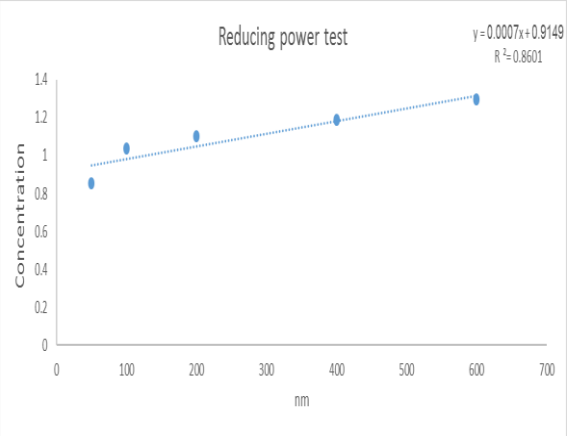

Fig 2 Standard gallic acid curve in FRAP test

2-3-3- Measurement of DPPH free radical inhibition activity

Antioxidant activity was measured by DPPH active radical inhibition method according to the presented method [19]. First, one milliliter of methanolic DPPH solution was mixed with 3 milliliters of the extracts and the resulting mixture was kept for 30 minutes at room temperature and in a dark place. Then its absorbance was read at the wavelength of 517 nm and finally, the activity was calculated in terms of DPPH inhibition percentage according to the following formula.

= % DPPH free radical inhibition

100× Absorption of DPPH solution containing extracts - absorption of DPPH solution (control)

Attraction of DPPH solution (see)

2-3-4- Determining the total antioxidant capacity

In order to measure the antioxidant activity by the total antioxidant capacity method [18], first 500 ml reagent solution (mixture of sulfuric acid, sodium phosphate, ammonium molybdate) was prepared. Half a milliliter of the extract was mixed with 5 milliliters of the reagent solution, then it was placed in a Bain-Marie at 95°C for 90 minutes, and after cooling to room temperature, the absorbance of the samples was read at a wavelength of 695 nm with a spectrophotometer. Gallic acid was used to prepare the standard chart [18].

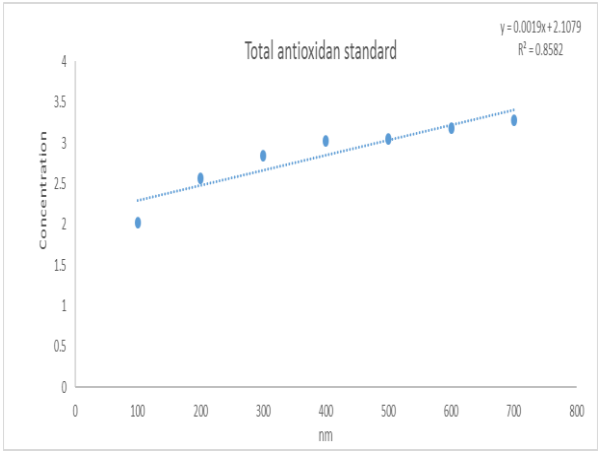

Fig 3 Standard gallic acid curve in total antioxidant test

2-4- Investigating the effect of produced extracts on Protoscolex*Echinococcus granulosus*

From From September to December 1400, the lungs and livers of sheep infected with*Who are you?*It was collected from the industrial slaughterhouse of Hamedan city in 20 times and transferred to the parasitology laboratory of the Faculty of Paraveterinary Medicine of Bu Ali Sina University. In sterile conditions, the contents of the cysts were transferred into Falcon tubes with a syringe, and after transversal cutting and draining the cyst fluid,

the germinal membrane was transferred into the plate using sterile forceps. Then, the germinal membrane was washed with physiological serum to obtain more protoscolex. The cyst fluid was observed under a microscope and checked for fertility or not. In this way, 20 microliters of liquid containing protoscolex was mixed with 20 microliters of 0.1% eosin dye on a microscope slide and observed under the microscope. Dead protoscolexes remain red and living protoscolexes remain colorless [8].

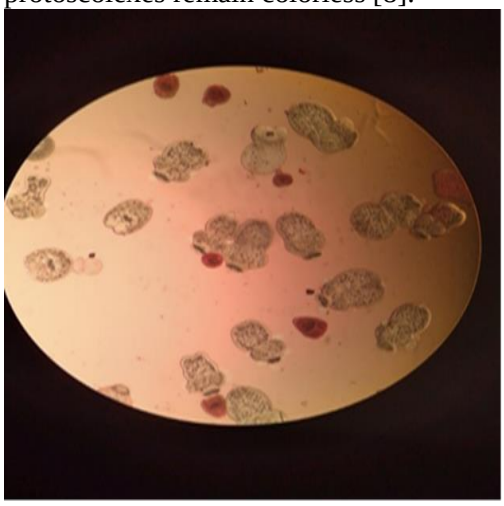

Fig 4 Protoscolices expoused with eosin (after exposure to the stain, dead protoscolices absorbed eosin and colored red, alive protoscolices remained colorless).

5-2- Statistical analysis and optimization

Response surface method was used to optimize the extraction conditions of plant extracts under the influence of solvent concentration and extraction time. For this purpose, the central composite plan ([1]CCD) with 3 levels and 5 repetitions at the central point was used to investigate the effect of extraction conditions on the antioxidant properties of the resulting extracts (+1, 0, -1). In this research, the range of independent variables of solvent concentration used ($_1$X) and extract extraction time ($_2$X) was deduced from the preliminary tests (Table 1). The experimental treatments were randomized in order to minimize the effects of unanticipated changes in the observed responses. Design Expert 11.0.2 software was used to draw 3D diagrams and optimize data. Also the results*Protoscolexation of the extracts was done based on a completely randomized design with 4 replications. To analyze the data from the software (2001)*SAS was used. The comparison of the mean of the measured traits was done using Duncan's multi-range test and at the probability level of 5% [20].

Table 1 Independent variables and levels used to optimize the antioxidant properties of ethanolic extracts of *Echinophora platyloba* extract under different extraction conditions.

Independent variables	Levels and limits of variables		
	-1	0	+1
Extraction time (hr)	24	48	72
Solvent concentration (v/v)	0	50	100

3- Results and discussion

3-1- Investigating the antioxidant properties and phenolic compounds of Khoshareze plant extracts

3-1-1- Total phenolic compounds of extracted extracts

The process of changes in the total phenolic compounds of Khosharizah plant extracts under the influence of different extraction conditions, including extraction time and different concentrations of ethanol solvent, is presented in Figure 5. As can be seen, with the increase in the extraction time, the amount of total phenolic compounds goes through an increasing trend, while with the increase in the concentration of the ethanol solvent, the amount of extracted phenolic compounds decreases. It has already been shown that increasing the concentration of the ethanolic solvent decreases the polarity of the extraction solvent (amount of water in the solvent) and therefore the solvent cannot extract more polar and phenolic compounds [23].

[1]. Central Composite Design

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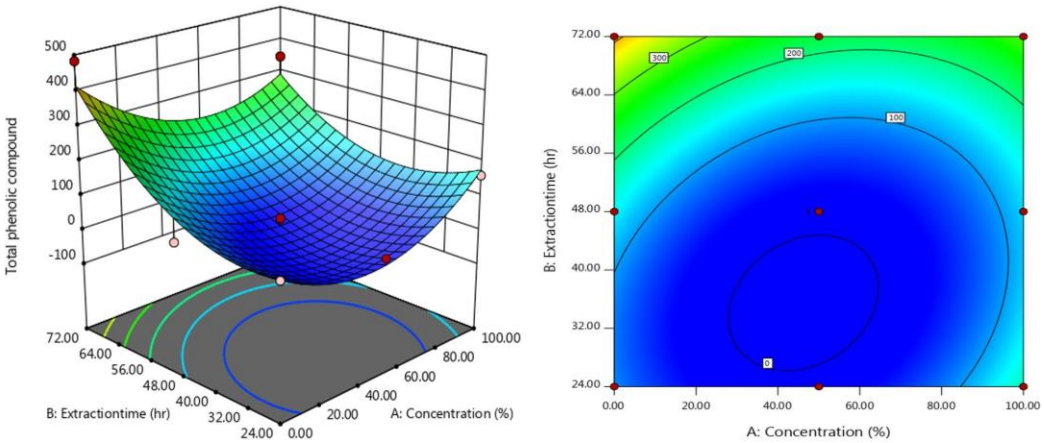

Fig 5 3D and 2D diagram of changes in total phenolic compounds (mg Gallic acid per 100 g of *Echinophora platyloba*) of ethanolic extracts of a *Echinophora platyloba* under the influence of ethanol solvent concentration and extraction time (extraction temperature of 40°C).

Also, according to the results of the present study and in line with previous studies such as Morello et al. (2004), the extraction time had an important effect on the extraction rate of total phenolic compounds [12]. to penetrate the plant tissue and phenolic compounds have enough time to separate from their substrate and enter the surrounding solvent [22]. On the other hand, the application of extraction combined with heat can easily destroy plant components and plant compounds enter the environment [25]. For this reason, the amount of extracted phenolic compounds increases with the application of hydrothermal extraction process. In general, thermal methods, especially those that are performed in a humid environment, cause the release of more and more phenolic compounds by breaking the cell wall [18]. Therefore, this method is suggested for future research.

Houshmand and Mahdian (2013) studied the possibility of extracting the extract of Khoshareze plant with ultrasound waves and checked the antioxidant activity of the produced extracts by RFAP method. The results showed that with the increase of temperature and time of the process, the amount of iron recovery power of the extract increases, but it decreases at high temperature and times of the extraction process [21].

In the study of Moshiri et al. (2019), the extract of zenian seeds under different extraction conditions with ethanol solvent in concentrations (0, 50 and 100%), extraction time (0.25, 12 and 24 hours) and extraction temperature 20, 50 and 80 °C was prepared. The results showed an increase in extraction efficiency by adding water to the extracts. Also, it was found that with the increase in the extraction time, higher amounts of phenolic and antioxidant compounds were extracted, but at higher temperatures, due to the thermal degradation of phenolic compounds, the antioxidant properties of the extracts decreased [18].

Shabanian et al. (2021) in a study of green walnut skin extract with ethanol solvent (in concentrations of 0, 50 and 100%) in different microwave powers (90, 450 and 900 watts) and different time intervals (1, 8 and 15 minutes) extracted and in order to determine the optimal extract to add to the oil, the total amount of phenolic compounds and the antioxidant activity of the extracts were measured by methods of reducing power, DPPH active radical inhibition and total antioxidant capacity. The results showed an increase in the extraction of phenolic and antioxidant compounds with increasing microwave power and extraction time, while with an increase in ethanol concentration (use of pure ethanol), the extraction rate of total phenolic compounds decreased due to the decrease in solvent polarity compared to hydroalcoholic extract. Also, in high microwave powers, with the prolongation of the extraction time, the amount of total phenolic compounds and antioxidant properties decreased, which can be due to the destructive effect of the produced heat on the phenolic compounds during long extraction times [22].

In the present study, the results of the analysis of variance of the data obtained from the measurement of the amount of total phenolic compounds for an ethanolic extract with a quadratic statistical model were significant at the statistical level of 5% (Table 2). According

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to the results of variance analysis (table 2) and the resulting regression coefficients (table 3), a quadratic equation of the statistical model to calculate the amount of total phenolic compounds of the ethanolic extract is given in equation number 2. In this equation, A is equivalent to the solvent concentration (v/ v) and B is the extraction time in hours.

Total phenolic compounds = +12.73 -9.01 A +109.37 B -52.07 AB +121.39 A^2 +108.50 B^2

According to the results of regression analysis, using equation 2, the amount of phenolic compounds of the extracts can be predicted with an accuracy of 86.01%.

Table 2 The analysis, variance of the regression coefficients of predicted linear and quadratic polynomial models for predicting antioxidant properties of *Echinophora platyloba* extract under different extraction conditions.

Response	Source	Sum of Squares	DF	Mean of Squares	F-value	p-value	significance
Total phenolic compounds	Model	2.012E+05	5	40233.35	8.61	0.0067	significant
	A-Concentration	486.90	1	486.90	0.1042	0.7563	
	B-Extractiontime	71769.47	1	71769.47	15.36	0.0058	
	AB	10844.10	1	10844.10	2.32	0.1715	
	A^2	40700.52	1	40700.52	8.71	0.0214	
	B^2	32514.76	1	32514.76	6.96	0.0335	
	Residual	32717.14	7	4673.88			
	Lack of Fit	32717.11	3	10905.70	1.363E+06	< 0.0001	significant
	Pure Error	0.0320	4	0.0080			
	Cor Total	2.339E+05	12				
Total antioxidant capacity	Model	82247.08	5	16449.42	36.21	< 0.0001	significant
	A-Concentration	5489.53	1	5489.53	12.08	0.0103	
	B-Extractiontime	24249.73	1	24249.73	53.38	0.0002	
	AB	5176.00	1	5176.00	11.39	0.0118	
	A^2	23574.74	1	23574.74	51.90	0.0002	
	B^2	7059.22	1	7059.22	15.54	0.0056	
	Residual	3179.89	7	454.27			
	Lack of Fit	3179.73	3	1059.91	26303.74	< 0.0001	significant
	Pure Error	0.1612	4	0.0403			
	Cor Total	85426.97	12				
FRAP	Model	6095.52	5	1219.10	3.52	0.0657	not significant
	A-Concentration	58.88	1	58.88	0.1700	0.6925	
	B-Extractiontime	0.1727	1	0.1727	0.0005	0.9828	
	AB	771.95	1	771.95	2.23	0.1792	
	A^2	3685.14	1	3685.14	10.64	0.0138	
	B^2	185.47	1	185.47	0.5353	0.4881	
	Residual	2425.22	7	346.46			
	Lack of Fit	2424.72	3	808.24	6410.16	< 0.0001	significant
	Pure Error	0.5043	4	0.1261			
	Cor Total	8520.74	12				
DPPH	Model	178.23	2	89.11	2.52	0.1299	not significant
	A-Concentration	17.44	1	17.44	0.4934	0.4984	
	B-Extractiontime	160.79	1	160.79	4.55	0.0588	
	Residual	353.51	10	35.35			
	Lack of Fit	353.51	6	58.92	1.178E+05	< 0.0001	significant
	Pure Error	0.0020	4	0.0005			
	Cor Total	531.74	12				

Table 3 The regression coefficients of predicted quadratic polynomial and liner models for predicting different antioxidant activity of *Echinophora platyloba* extract under different extraction conditions.

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Response	Factor	coefficients	R^2	R^2 adjusted
Total phenolic compound	Intercept	12.73	0.8601	0.7602
	A-Concentration	-9.01		
	B-Extraction time	109.37		
	AB	-52.07		
	A^2	121.39		
	B^2	108.50		
Total antioxidant capacity	Intercept	19.14	0.9628	0.9362
	A-Concentration	30.25		
	B-Extractiontime	63.57		
	AB	-35.97		
	A^2	92.39		
	B^2	50.56		
FRAP	Intercept	28.97	0.7154	0.5121
	A-Concentration	-3.13		
	B-Extractiontime	0.1697		
	AB	-13.89		
	A^2	36.53		
	B^2	8.19		
DPPH	Intercept	15.57	0.3352	0.2022
	A-Concentration	1.70		
	B-Extractiontime	-5.18		

3-1-2- The revitalizing power of extracts

Figure 6 shows the three-dimensional and two-dimensional curves of the changes in the revitalization power of the hydro-ethanolic extracts of Khoshariz plant under the influence of different extraction conditions (extraction time and different concentrations of ethanolic solvent).

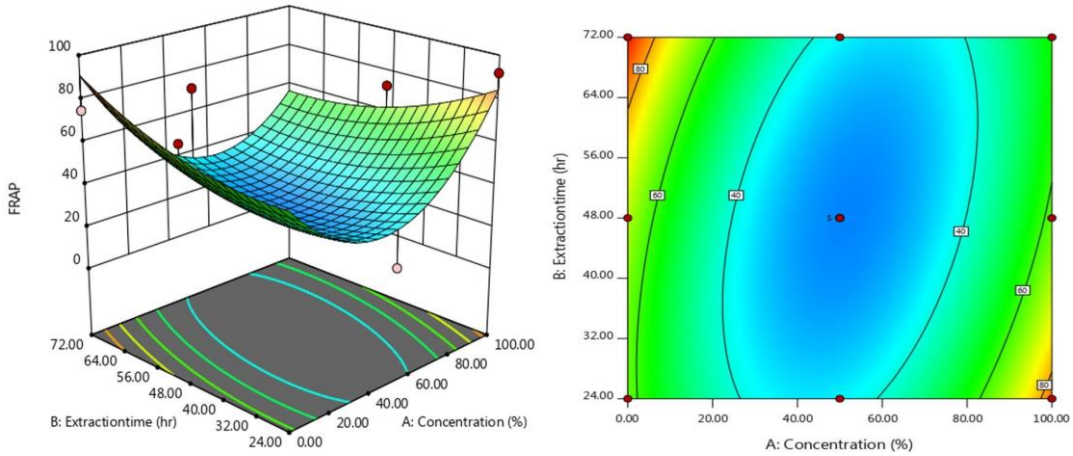

Fig 6 3D and 2D diagram of changes in reducing power of ethanolic extracts of a *Echinophora platyloba* under the influence of ethanol solvent concentration and extraction time (extraction temperature of 40°C).

As it can be seen, with the increase of ethanol concentration up to 50%, the revitalization power of the extracted extracts has a decreasing trend, and then with the increase of the ethanol solvent concentration, the revitalization power of the extracts shows an increasing trend. As it can be seen, with the increase of the extraction time, the revitalization power of the extracts goes through an almost constant and increasing process. By increasing the concentration of ethanol solvent, the reducing power of the extracts decreases because the amount of phenolic compounds in the extracted extracts had a downward trend with increasing the concentration of the ethanol solvent [26] and the reducing power has a direct relationship with the amount of phenolic compounds [27,28]. The increase in the revitalization power with the increase in the extraction time can also be due to the increase in the amount of total phenolic compounds of the extracts under long-term extraction conditions [29].

A quadratic equation of the statistical model to calculate the antioxidant activity according to the revitalizing power of extracted extracts is presented in equation (3), in this equation, A is equivalent to the solvent concentration (v/v)

and B is the extraction time in hours. According to the results of the regression analysis, if equation 3 is used, by using equation 3, the antioxidant activity can be predicted with 71.54% accuracy in terms of the revitalization power of the extracted extracts (Table 3).

FRAP = +28.97 -3.13 A +0.1697 B -13.89 AB +36.53 A^2+8.19 B^2

3-1-3- Antioxidant property of DPPH active radical inhibition of extracted extracts

As in the three-dimensional and two-dimensional curves, the process of changes in the antioxidant property of inhibiting the active radical DPPH of the alcoholic extracts of Khoshareza plant under the influence of different extraction conditions (extraction time and different concentrations of ethanol solvent) in Figure 7 can be seen with the increase in the concentration of ethanol, the amount of the antioxidant property of inhibiting the active radical DPPH of the resulting ethanol extracts increases.

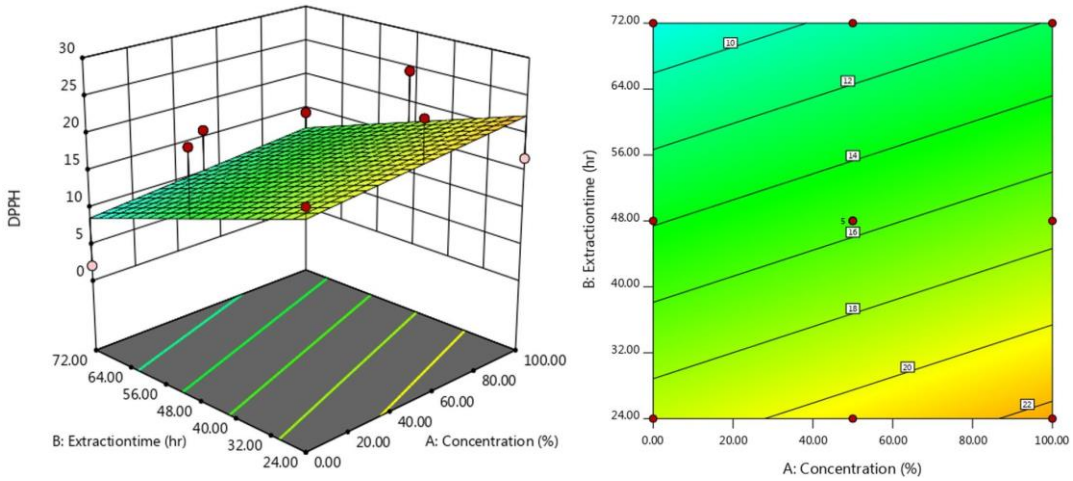

Fig 7 3D and 2D diagram of changes in antioxidant properties of DPPH activated radical inhibition of ethanolic extracts of a *Echinophora platyloba* under the influence of ethanol solvent concentration and extraction time (extraction temperature of 40°C).

The highest value of DPPH active radical inhibition antioxidant property (26.40) was observed in the range of extraction time of 24 hours and concentration of 50% alcohol solvent. Also, with the increase of extraction time, the antioxidant properties of DPPH active radical inhibition of the extracts have a downward trend. The results of data analysis showed that the solvent concentration had a significant effect on the amount of inhibition of free radicals. Also, the results indicated that the ability of the extracts to inhibit free radicals depends on the concentration of the solvent, and with the increase of the concentration of the solvent, their free radical absorption activity increases. Since there is a direct relationship between the radical scavenging activity and the amount of phenolic compounds in fruits [27, 30], therefore, by increasing the amount of total phenolic compounds in the extracted extracts, it is expected that the percentage of active DPPH radical inhibition of the extracts will increase, but in the present study, despite the increase The amount of phenolic compounds under the effect of increasing the extraction time, the DPPH active radical inhibitory power of the extracts decreased, which is different from the results of other researchers. The difference in the antioxidant activity of different extracts is related to the amount of hydrophilic and hydrophobic phenolic compounds present in the extract. Basically, the DPPH test measures the antioxidant activity of water-soluble phenolic compounds. Chen et al. (2005) reported that aqueous and methanolic extracts inhibit DPPH by 80-82% [31].

Equation 4 shows the statistical model of the antioxidant properties of DPPH active radical inhibition of extracts. As can be seen in Tables 2 and 3, the antioxidant property of inhibiting the active DPPH radical of the extracted extracts was linear, which does not have enough accuracy for prediction, and if this relationship is used, only 33.52% accuracy can be expected.

DPPH = +15.57 +1.70 A -5.18 B

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3-1-4- Antioxidant properties of all extracts

As the two-dimensional (meter) and three-dimensional curves show the changes in the antioxidant properties of the total ethanolic extracts of Khoshareza plant under the influence of different extraction conditions (different extraction times and different concentrations of the ethanolic solvent), the antioxidant activity of the total ethanolic extracts increases with the increase of the extraction time. However, with the increase in the concentration of ethanol solvent up to 50%, the antioxidant activity of the ethanolic extracts has a downward trend, and then with the increase in the concentration of the ethanol solvent, the antioxidant activity of the total extracts increases (Figure 8). This observation can be due to the fact that with the increase in the extraction time, the amount of mass transfer in the extraction process is increased and the compounds effective in antioxidant activity are better removed from inside the cells [32].

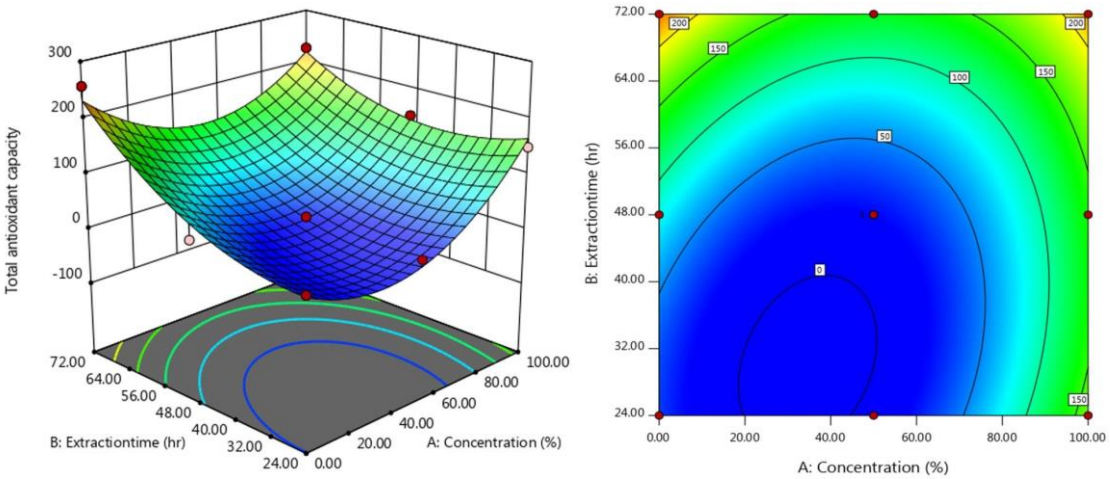

Fig 8 3D and 2D diagram of changes in total antioxidant capacity of ethanolic extracts of a *Echinophora platyloba* under the influence of ethanol solvent concentration and extraction time (extraction temperature of 40°C).

The results of the analysis of variance of the data obtained from the measurement of the total antioxidant property for the ethanolic extract with the quadratic statistical model were significant at the statistical level of 5% (Table 2). According to the results of variance analysis (Table 2) and the resulting regression coefficients (Table 3), a quadratic equation of the statistical model to calculate the antioxidant properties of the total ethanolic extract is given in equation (5). In this equation, A is equivalent to solvent concentration (v/v) and B is extraction time in hours. According to the regression analysis results, if equation 5 is used, it is possible to predict the antioxidant properties of all extracts with 96.28% accuracy.

Total antioxidant capacity = +19.14 +30.25 A +63.57 B -35.97 AB +92.39 A^2 +50.56 B^2

2-3- Optimizing the extract extraction process from Khosharizah plant under different solvent extraction conditions

Table 3 shows the conditions determined for the independent variables to optimize the extraction of the extract from the khushariza plant and the optimized conditions. Since in extraction processes, the goal is to achieve the highest antioxidant activity and preserve active compounds and minimize thermal damage during long extraction times, therefore, as seen in Table 4, the independent variables of solvent concentration in the applied range (0-100%) and the extraction time has been kept to a minimum to reduce the adverse effect of heat on phenolic and antioxidant compounds.

Table 4 The constrain used to optimize the antioxidant properties of ethanolic extracts of *Echinophora platyloba* under the influence of different extraction conditions

name	Goal	Lower limit	upper limit
A:Concentration	is in range	0	100
B:Extractiontime	minimize	24	72

Total phenolic compound	maximize	30.016	483
Total antioxidant capacity	maximize	18.112	257.1
FRAP	maximize	19.48	91.98
DPPH	maximize	2.07	26.4

Also, dependent variables such as the amount of total phenolic compounds, the amount of total antioxidant capacity, the amount of inhibitory power of DPPH active radical and the amount of revitalizing power of the extracts have been considered as maximum. In the optimization process, all independent parameters were given the same weight and importance. According to the desired conditions, the solution with the highest desirability, the most appropriate and the best conditions will be the first solution with the conditions of extraction time of 24 hours and the concentration of ethanol solvent 100% as the best conditions to achieve the optimal conditions. If the conditions of the first solution are applied, the antioxidant properties and phenolic compounds of the extracts obtained from Khoshareze plant are optimally preserved (Figure 9).

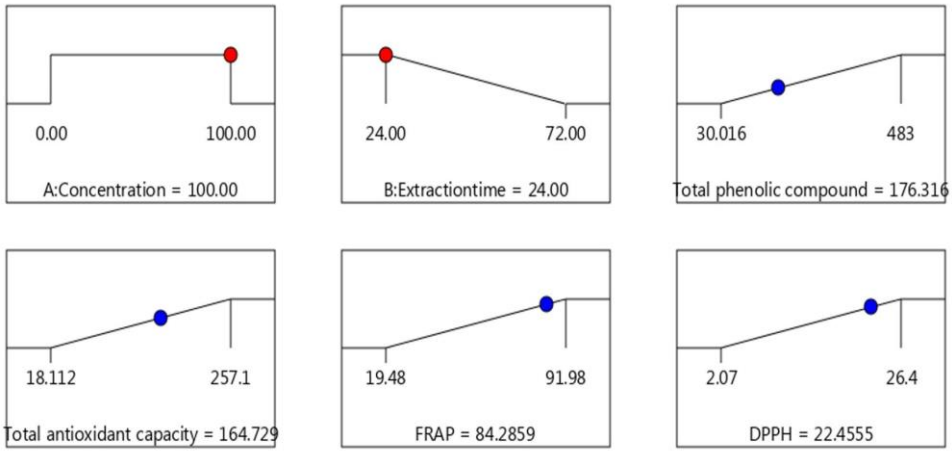

Fig 9 Results of optimizing the extraction of ethanolic extracts of *Echinophora platylob*

3-3- The lethal effect of the optimal extract on Protoscolex*Echinococcus granulosus*

In the present study, 100% ethanolic extract of Khoshareze plant had a high lethality percentage at 5, 15 and 30 times.

So that the lethality percentage of this extract was 100% on average in all three times and all 4 repetitions of the experiment (Figure 10).

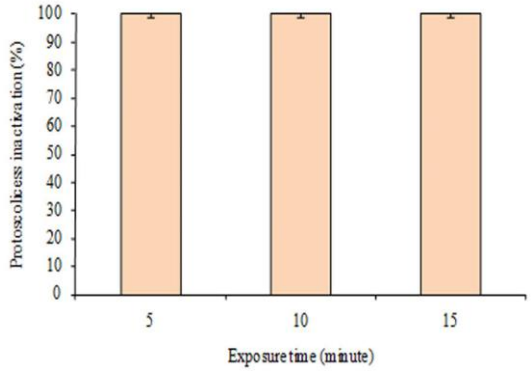

Fig 10 Effect of *Echinophora platylob* extract on protoscolicess inactivation (%)

In previous studies, the anti-fungal and anti-bacterial effects of the plant have been investigated and its positive effect has been shown. For example, the antifungal effect of the extract of Khosharize plant on the common dermatophytes Trichophyton Schoeneline and Trichophyton verrucosum was shown at concentrations of 35, 50 and 150 mg/ml [33]. In another study, a significant inhibitory effect of the methanolic extract of Khoshariza against the pathogenic Nocardia strain was reported [34]. Also, the strong antibacterial effect of Khosharizeh essential oil on Staphylococcus aureus bacteria has been reported [35]. It seems that the beneficial antimicrobial, antifungal and protoscolexicidal activity is related to the presence of alkaloids and flavonoids in this plant [14, 28].

4- General conclusion

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The analysis of the data showed that by adding water to the studied solvents, their efficiency in extracting phenolic compounds increased and the antioxidant property of the resulting extracts increased, which is due to the increase in the polarity of the solvent. In general, it can be said that natural antioxidants can be used as a substitute for synthetic antioxidants. Also, the results of in vitro tests showed that 100% ethanolic extract of Khoshareze plant can be used as a suitable candidate in in vivo environments. For this purpose, it is suggested to evaluate the effectiveness of this extract in the experimental model of infection with hydatid cyst, as well as to investigate possible side effects in animals and subsequently in humans.

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بهینه سازی شرایط استخراج(غلظت حلال و زمان استخراج) عصاره آبی، الکلی و هیدورالکلی گیاه خوشاریزه (*Echinophora platyloba*)، خصوصیات آنتی اکسیدانی و اثر کشندگی آن بر پروتواسکولکس‌های کیست هیداتید در شرایط آزمایشگاهی

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چکیده

اکینوکوکوزیس سیستیک (هیداتیدوزیس) یک بیماری مهم و شایع مشترک بین انسان و حیوان است که توسط مرحله لاروی سستودهای جنس *اکینوکوکوس* ایجاد می‌گردد. اگرچه در بسیاری از موارد بیماری در انسان، درمان انتخابی جراحی است ولی به طور ثانویه ممکن است در مرحله تخلیه کیست باعث انتشار نوزادان انگل (پروتواسکولکس‌ها) و مسبب رشد کیست‌های جدید شود. در حال حاضر از محلول‌هایی نظیر نمک هایپرتونیک و نیترات نقره برای غیر فعال کردن محتویات کیست استفاده می‌شود که عوارض جانبی برای بیماران به همراه دارند. بنابراین نیاز به یافتن ترکیبات پروتواسکولکس‌کش جدید با تأثیر بیشتر و عوارض کمتر همیشه مورد توجه بوده است. هدف از این تحقیق، بهینه‌سازی شرایط استخراج عصاره گیاه خوشاریزه و بررسی ویژگی‌های آنتی‌اکسیدانی و ترکیبات فنولی حاصل از آن و نیز خاصیت کشندگی پروتواسکولکس‌های کیست هیداتید در شرایط آزمایشگاهی بود. برای این هدف، ابتدا عصاره هیدروالکلی گیاه خوشاریزه تحت تأثیر غلظت‌های ۰، ۵۰ و ۱۰۰٪ اتانول و طی مدت زمان‌های ۲۴، ۴۸، ۷۲ ساعت استخراج گردید. برای تعیین بهترین شرایط استخراج عصاره‌ها از روش سطح پاسخ استفاده گردید و عصا ره‌گیری در شرایط بهینه صورت گرفت. نهایتاً از عصاره‌های استخراجی در شرایط بهینه غلظت‌های ppm ۶۰۰، ۴۰۰، ۲۰۰ تهیه گردید و تأثیر آن‌ها بر پروتواسکولکس‌های زنده استحصال‌شده از کیست‌های هیداتید کبد گوسفند و بز از کشتارگاه همدان بررسی گردید. نتایج نشان داد عصاره خوشاریزه حاصل از ۲۴ ساعت استخراج با اتانول خالص (۱۰۰٪) در زمان‌های ۵، ۱۵ و ۳۰ دقیقه می‌تواند ۱۰۰ درصد پروتواسکولکس‌ها را از بین ببرد. نتایج آزمایشات بیرون‌تنی (*in vitro*) حاضر پیشنهاد می‌دهد که عصاره اتانولی گیاه خوشاریزه می‌تواند به عنوان یک کاندیدای مناسب جهت استفاده در محیط‌های درون‌تنی (*in vivo*) استفاده شود.