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Impact of ultrasound pretreatment with different solvents on the antioxidant activity, phenolic and flavonoid compounds of the St. John's wort (*Hypericum perforatum* L.) extract

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ABSTRACT

In this study, the effect of ultrasound pretreatment (at 40 kW power, for 20 min) with single partial solvent including water and ethanol and two partial solvents containing water/ethanol in the form of 30% (water): 70% (ethanol) and 60% (water): 40% (ethanol), were considered on the extraction efficiency, total phenolic and flavonoid compounds, as well as the free radical inhibition rate of the St. John's wort (*Hypericum perforatum* L.) extract. In order to analyze the data, a completely randomized design was used in three replications, and the comparison of means was done by Duncan's multi-range test, at the probability level of α=1%, and using SPSS 14. Based on the results, the highest amount of extraction efficiency (18.3 %), total phenolic (0.295 mg GAE/g dw) and flavonoid (0.105 mg QE/g dw) compounds and the inhibition rate of free radicals (84.21 %) was measured in the samples pre-treated with ultrasound along with two partial solvents [30% (water): 70% (ethanol)] and the lowest amount of extraction efficiency (4.21%), total phenolic (0.051mg GAE/g dw) and flavonoid (0.0115 mg QE/g dw) compounds as well as free radical inhibition rate (47.53 %) was specified in water (100 %) solvent.

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1- Introduction

Nowadays, due to the toxicity and carcinogenicity of chemical and synthetic compounds and the side effects of their consumption as well as the development of drug resistance, the use of plant extracts rich in bioactive compounds has attracted the attention of many researchers, therefore the necessity of replacing synthetic antioxidants with natural ones. And finding natural sources with suitable antibacterial activity seems essential in order to increase the shelf life of food. Several reports show that some plant products such as extracts have many biological and pharmacological effects and have antimicrobial and antiseptic effects.]1 and 2[. Antioxidants are compounds that delay or slow down the oxidation of food. These materials can be oxidized through autoxidation. Antioxidants, as hydrogen donors or free radical acceptors, delay autoxidation by increasing the induction period. Some natural antioxidants can be used as a substitute for synthetic antioxidants in food. Also, due to the high cost of synthetic antioxidants and the availability of many natural antioxidants, the use of essential oils and natural and herbal extracts is more affordable.3 and 4[.

Hofarikun, tea grass or raei flower (*Hypericum perforatum* L.) a medicinal plant from the familyHypericaceae is considered. This plant has vegetative growth in the first year, but its flowering starts from the second year onwards. Hofarikon produces two types of stems. Flowering stems and vegetative stems that are without flowers and are removed from the crown in late winter and early fall, respectively. Also, the flowers are bright yellow and have radial symmetry. The leaves of this plant are petioleless, with rounded ends and no cuts. There are many dark and light spots on the leaves. The dark spots are on the edge of the leaves, but the bright spots are scattered on the entire surface of the leaf, which are the places of accumulation of hypericin (the most important active ingredient of Hofariquon) and essential oil, respectively [5]. It is noteworthy that the concentration of effective substances in the leaves of thin leaf varieties is 2 to 3 times higher than that of wide leaf varieties. According to the conducted researches, various biologically effective compounds have been identified in this plant, which include: anthraquinone derivatives that are present in this plant at the rate of 0.1 to 0.15% and include hypericin and pseudohypericin (the amount of which is more in the flower than in the stem and their precursors are protopesudohiprisin, protopsudohiprisin and the rare compound cyclopseudohiprisin. Under the influence of light, the mentioned precursors are converted into hypericin and pseudohypericin cyclic compounds, so the extraction should be done in the dark [6]. Flavonoids, which include flavonols (such as kaempferol), flavones (such as quercetin and rutin), biflavonoids, aminoflavones, and catechins. Also, tannins amount to 8-9%, whose type is not exactly known [7]. Other phenols include caffeic, chlorogenic, and ferulic, followed by volatile oils, most of which include methyl-2-octane anda Andb It also contains pinene, geraniol, and small amounts of myrcene and limonene.8 and 9[. Now it seems that the extract of the hofarikon plant because of its

compoundsTerpenoid, flavonoid, polyphenol and tannins have antioxidant, antiviral, antibacterial and antifungal properties. In this regardIn order to obtain these bioactive compounds, the process of extracting and purifying natural extracts of plant origin seems to be the first and most necessary step. One of the important points in extracting effective compounds from medicinal plants is choosing a suitable method for extracting bioactive compounds from the matrix, with minimum impurity and also preserving their biological activity.This variability depends on the difference in the internal bonds of these compounds and especially on the polarity of the molecules that make up the solvent, therefore in the extraction processFactors such as solvent type, sample to solvent ratio, sample particle size, extraction time and temperature are very important [10]. Currently, extraction is done traditionally through methods such as Soxhle, distillation, soaking and percolation and using water and organic solvents. It is obvious that according to the method and solvent used, the obtained extracts show different properties. What is certain is that traditional methods are time-consuming and require a large amount of various solvents. Also, many active substances may be destroyed during extraction [4].But in recent years,extraction with the help of modern technologies such as microwaves, ultrasonic waves or with supercritical fluid becauseReducing the duration of extraction, reducing the amount of solvent used, increasing the extraction efficiency and improving the quality of the resulting extracts, has been expanded. Through the phenomenon of cavitation, ultrasonic waves lead to a series of physical, mechanical, chemical and biochemical changes, and therefore it can be used in processes such as extraction. The mechanical effects of ultrasound waves and the cavitation phenomenon caused by these waves increase the permeability of the solvent into plant cells, increase the mass transfer, and then increase the extraction efficiency at lower temperatures. Due to the mechanical effects of ultrasound waves, it is often used as a pre-treatment in the extraction process. Also, ultrasonic waves enable the extraction of heat-sensitive compounds [11].Investigations show that various researches have been conducted regarding the effect of ultrasound pretreatment on the amount of extract extracted from different plants.[12,13 and 14].

As mentioned, tea grass can grow in a wide range of climatesContains bioactive substances with strong antioxidant and antibacterial propertiesIt is considerable. But the most important feature of the Iranian species of this plant is the high percentage of hypericin as the most important effective substance in its leaves and flowers, which plays a very important role in the treatment of various diseases. According to the investigations, there has been no research related to the preparation of aqueous and alcoholic extracts of this plant with the help of ultrasound waves. Therefore, in this research, the effect of different solvents along with ultrasound pretreatment on antioxidant activity and the amount of phenolic and flavonoid compounds extracted from this plant was investigated.

2- materials and methods

2-1- Raw materials

The tested Hofarikun plant was obtained from the farm of the research collection of medicinal plants of Jihad University, in the spring of 1402. In the following, the flowering branches of this plant were kept in polyethylene bags and in a home freezer (temperature -18 degrees Celsius) immediately after collecting and separating the impurities in dry shade and until the experiment. In the following, during the extract extraction process, dried samples using a mechanical mill (Bush model millBOSCH TSM6A01) were converted into very fine particles and passed through a 40 mesh sieve. All reagents and standards, as well as all chemicals and solvents, were purchased from Sigma and Merck respectively and with the highest purity.

2-2- Methods

2-2-1- Extraction of the extract by flooding method and with the help of ultrasound pretreatment

To perform the test, 1 gram of hofarikon plant was powdered by a digital scale with an accuracy of 0.01 gram (Sartorius, model PT210, Germany) weighed and poured into an Erlenmeyer flask with a capacity of 500 ml. In all experimental treatments, the ratio of dried powder to solvent was 1:10 (wt–volume) was considered. Next, different ratios of solvents (30% (water): 70% (ethanol), 60% (water): 40% (ethanol), 100% (ethanol) and 100% (water)) were poured into Erlenmeyer flask and powdered which was already poured into the Erlenmeyer flask, was mixed for 12 hours with a shaker at room temperature. The treatments that should be applied to them before the ultrasound treatment were exposed to ultrasound waves (40 kW) for 20 minutes before placing the earlen on the shaker. In this regard, an ultrasonic device with a probe (Top Sonic Company) and a cylindrical titanium probe with a diameter of (2.5) cm were used. Next, the solvent mixture containing the extract and plant solids were separated from each other using Whatman filter paper number one. After this step, the extracted extract was centrifuged at 7800 rpm for 30 minutes and then the clear part above the mixture was separated. Extracts obtained by vacuum rotary evaporator (FINGER-RV05، Germany (at a temperature of 40 degrees Celsius)with the aim of preventing damage to phenolic compounds) and at 200 drav/min, concentrated and finally by lyophilization (Operun-FDB550, South Korea) and dried at -50 degrees Celsius and turned into powder. The obtained powders were packed in polyethylene bags impermeable to air and moisture and stored in a home freezer (temperature -18 degrees Celsius).4 and 15[.

Table1. The type of solvent and pretreatment used in the study

Treatment types	Code
70% (ethanol)/30% (water)	E70.W30
40% (ethanol)/60% (water)	E40.W60
Ultrasound pretreatment (40 kW, 20 min) + 70% (ethanol)/30% (water)	P40.E70.W30
Ultrasound pretreatment (40 kW, 20 min) + 40% (ethanol)/60% (water)	P40.E40.W60
70 % ethanol	E70
Water	IN

2-2-2- Total efficiency of extraction

Each of the obtained extracts were placed in a vessel with a temperature of 50 degrees Celsius for 16 hours. After desiccating and reaching a constant weight, the extraction efficiency was calculated using equation 1 [16].

(1)

$$Extraction\ efficiency\ (\%) = \frac{A \times 100}{B}$$

where in:A = Weight of dried ingredients (g) AndB= The weight of the primary ingredients (g) Is.

2-2-3- Measuring the total amount of phenolic compounds

Amount of phenolic compounds of hofarikon extract by Folin-Ciocalto method(Folin-Ciocalteu) It was measured. To measure the amount of phenolic compounds, one milliliter of extract solution was mixed with one milliliter of hydrochloric acid solution (6 M) and 5 milliliters of 75° methanol solution in water. The resulting solution was shaken for 2 hours in a water bath with a temperature of 90 degrees Celsius. After leaving the water bath and cooling, the solution was diluted with distilled water to reach a volume of 10 ml. Then, one milliliter of the resulting solution was mixed with 5 milliliters of Folin solution, which was diluted 9 times, and 15 milliliters of sodium carbonate and brought to a volume of 100 milliliters. Next, the absorbance value of the solution at the wavelength of 760 nm was read with a spectrophotometer. In order to draw the calibration curve, a basic solution of gallic acid with a concentration of 100 mg/ml was prepared and different concentrations were prepared using it. In the following, all the above steps were performed by different concentrations of gallic acid (instead of extract) and the absorbance value of the samples was read. Now, according to the obtained numbers, the absorption curve was drawn against different concentrations of gallic acid (mg/ml) and its equation was determined. Next, by putting the absorption value of each sample in the obtained equation, the total amount of phenol in the extract is determined and calculatedmg of gallic acid per gram of dried extract was reported]17[.

2-2-4- Measuring the amount of flavonoid in the whole extract

The total amount of flavonoid compounds of each extract was measured by aluminum chloride colorimetric method. 0.5 ml of the extract was dissolved in 1.5 ml of methanol. Then 0.1 ml of 10% aluminum chloride and 0.1 ml of 1 M potassium acetate solution were added to it. Next, 2.8 ml of distilled water was added to it and kept at room temperature for 30 minutes. Then the absorbance value of the solution at 415 nm wavelength was read with a spectrophotometer. The amount of flavonoid was reported in terms of mg of quercetin per gram of extract [18].

2-2-5- Measurement of free radical inhibition(DPPH)

2,2 Diphenyl-1-picrylhydrazyl is a stable radical compound with purple color, which is converted to yellow diphenylpicrylhydrazine by reduction by electron-donating elements or hydrogen. To measure the free radical scavenging power, 3 ml of each extract extracted in 80% methanol with 1 ml of 1 mM methanolic solution.DPPH The mixture was vigorously shaken, then the obtained mixture was

placed in the dark for 30 minutes at ambient temperature (25-26 degrees Celsius) until the color change took place, and then the absorbance at a wavelength of 517 nm with the device Spectrophotometer was measured. Now whatever the amountDPPH obtained was higher, it showed that the desired extract has more antioxidant potential [19].

$$Inhibition\ (\%) = \frac{(A_0 - A_{30}) \times 100}{A_0}$$

(2)

where in:A_0 = Attracting the witness sample andA_{30}= Sample absorption after 30 minutes.

2-2-6- data analysis

In order to analyze the data obtained from the research, a completely random design was used in three replications and the averages were compared by Duncan's multiple range test at the level of probability.a=5% And using softwareSPSS Version 14 is done.

3- Results and discussion

The results of analyzing the variance of the data obtained from the effect of different treatments on the total extraction efficiency,The total amount of phenolic compounds, total flavonoid content of the extract and free radical inhibition rate are shown in Table 2.

Table2. The variance analysis of data

	TEE			TPC			TFC			DPPH		
	df	MS	F	df	MS	F	df	MS	F	df	MS	F
Treatment	5	28.92	12.52*	5	25.28	10.32*	5	21.01	11.42*	5	40.76	24.12*
Error	12	2.31	-	12	2.45	-	12	1.84	-	12	1.69	-
Total	17	-	-	17	-	-	17	-	-	17	-	-

TEE: Total extraction efficiency (%), TPC: Total phenolic compounds (mg GAE/g dw), TFC: Total Flavonoids compound (mg QE/g dw), DPPH: 2,2-diphenyl-1-picrylhydrazyl (%); **Significant difference at α = 1% probability level.

3-1- Evaluation of the total efficiency of extraction

According to Table 2, the effect of different treatments on the efficiency of extracted extracts is significant ($p\leq 0.01$). Also, according to Figure 1, a significant difference was observed between different treatments in terms of the effect on the total yield of the extracted extract ($p\leq 0.01$). In this regard, the highest (18.3 percent) and the lowest (4.21 percent) extraction efficiency in the treatments, respectivelyP40.E70.W30 AndIN Obtained.

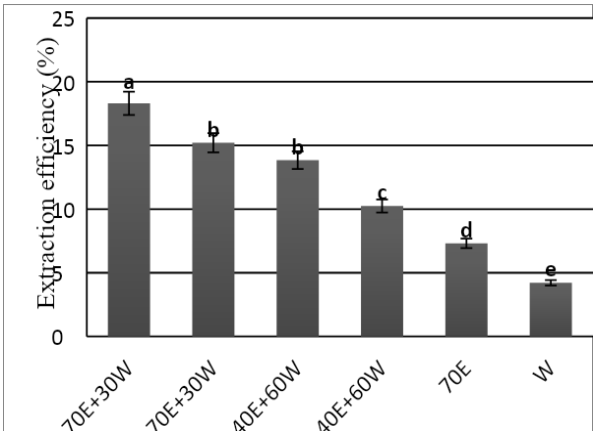

Fig 1. The effect of ultrasound pretreatment with different solvents on the extraction efficiency of *Hypericum perforatum* L. extract

GenerallyThe amount of extraction efficiency depends on various factors such as the type of solvent, compounds in the sample, temperature, time, power and extraction method]20[. According to the results, treatmentsP40.E70.W30 ،P40.E40.W60 ، E70.W30 AndE40.W60 compared to treatmentsE70 AndIN They had a higher extraction efficiency because solvents like ethanol have less polarity compared to water, so by reducing the dielectric constant of the solvent, they cause more dissolution and diffusion of phenolic compounds. Of course, the use of appropriate amounts of water along with organic solvents, in addition to causing the extraction of polar compounds, causes the swelling of plant tissues to increase, as a result of which, the contact between the plant matrix and the solvent increases and the amount of extraction increases.[21 and 22].On the other hand, extraction efficiency increased in all samples treated with ultrasound. BecauseUltrasonic waves improve the process of extracting plant compounds by creating porosity and pores in the walls of cells and facilitate and accelerate mass transfer. Also, the produced cavitation bubble is disintegrated in the vicinity of the surface of the plant material during the contraction cycle and directly introduces the microjet to the surface of the material. The high pressure created during this process tears the cell walls of the plant matrix and releases their contents into the environment.]23[. On the other hand, the use of high power ultrasound increased the temperature of the solution Therefore, the increase in the yield of extracted extract due to the increase in temperature can be due to the improvement of mass transfer as a result of increasing the solubility of the extract and reducing the viscosity of the solvent.]21[.

3-2- Evaluation of the total amount of phenolic compounds

According to Table 2, the effect of different treatments on the total amount of phenolic compounds extracted from Hofarikun plant is significant ($p\leq 0.01$). In this regard and according to Figure 2, the highest amount of phenolic compounds (mg GAE/g dw 0.295) in treatmentP40.E70.W30 and its lowest value (mg GAE/g dw 0.051) in the treatment

containing water solvent (IN) seen. In this regard, a significant difference was observed between different treatments in terms of the effect on the total amount of phenolic compounds in the extracted extract.

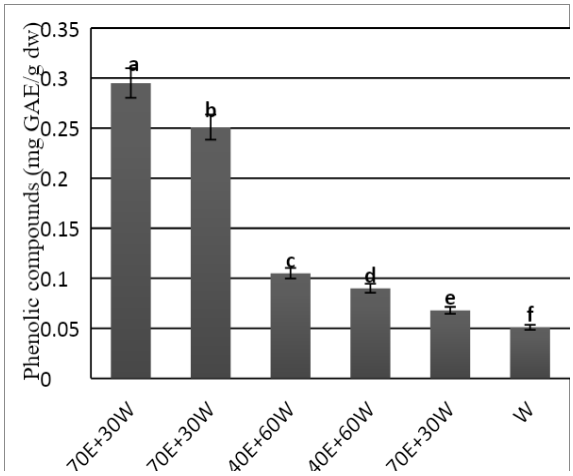

Fig 2. The effect of ultrasound pretreatment with different solvents on the total phenolic compounds of *Hypericum perforatum* L. extract

Phenolic compounds are pentose phosphate and phenylpropanoid derivatives in plants. These secondary metabolitesThey are capable of neutralizing free radicals, forming complexes with metal ions and quenching single and triple oxygen molecules. The extraction of these compounds from plants depends on the solubility of these compounds in different solvents, and the polarity of the used solvents also plays a major role in increasing the solubility of these compounds. The solubility of phenolic compounds varies depending on the type of solvent, their degree of polymerization and their interaction with other compounds in plant tissues.]24[. According to Figure 2, treatmentsP40.E70.W30 ،P40.E40.W60 ،E70.W30 AndE40.W60 compared to treatmentsE70 AndIN They had more ability to extract phenolic compounds. The reason for the result is that the use of water alone creates a completely polar environment, as a result of which some phenolic compounds with a low degree of polarity are less extracted, but adding water to organic solvents creates a relatively polar environment that can change the values and spectrum. extract a wider range of phenolic compounds with different polarities. According to the results of some researches, more phenolic compounds were observed in higher proportions of water than organic solvents.[21 and 22].However, in this study, the amount of phenolic compounds was measured at higher proportions of ethanol, which is also reported in the studies of some other researchers.[25 and 26].It seems that the cause of the result can be attributed to the decrease in the dielectric constant of the solvent due to the increase in the amount of ethanol, which causes the solute molecules to be placed between the solvent molecules more easily and dissolve.]27[. On the other hand, based on Figure 2, the use of ultrasound pretreatment increased the phenolic compounds of the extracted extract. The reason for the increase in the amount of extraction can be attributed to the phenomenon of cavitation. In fact, as a result of the propagation of ultrasound waves in the solid-liquid phase, a cycle of contraction and expansion is created in the environment, and this process causes the formation of bubbles. These bubbles gradually grow and eventually collapse. The high energy of the generated waves causes the cell wall to break and disintegrate, thereby increasing the possibility of releasing the contents of the cell into the

environment.Also, the shearing force created by ultrasonic waves can reduce the size of the particles, so it increases the contact surface of the particles with the solvent and as a result, the diffusion of the solvent inside the particles increases. In summaryThe increase in the extraction rate of phenolic compounds is the intensification of mass transfer caused by the collapse of cavitation bubbles near the cell walls. On the other hand, the use of ultrasound pretreatment, especially at high powers, increases the temperature of the product, and this increase in temperature has an effect on the destruction of the cell wall, and as a result, increases the permeability of the solvent into the cell and, as a result, increases the extracted compounds.]28[. Other researchers have also achieved similar results in their research[13, 14 and 23].

3-3- Evaluation of total flavonoid content of the extract

Flavonoids are derivatives of phenolic compounds that have the ability to form complexes with protein compounds, including enzymes effective in oxidation such as lipoxygenase and alcohol dehydrogenase, and thus disrupt the oxidation process.]15[. According to Table 2, the effect of different treatments on the total amount of compoundsFlavonoid extracted from Hofarikon plant is significant ($p\leq 0.01$). In the same direction and according to Figure 3, the highest amount of compoundsFlavonoid (mg QE/g dw 0.105) in treatment P40.E70.W30 and its lowest value (mg QE/g dw 0.0115) in the treatment containing water solvent (IN) was measured. It is noteworthy that there is a statistically significant difference between all treatments in terms of the amount of compoundsFlavonoids seen.

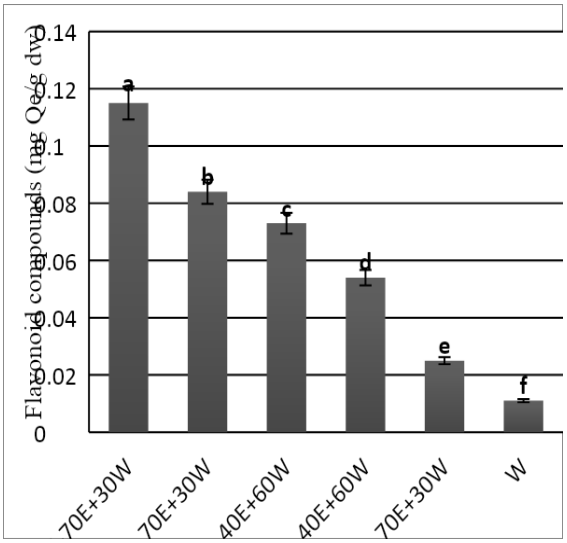

Fig 3. The effect of ultrasound pretreatment with different solvents on the total flavonoid compounds of *Hypericum perforatum* L. extract

According to Figure 3, treatmentsP40.E70.W30 ،P40.E40.W60 ،E70.W30 AndE40.W60 compared to treatmentsE70 AndIN from greater ability to extract compoundsFlavonoids had Because, as previously stated, using water for extraction creates a completely polar environment, as a result compounds with low polarity are extracted less. Also, aqueous extracts contain impurities such as organic acids, proteins, and soluble sugars that interfere with the identification and determination of flavonoid compounds.]29[. On the other hand, in presenting the reasons for the result, it can be said that when water is used alone as a solvent, part of it is absorbed by plant cells, therefore, it causes a decrease in the ratio of solvent to dried material, which results in a decrease in the amount of extraction.]30[. On the other hand, according to Figure 3, the use of ultrasound pretreatment increased the extraction rate of

flavonoid compounds. One of the reasons for the result is that the use of ultrasound at high power increases the temperature of the solution, which can increase the solubility and increase the diffusion coefficient.]25[. Also, the increase in temperature may cause softening and weakening of plant tissues and decrease the resistance of the cell wall, and on the other hand, cause the hydrolysis of bonds between phenol-protein and phenol-polysaccharide compounds, and as a result, cause more extraction. Among the other reasons, we can mention the shear stress resulting from ultrasound waves, which may have caused the breaking of large polymer molecules and, as a result, more favorable extraction of flavonoid compounds.]28[. It is noteworthy that other researchers have also achieved similar results in their research[23 and 24].

3-4- Evaluation of free radical inhibition rate(DPPH)

According to Table 2, the effect of different treatments on the amount of free radical inhibition of extracted extracts was significant ($p\leq 0.01$). Also, according to Figure 4, a significant difference was observed between different treatments in terms of the effect on free radical inhibition ($p\leq 0.01$). In this regard, the highest (84.21 percent) and the lowest (47.53 percent) rate of free radical inhibition, respectively, in the treatmentsP40.E70.W30 AndIN It was measured.

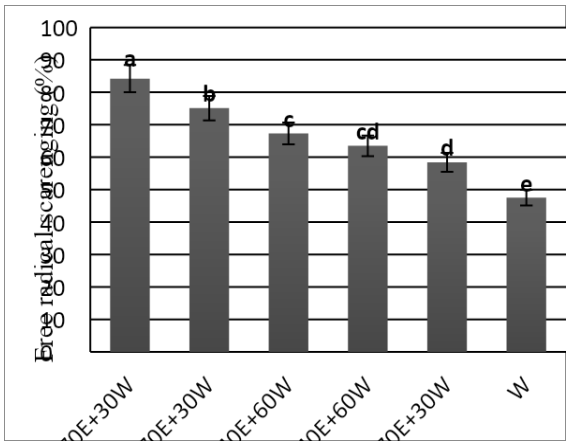

Fig 4. The effect of ultrasound pretreatment with different solvents on the free radical scavenging of *Hypericum perforatum* L. extract

In general, the inhibitory power of different extracts depends on the number and position of hydroxyl groups and the molecular weight of phenolic compounds. In phenolic compounds with lower molecular weight, hydroxyl groups are more easily accessible. In addition, phenolic compounds, after donating their hydrogen, turn into phenoxyl free radicals. The stability of these radicals can affect the antioxidant capacity of phenolic compounds]27[. According to the results, treatmentsP40.E70.W30 ،P40.E40.W60 ، E70.W30 AndE40.W60 compared to treatmentsE70 AndIN They had a higher level of free radical inhibition, which can be attributed to the high level of phenolic compounds extracted by these treatments and the presence of hydroxyl groups in the chemical structure of the phenolic compounds of Hofarikon plant extract. In other words, by increasing the concentration of phenolic compounds or the degree of hydroxylation of phenolic compounds, the radical scavenging activity of the extract also increased.]31[. Many researchers have reported similar results regarding the existence of a high correlation between the

ability to inhibit free radicals and the amount of phenolic compounds.[20 and 32].Of course, in some studies, researchers have mentioned that the relationship between phenolic compounds and the neutralizing power of free radicals is not necessarily a direct relationship because some phenolic compounds have antiradical power.]33[.

4- Conclusion

Extraction and preparation of extracts is considered the first and most important step in the research of medicinal plants. Therefore, choosing a suitable method for extracting bioactive compounds from the plant matrix with minimal impurities and maintaining their bioactive properties is of particular importance. In this regard, in the current study, the effect of different solvents along with ultrasound pretreatment on the extraction efficiency, the total amount of phenolic and flavonoid compounds, as well as the inhibition of free radicals was investigated. According to the results, the highest extraction efficiency (18.3 percent), the total amount of phenolic compounds (mg GAE/g dw 295/0), the total amount of flavonoid compounds (mg QE/g dw 105/0) and the level of inhibition of free radicals (84.21 percent(in samples pre-treated with ultrasound and solvent)30% (water): 70% (ethanol)) was observed. The results show that ultrasound pre-treatment due to the creation of mechanical fluctuations, along with high percentages of ethanol, in a mixed solvent (water/ethanol), can be used as an effective method in extracting and preserving heat-sensitive bioactive compounds of Hofarikon plant.

5- Resources

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تاثیر پیش تیمار اولتراسوند به همراه حلال‌های مختلف بر فعالیت آنتی‌اکسیدانی و مقدار ترکیبات فنلی و فلاونوئیدی عصاره استخراج شده از گیاه هوفاریقون (*Hypericum perforatum* L.)

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چکیده

در پژوهش حاضر تاثیر پیش تیمار اولتراسوند در توان ۴۰ کیلو وات و در مدت زمان ۲۰ دقیقه به همراه حلال‌های تک جزئی آب و اتانل و دو جزیی آب/اتانل به صورت ۳۰٪ (آب):۷۰٪ (اتانل) و ۶۰٪ (آب):۴۰٪ (اتانل)، بر بازدهی استخراج، مقدار کل ترکیبات فنولی و فلاوونوئیدی و همچنین میزان مهار رادیکال‌های آزاد عصاره استخراج‌شده از گیاه هوفاریقون مورد بررسی قرار گرفت. در ادامه به‌منظور تجزیه و تحلیل داده‌ها، از طرح کاملاً تصادفی و در سه تکرار استفاده شد و مقایسه میانگین‌ها توسط آزمون چند دامنه‌ای دانکن، در سطح احتمال $\alpha=1\%$ و با استفاده از نرم‌افزار SPSS نسخه ۱۴ انجام پذیرفت. طبق نتایج، بیشترین میزان استخراج (۱۸/۳ درصد)، مقادیر کل ترکیبات فنولی (۰/۲۹۵ mg GAE/g dw) و فلاوونوئیدی (۰/۱۰۵ mg QE/g dw) و میزان مهار رادیکال‌های آزاد (۸۴/۲۱ درصد) در نمونه‌های پیش تیمار شده با اولتراسوند به همراه حلال دو جزیی آب / اتانل [۳۰٪ (آب):۷۰٪ (اتانل)] و کمترین میزان استخراج (۴/۲۱ درصد)، مقادیر کل ترکیبات فنولی (۰/۰۵۱ mg GAE/g dw) و فلاوونوئیدی (۰/۰۱۱۵ mg QE/g dw) و همچنین میزان مهار رادیکال‌های آزاد (۴۷/۵۳ درصد) در حلال تک جزئی آب (۱۰۰٪) اندازه‌گیری شدند.