Original Article

Laboratory Experiment of Extraction of Active Pharmaceutical Ingredients of Oliveria Decumbens Plant by Supercritical Method Using Ultrasound Process

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ABSTRACT

In this research, the Oliveria decumbensa, a native and medicinal plant has been used. The extract of this plant contains anti-inflammatory compounds, very strong antioxidants, allergic reduction compounds and antiviral and anticancer compounds. Extraction of this plant was investigated via supercritical extraction system using supercritical CO_2 fluid in two stages with ultrasound waves and without ultrasound waves. The research showed that the highest percentage of effective compounds was extracted by supercritical fluid through the ultrasound process. In this study, the extractive effect of two compounds of Octanoic acid and Nonadecanoic Acid as two compounds with the highest drug effect was investigated due to the anticancer and antiviral properties. This amount for the active substance Octanoic Acid in SCF state is 0.02 and in the USCF is 0.14. and for Nonadecanoic Acid in the SCF state is 0.11 and in the USCF state it is 0.86. Also, the extraction in the supercritical device was investigated in both states with ultrasound and without it, at pressures of 190, 170 and 210 bars and the temperatures of 55,45 and 65 Centigrade and frequencies of 37 and 80 kHz. The results showed that the percentage of product (gram of active ingredient / gram of sample) at the pressure of 210 bar and 37 kHz frequency had the highest extraction rate.

Introduction

Oliveria decumbens is an aromatic and fragrant plant which grows frequently and automatically in Iran from the early spring in the plains around Kazerun and Dehdasht, especially the lands and plains of the north and west of the city of Kazerun from the vicinity of the oil well to the plain of Devon, and it is harvested from the late spring to the first two months of the summer.

Oliveria decumbens (Gehanneh or Den) is one of the medicinal herbs that has many medicinal properties, including blood purification and relief of inflammation and bellyache relief.

There are many drug combinations in this plant, some of which are used as anti-inflammatory agents for the treatment of rheumatism, and some of these compounds contain antioxidants and allergic reduction or

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treatment compounds as well as antiviral and anticancer drugs.

Considering the importance of the compounds in this plant, the proper method of extracting them should be applied. Supercritical fluid extraction technology (SFE) is a replacement for conventional methods for extracting and breaking active components. Among the most commonly used supercritical fluids, carbon dioxide (CO_2) is observed, which has advantages in extraction processes, including low cost, non-toxic, non-flammable, stagnant, and appropriate extraction capability.

Solvent extraction is one of the oldest methods of separation, and undoubtedly its use dates back to the BC. The science of solvent extraction has been developed over a long time and has been the most advanced advancement in solvents and fluids used in extraction processes. Extraction techniques such as Sonication, Soxhlet, Solid-phase extraction and Liquid-Liquid extraction which have been invented a long time ago, are also used today, in the same way, to prepare the sample. In addition, methods for extracting with liquid solvents such as Soxhlet have various limitations such as environmental pollution due to the presence of dispersed solvents, incomplete reopening of specimens, time consuming process, high solvent consumption and etc. [1-3]. Conventional separation methods such as solvent extraction and distillation have drawbacks such as leaving small amounts of solvent or thermal degradation [2-4]. Non-traditional methods, which are environmentally friendly are developed over the past years due to reducing the use of chemicals, reducing the operating time, and better performance and high-quality extracts. Therefore, ultrasound methods, pulsed electric field, enzymatic digestion, extrusion, microwave heating, ohmic heating, supercritical fluids, and accelerator solvent have been studied as traditional methods. Ultrasound-assisted extraction (UAE), Microwave-assisted extraction (MAE) and supercritical fluid extraction (SFE) are probably more feasible on a laboratory or industrial scale, though critically criticized by several researchers [5-7].

Thus, the researchers were thinking of developing a new extraction method, which, in addition to not having the above disadvantages, also has several advantages. One of these methods is the supercritical fluid extraction (SFE), which has many advantages, the most important of which is the reduction of extraction time and environmental pollution.

The supercritical fluid is fluid under conditions of temperature and pressure above the critical point. These fluids have high permeability and low viscosity in terms of transition properties such as gases and in terms of solvent power; they are similar to liquid solvents. In an area above the critical point where the fluid is called supercritical, there is a stage in which there is no distinction between the gas and the liquid phase, and the density of the liquid is equal to the density of the gas. Since in scf extraction processes, the pressure is higher than the critical pressure, unlike the operations performed in the liquid phase, the variable pressure is effective in controlling the process. It should be noted that, near the critical point, small changes in pressure and temperature can make a lot of changes in the density field. Due to the very good properties of these fluids, they are extracted and separated for a wide range of materials in purification. Carbon dioxide and water are the most commonly used and most widely used supercritical fluids.

By changing the temperature and pressure, the fluid can be led to a greater degree of fluid or gaseous properties. The most important property of these fluids is their solubility, which is directly related to their density at constant temperature, and as the density increases, this capacity increases, and since density has a direct relationship with pressure, it can be said that their solubility increases with increasing pressure. The relationship between solubility and temperature is a little more complicated [8-10]. In constant density, solubility increases with increasing temperature, while in the vicinity of the critical point, with a slight change in temperature, many changes can be seen in density. Therefore, near the critical point, the solubility of these fluids usually decreases with increasing temperature, and then increases again.

In this research, the aim was to investigate and obtain Oliveria decumbens extracts by supercritical fluid extraction (SFE) with ultrasonic waves. One of the advantages in this method is that the ultrasound technique is based on the formation of high frequency ultrasonic waves that can create holes which have been caused by the expansion and diffusion cycles. Such cycles cause disturbances in the cell walls of the solid matrix, which penetrates the solvent and mass transfer, thus increasing the extraction result. On the other hand, ultrasonic extraction technique is an attractive method because the equipment used is simple and cheap. This device utilizes energy from ultrasound waves (sound waves frequency higher than 20 kHz) to facilitate the extraction of the species by organic solvent from solid samples. This technique has been used to extract various organic compounds from different materials. Increasing the extraction efficiency of organic compounds by ultrasonic is attributed to the formation of a bubble caused by ultrasound passing through the solvent. During the application of ultrasound waves, bubbles are created and compressed. Increasing pressure and temperature leads to bursting and splashing of bubbles. The bursting of bubbles, which triggers pulsation in the solvent, increases the mixing speed [11].

Ultrasonic also provides a mechanical effect and allows the solvent to penetrate more in the sample tissue and increase the contact between the solid and the liquid phase. This action, coupled with an increase in mass transfer and significant cellular degradation by bursting bubbles, results in the release of the compound from the cell to the solvent.

In this perspective, this paper provides a comparative analysis of the technical aspects of these two innovative and eco-friendly techniques: Ultrasound-assisted supercritical extraction (USCF) and supercritical fluid extraction (SFE). With the help of these two methods, the effective materials of the Oliveria decumbens plant were investigated at pressures of 210, 180, 150 bars and also at 65, 55, 45 °C and a mass fraction of 60, 40 and 80 µm.

Figure 1 The image of Oliveria decumbens plant

Materials and Methods

About 200 grams of Oliveria decumbens that were collected from the plains near the city of Kazerun were divided into three parts after drying by smelt particles as fine particles and uniformity of particle size with 40, 60 and 80 micrometers. The supercritical solvent used in this study was carbon dioxide with a purity of %99.

Supercritical Fluid Extraction

The flow diagram of the test device is shown in Figure 2. To enhance purity, CO_2, which is stored in a CO_2 cylinder, passes through a column of molecular sieve seeds. The CO_2 is then cooled down to -15 °C in a cooler and then pressurized by a piston pump. The valve is located at the outlet of the pump, and therefore the CO_2 flow is easily controlled and retained for further use. The carbon dioxide is heated before being introduced into the column using a pre-heating coil. The pressure of the system is controlled and controlled by a return pressure regulator and a high-pressure pump. In the exhaust of the return pressure regulator, a limiting heater will increase the outlet output temperature up to 40 °C, to prevent a potentially serious safety problem caused by the Jolly-Thompson effect, which may result in the freezing of carbon dioxide. The stainless-steel extraction column is made of two sides (height = 23 cm, internal diameter 2.8 cm and outer diameter 5.3 cm). The heat of the exterior wall is heated and pumped by a hot water stream by a heater. This extraction column is located in an ultrasound wavelength bath that produces wavelengths of 37 to 80 kHz and power up to 310 watts.

The sample size used in each step was 5 g. In both cases, using ultrasonic and without it, the effects of pressure were investigated at three pressures of 180, 150, 210 and 55, 45 and 65 °C, and the particle size of 40, 60 and 80 micro meters, and the results are shown in Figures 1 to 3.

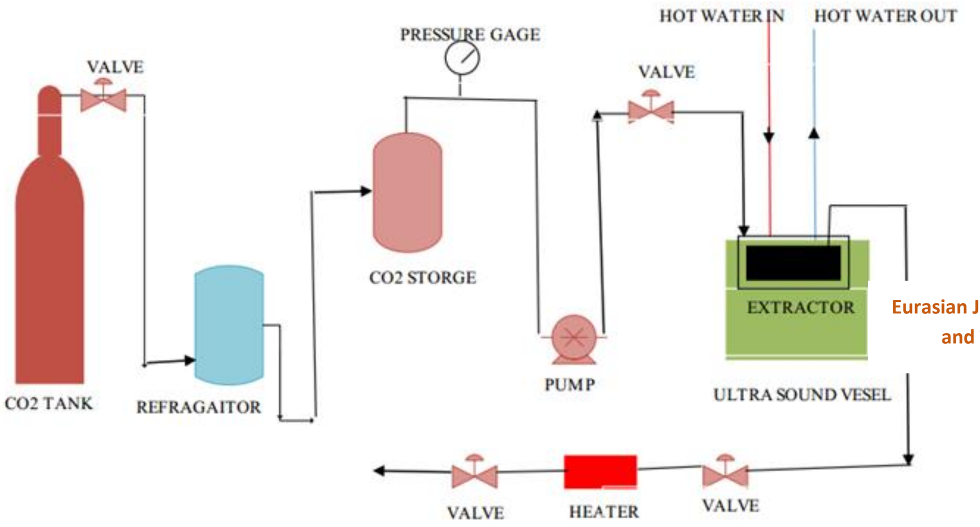


Figure 2 Supercritical Extraction Device Diagram

Ultrasound-Assisted Supercritical Extraction

When the equation is T> Tc, the materials are not able to convert to the liquid by increasing pressure. Condensates (fluid) lose their energy in the ultrasonic state and they tend to separate from each other. Fluids lose their molecular adhesion under ultrasonic conditions and increase their diffusivity over the rest of the material. Therefore, they can penetrate the porous solids more quickly, which is not the case with other solutions. Extracted extracts (Octanoic acid) were at 2% in the first 40 minutes of extraction without ultrasound, while the result obtained at the same time for ultrasound at 100 watts was close to 14%. And for another extracted substance (Nonadecanoic acid) was 11% in no ultrasound state and for ultrasonic was 86%. Over the course of periods of decreasing rates of extraction and reduction of emissions, the application of ultrasonic waves increased the product. As shown in Figure 2, in order to investigate the effect of ultrasound waves on the extraction vessel inside an ultrasound bath (ELMA), made in Germany, an element was produced at 310 watts, which was extracted at frequencies of 37, 80 kHz and 50 and 100 watts [12].

Gas Chromatography Device Specification- Mass Spectrometer (GC / MS)

In this study, the GC-MS system, including the Agilent 5975 C sensor with electron ionization source (EI), was coupled with a 7890 Agilent gas chromatography apparatus, which was used for HP-5MS column with a length of 30 m, internal diameter of 0.25 mm, film thickness of 0.25 µm.

The inlet temperature (INLET) of the gas chromatography system was 28 °C, the temperature of the ionization source of the mass detector was 150 °C, the analyzer temperature was 230 °C, and the temperature of the interface between the GC MS was adjusted to 280 °C. The measurements of the volatile compounds were performed using head space experiment.

The product of both extraction modes, using ultrasound and without it, was tested by the GC device (gas chromatography) and (MS) mass spectrometry and the graphs are shown in Figures 3 and 4.

According to Figure 3 (Sample without ultrasound), we see that in the 30th and 40th minutes, scanning gas chromatography-the mass spectrometry of the first active substances is found. In the 43rd to 49th minutes, the scanned objects by the device tend to climb, but their amount is negligible. In the 51.8 and 52.59 minutes, the two substances studied in this study (Octanoic acid and Nonadecanoic acid) are scanned with percentages specified in the graph. In Figure 4 (Sample using ultrasound), the first active substances are scanned at a significant percentage in the minutes of 10/17 and 16/59, and in the minutes of 27.99 and 28.30, the two substances of Octanoic acid and Nonadecanoic acid are scanned in high concentrations. By comparing these two graphs, the difference between the graphs at the time of scanning and the percentage of scanned substances in two states (using ultrasound and without using it) is clearly visible.

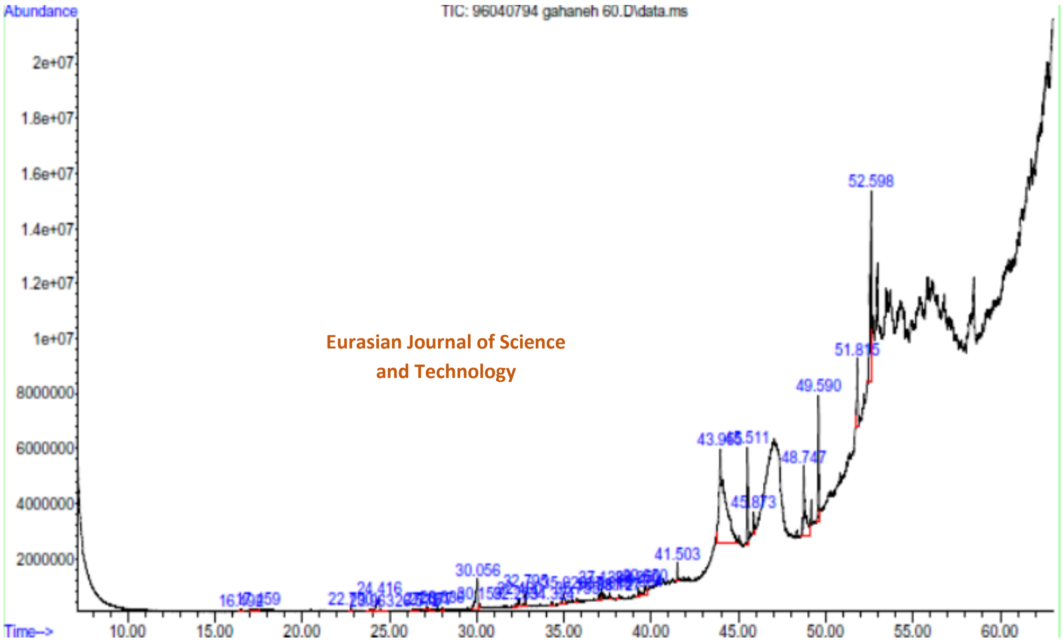


Figure 3 Gas chromatography diagram of Oliveria decumbens extract without ultrasound

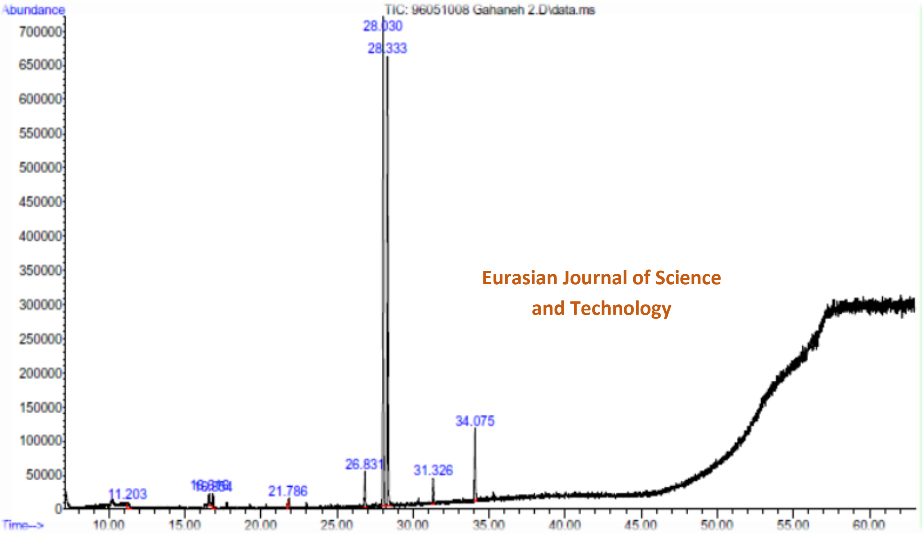
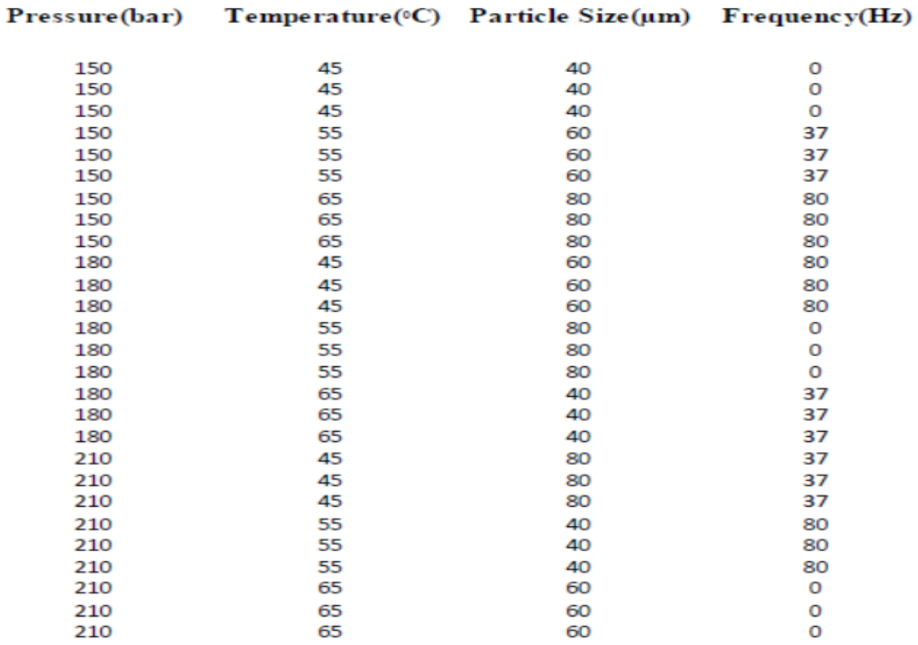


Figure 4 Gas chromatography diagram of Oliveria decumbens extract using ultrasound

Results and Discussion

In this study, the parameters considered were pressure, temperature, particle size and ultrasonic variable frequency. The reason for this is that in extracurricular extraction study, the most fundamental thermodynamic parameters are two parameters of pressure and temperature, which determines the cost of equipment and energy on the one hand as well as the factor where the slightest change in extracurricular extraction determines the quality and quantity of the extraction product. The size of the particle, depending on the type of plant, can play a very significant role in the extraction of the active ingredient. Frequency and its relation to wavelengths are very effective in determining the amount of energy and its effect as an external parameter in extraction. The design of the testing stages is done with the MINITAB software by Taguchi method, which can be considered in Table 1.

Table 1 Designing of Taguchi Test Procedures



Pressure(bar)	Temperature(°C)	Particle Size(μm)	Frequency(Hz)
150	45	40	0
150	45	40	0
150	45	40	0
150	55	60	37
150	55	60	37
150	55	60	37
150	65	80	80
150	65	80	80
150	65	80	80
180	45	60	80
180	45	60	80
180	45	60	80
180	55	80	0
180	55	80	0
180	55	80	0
180	65	40	37
180	65	40	37
180	65	40	37
210	45	80	37
210	45	80	37
210	45	80	37
210	55	40	80
210	55	40	80
210	55	40	80
210	65	60	0
210	65	60	0
210	65	60	0

Pressure Effect

In the study of the effect of pressure, the laboratory conditions are similar to those mentioned above, except that the extraction pressure was considered as a variable and the temperature for the SFE process and for the USFE process was considered at different stages of 45, 55 and 65 ° C. As shown in Figure 5, at pressures between 180 and 210 bars, the extraction efficiency increased explicitly with increasing extraction pressures, and when the pressure for the USFE process was above 150 bars and for the SFE process was higher than 200 bars, the trend was decreasing. This phenomenon shows that the dissolving solubility is highly dependent on the density of the supercritical fluid, which is very similar to the pressure at a temperature range of 45-55 ° C. Therefore, increasing the pressure does not lead to a significant impact on efficiency. In our study, the preferred pressure for the USFE process is 150 bars, which at this pressure has an extraction efficiency of about 93%. In contrast, the preferred pressure for the SFE process is 210 bars, at which the extraction efficiency for components is about 81%. The results in Figure 5 show that in the supercritical extraction process, the presence of ultrasound waves can increase mass propagation, and also show that the ultrasonic effect will be more effective in milder conditions. These results can also be interpreted as follows: In the USFE process, to achieve the same efficiency with the SFE process, the extraction pressure can be reduced.

Figure 5 Effect of extraction pressure on the yield

Temperature Effect

Temperature is a major factor in the extraction of compounds. In general, higher temperatures will result in increased speed and extraction efficiency. However, at high temperatures due to the phenomenon of degradation of compounds, the amount of recycling is reduced. In the study of the effect of temperature on USFE and SFE, the experimental conditions of extraction pressure of 150 and 40 minutes for testing, USFE ultrasound of 37 kHz and W100 were used. When ultrasound is applied, it may occur due to the proliferation of ultrasonic waves of high intensity and local heating effects. If the CO 2 stream is so slow that it cannot remove the heat at the right time, due to the irregular ultrasonic focusing on a particular part of the extractor, clusters of material burnt in the tank are formed. Therefore, CO_2 flow in all USFE tests should be equal to 1.5 L/h or greater. The SFE without ultrasound was also performed for comparison, and all tests were performed at three times with a reset ability of ± 5%. Figure 6 shows that in the USFE and SFE, the effect of temperature on extract extraction is completely different. Each data point in this figure (and other subsequent shapes) is the average value obtained from three experiments. The EY obtained by USFE is higher than the EY obtained by SFE in the same circumstances. This result can be interpreted as in the USFE process, to achieve a similar EY to the SFE process, a much lower temperature is required. On the other hand, the extraction temperature affects the extraction process in two respects: The solubility of propagation or release, and the solubility of solubilization. When the temperature rises, the soluble playback capability increases, so EY improves. However, the effect of the solubility is complicated due to temperature variations. When the temperature rises, the density of the CO_2 decreases, which reduces its solubility; on the other hand, the solvent vapor pressure also increases with temperature, which leads to Increases its ability to dissolve. In general, each solvent-soluble system has a certain apparent behavior against temperature. The optimum points for the USFE and SFE processes reflect the combined effects of temperature on the solubility and the solubility of translucency. Operations performed at a higher or lower temperature than the optimal values will have a lower EY. This conclusion also shows that ultrasound can increase the total mass emission (propagation) within the supercritical system (9).

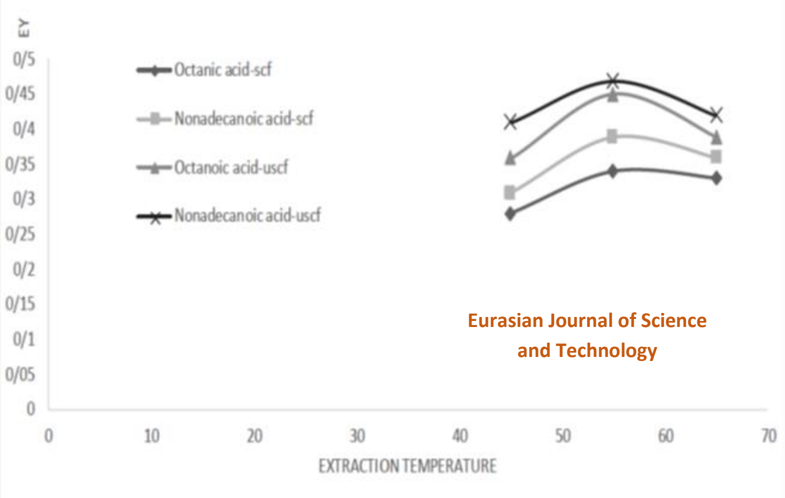


Figure 6 Effect of extraction temperature on the yield

Particle size Effect

The smaller the particle size is, the greater the percentage of recovery will be, but usually an optimal amount should be considered. Increasing the particle size will have a reverse effect on the efficiency of the extraction process or, simply reducing the size of the core, will increase the extraction efficiency. As shown in Figure 7, with increasing particle size, the efficiency is reduced, or the extraction efficiency is proportional to the size of the particle. Several theorists have suggested the idea of increasing the rate of efficiency by reducing the size of the nucleus, U. Salgin et al. (2005) investigated this issue. He considered the effects of in-core infiltration on larger particles far more than small-sized particles, and thus considered resistances in larger particles considerably larger, making them a major factor in reducing the extraction efficiency in larger particles. In general, it can be said that with increasing core size or diameter, the amount of core resistance increases, which reduces the amount of extraction and yield. As the core radius increases, the path to be reached by the extract to reach the surface increases, and as a result, time increases, and the amount of extraction decreases.

Ultrasound Frequency Effect

The increase in the extraction efficiency of organic compounds by ultrasonic is attributed to the formation of a bubble formed by ultrasonification of the solvent. During the application of ultrasound, bubbles are created and compressed. Increasing pressure and temperature leads to bursting and splashing of bubbles. The bursting of bubbles that triggers pulsation in the solvent increases the mixing speed. It is quite clear that the lower the frequency is, the higher the cavity bubbles will be. Therefore, the lower frequencies of ultrasound power waves (About 37 kHz) intensely result in bubble burst and thus are more efficient for extraction processes. If the extraction system has a heterogeneous and complex environment, the impact of the size of the bubbles on the extraction efficiency should be considered. The effect of the frequency may be related not only to the size of the cavitation bubbles, but also may be related to its effect on the internal and external resistance of mass transfer. Although this issue has not been investigated in extraction studies, this work has been carried out in the fields related to drying. In this process, solid structure plays a major role in the effects of using ultrasound waves [13].

Ultrasonic also provides a mechanical effect and allows the solvent to penetrate more in the sample tissue and increase the contact between the solid and the liquid phase. This action, coupled with an increase in mass transfer and significant cellular degradation by bursting bubbles, results in the release of the compound from the cell to the solvent. Using higher temperatures in the ultrasonic technique leads to an increase in the number of bursting bubbles and increases the extraction efficiency. On the other hand, the higher the power of the ultrasound is, the stronger the vibrations will emerge. Therefore, EY will also increase. Under super-critical conditions, static pressure above the tip of the ultrasound detector is usually very high. In such a system, part of the ultrasound power is used to overcome static pressure. Only when the ultrasound power is high enough, turbulent vibrations, which are one of the well-known ultrasonic phenomena, are created [14].

Conclusion

In this study, two SFE and USCF methods under different thermodynamic conditions at pressures of 150, 180 and 210 bars and temperatures of 55, 45 and 65 °C were investigated for the extraction of the effective substance. The results of the study showed that the use of USCF, which increases the mixing speed due to the increase of cavitation bubbles and bubble bursting that triggers pulsation in the solvent, has increased the extraction efficiency up to 12% compared to SFE. Also, the use of ultrasound process has been more effective compared with increasing the pressure to increase extraction efficiency with supercritical fluid. Moreover, in extracting with ultrasound process, the lower frequency and more power, *i.e.*, increasing the size of the cavitation bubbles, have had the best effect on the extraction rate. In the study of the effect of pressure on the use of the ultrasonic process, even at low pressures, the ultrasonic effect was more effective in increasing the efficiency, and the number of compounds scanned by GC / MS gas chromatography was higher and the proportion of their active ingredient compounds was much higher than that used solely by pressure alone. Regarding the effect of temperature in this study, it was also shown that the use of higher temperatures in ultrasonic techniques leads to an increase in the number of bubble burst and increases the extraction efficiency.

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