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Trabajo científico

Ultrasound-assisted extraction of coumarin from *Justicia pectoralis* Jacq

Extracción asistida por ultrasonido de cumarina a partir de *Justicia pectoralis* Jacq

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Abstract

Justicia pectoralis Jacq, Acanthaceae, is used in popular medicine in different countries for the treatment of respiratory diseases and central nervous system diseases. The main bioactive components in the aqueous and hydroalcoholic extract are coumarin and 7-hydroxycoumarin. These conventional extraction methods have some limitations. In the present study ultrasound-assisted extraction is evaluated as a simple and effective extraction method. The influence of the low and high ultrasonic frequency was evaluated. The results showed that the increase of the frequency diminishes the coumarin extraction. This doesn't cause degradation of the coumarin but affected the efficiency of the extraction process among a 15.7% and 30.8%. Finally the ultrasonic extraction does not affect the yield of the drying by spray dried. In conclusion, the best results were obtained when low frequency ultrasound equipment was used.

Resumen

Justicia pectoralis Jacq, Acanthaceae, es usada como medicina popular en diferentes países para el tratamiento de enfermedades respiratorias y del sistema nervioso central. Los principales componentes bioactivos en los extractos acuoso e hidroalcoholico son la cumarina y la 7 hidroxicumarina. Los métodos de extracción convencionales tienen limitaciones. En el presente estudio la extracción asistida por ultrasonido es evaluada por ser un método simple y efectivo. La influencia de la alta y baja frecuencia ultrasónica fue evaluada. Los resultados mostraron que la alta frecuencia disminuye la extracción de cumarina. Esto no causa degradación de la cumarina, pero afecta la eficiencia de extracción entre 15,7% y 30,8%. Finalmente la extracción ultrasónica no afecta el rendimiento en el secado por aspersión. En conclusión los mejores resultados fueron obtenidos cuando empleamos un equipo ultrasónico a baja frecuencia.

Key words: Ultrasound, extraction, spray dried, coumarin, *Justicia pectoralis* Jacq.

Palabras clave: Ultrasonido, extracción, secado por aspersión, cumarina, *Justicia pectoralis* Jacq

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Introduction

A variety of herbs and herbal aqueous and hydroalcoholic extract contain different phytochemicals with biological activity that can or have therapeutic effects. Coumarins are compounds widely distributed in nature. Coumarins have important effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors of toxic substances. The coumarins have been recognized to have anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities. On the other hand, the main metabolite of coumarin is 7-hydroxicoumarin. The hydroxycoumarins are typical phenolic compounds and, therefore, act as potent metal chelators and free radical scavengers.[1]

Justicia pectoralis Jacq. Acanthaceae is used in popular and folk medicine in different countries for the treatment of respiratory tract diseases (asthma, cough and bronchitis) and for the treatment of central nervous system diseases (as a sedative).[2-5] Pre-clinical experimental studies demonstrated that its extracts have a sedative effect.[6-8] While in a clinical trial sedative effect in patients was demonstrated upon administration of the decoction of *Justicia pectoralis.*[9] Dry extract has been used as active pharmaceutical ingredient in liquid and tablet formulation. They are chemical, physical and technologically stable in the time.[10,11]

Extraction techniques are widely employed for the isolation of bioactive substances from natural sources. However, they are usually time-consuming and, unless carefully controlled, are liable to cause degradation or unwanted chemical changes in the products.[12]

Justicia pectoralis extract is usually obtained by solid-liquid extraction with water by reflux process or with hydroalcoholic solution by repercolation extraction, from the plant foliage. The main bioactive components in the extract are coumarin and 7-hydroxycoumarin (umbelliferone).[3,13-15] These conventional extraction methods have some limitations regarding the high energy consumption, the long extraction time and the low of yield of the process. Ultrasound-assisted extraction is evaluated as a simpler and more effective alternative to conventional extraction methods for the extraction of bioactive compounds from natural product.[12,16-19]

When solid-liquid extraction is assisted by ultrasounds an intensification of mass transfer is favored. The mechanical effect of US promotes the release of soluble compounds from the plant body by disrupting cell walls facilitating solvent access to the cell content. This effect is much stronger at low frequencies (18–40 kHz) and practically negligible at 400–800 kHz. If the matrix has been previously dried, US accelerates its rehydration and swelling.[12,20,21]

This method has been applied in the extraction of many chemical constituents due to highly efficient, low energy required and reduced solvent- and time-consuming method.[17] Most of the work related to ultrasound assisted extraction has been carried out by using low frequency ultrasonic horn and ultrasound bath. The ultrasound baths are more widely used because the direct sonication by horn may affect the quality of ingredients being extracted and may prove harmful to thermally sensitive materials as they produce intense cavitation.[22] The present study evaluates the influence of the low and high frequency ultrasound assisted extraction of coumarin from *Justicia pectoralis* Jacq.

Materials and methods

Chemicals and reagents

Ethanol (pa) and methanol (HPLC quality) were from purchased Merck. Coumarin and 7-hydroxycoumarin standard were from Aldrich Chemical Co. HPLC-grade water was purified by use of Milli-Q system (Millipore, Bedford, USA).

Plant material

Justicia pectoralis Jacq (var. pectoralis) were collected in Artemisa province, Cuba. A voucher specimen (Nº 4636) was deposited at Medicinal Plants Experimental Station "Dr. Juan Tomás Roig" in the Drug Research and Development Center (CIDEM in Spanish). Before extraction process the vegetal drug were washed with water and 2% sodium hypochlorite solution and dried in recycled air stove at 45 °C. The vegetal material was ground in a steel grinder to a particle size of less than 0.5 mm. The material was stored in nylon bags until its use.

Conventional extraction methods

Percolation

Percolation is a common method of extraction in which test powder is packed into a percolator, and the solvent is added continuously to percolate through the powder packing and then it is collected. This procedure is used most frequently to extract active ingredients in the preparation of tinctures and fluid extracts.

A glass column (70 cm × 2.5 cm i.d.) was used. A 50 g of pulverized samples were filled in each column. An amount of 300 mL of thirty percent of hydroalcoholic solution was added.

The solid ingredients are moistened with an appropriate amount of the menstruum approximately for 4 h in a closed container. After the mass condenses, the percolator was closed. Additional menstruum is added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h.

After the maceration, the outlet is opened and the solvent is percolated at a controlled rate (2 mL min^{-1}) with continuous addition of fresh solvent. The collected volume was filtered under vacuum a cotton tissue (XX, 2 mm pore size, Filtronic, Brazil) and then the solvent was analyzed.

Reflux extraction

The dried plant material was extracted with water (300 mL) under reflux for 2 h. The ratio of plant material and extracting solvent was 1:20 w/v. The extracts were separated from the residues by filtering under vacuum a cotton tissue (XX, 2 mm pore size, Filtronic, Brazil) and then the solvent was analyzed.

Ultrasound-assisted extraction (US)

The extraction was conducted under certain conditions in the low frequency ultrasonic cleaning bath and in the high frequency ultrasound reactor.

Ultrasound bath

The ultrasound-assisted extraction was carried out in ultrasonic cleaning bath (SAKURA US-5 Japan, inner dimension: 300 mm × 150 mm × 150 mm) with ultrasound power of 150 W, and frequency of 28 kHz, with control temperature of 30 ± 2 ºC. The vegetal drug (15 g) dry and milled was mixed with 300 mL of 30% hydroalcoholic solution. Solid liquid ratio (g mL^{-1}) and extraction time (min) were fixed according to the experimental design. After being extracted, the mixture was filtered under vacuum a cotton tissue (XX, 2 mm pore size, Filtronic, Brazil) and then the solvent was analyzed.

Ultrasonic reactor (horn type)

An US multifrequency generator (Meinhardt Ultraschalltechnik) operating in continuous mode at frequencies of 580, 862 and 1142 kHz and variable electric power output, was connected to a stainless steel transducer (E 805/T/M, Ø 70 mm) and used in the sonication experiments. Reactions were performed in a 0.5 L cylindrical glass reaction vessel (internal Ø 75 mm) with the transducer placed at the bottom of the vessel in direct contact with the solution, at a distance of 68 mm from the liquid surface. Cooling of the reaction mixture was achieved by circulating water through the vessel jacket, so as to maintain an average temperature of 30 ± 1 °C.

A 15 g of vegetal drug was mixed with 300 mL of solvent (water or 30% hydroalcoholic solution according to the experimental design). The ultrasonic extraction was carried out during 60 min. After the extraction, the mixture was filtered under vacuum a cotton tissue (XX, 2 mm pore size, Filtronic, Brazil) and then the solvent was analyzed. Each extraction was performed according to the experimental design.

Analytical methods

High-performance liquid chromatography (HPLC)

The coumarin content was quantified by HPLC coupled with UV detection (HPLC-UV). The analysis was performed with a HPLC instrument Aluspher® 100 (RP-select B 5 mm) pre-column and Lichrospher® 100, RP 18 column (250 x 4 mm, 5µm, Merck, Germany). The chromatography conditions were methanol/water (40:60) as a mobile phase with a flow rate of 1.0 mL min^{-1} and 20 µL of injection sample. The calibration curve (R^2 = 0.999) was made with standard solutions of coumarin (Aldrich Chemical Co.) in the range of 5 ando 40 µg mL^{-1}, the measures were carried out at 274 nm. All analyses were performed in triplicate. The results were expressed as milligrams of coumarin per 100 gram of drug vegetal (mg 100 g^{-1}).[13]

Dry matter content

Dry matter content in the extracts was determined by drying at 105 ºC till constant weight in accordance with the Farmacopeia Brasileira.[23]

pH values

The pH (pHMeter PHSJ-3F) was determined according to Farmacopeia Brasileira.[23]

Experimental design

In the high-frequency ultrasound a factorial design with two variable at three level was used to determine the best combination of ultrasonic frequency (F) and power (P) for the coumarin concentration. The whole design consisted of 13 experimental runs, including five replicates at the centre of the design plan that were used for estimating the reproducibility of experiments. Extraction processes with water and with 30% hydroalcoholic solution were evaluated.

On the other hand, in the low frequency ultrasound a factorial design with two variables at three level was also used. In this case, two extraction variables were considered the sonication time (t) and the concentration of the hydroalcoholic solution (AC).

Effects of sonication time on the release of coumarin

The effect of sonication time on the release of coumarin to optimize extraction process was studied. The best extraction

conditions were considered according to the results of the experimental design. In parallel, an extraction in an ultrasonic bath using water as menstruum was carried out. Coumarin concentration was determined for each extract obtained after 15, 30, 60 and 120 min of sonication time.

In all the cases, the dried vegetal drug (15 g) was mixed with 300 mL of menstruum. After being extracted, the mixture was filtered under vacuum through a cotton tissue (XX, 2 mm pore size, Filtronic, Brazil) and then the solvent was analyzed.

Effects of sonication on the spray drying the extract

The effect of the ultrasonic extraction on the drying of the extract was evaluated. The best ultrasonic extraction process was selected for comparing with the traditional extraction method. The spray drying was carries out according to Rodriguez et al,[24] The extracts were fed to a mini spray dryer B 191 model (Büchi, Flawil, Switzerland). The spray dryer was operated at inlet temperature of 140 ºC and outlet temperature of 80 ºC. The air flow the rate of feeding and the atomization pressure were 600 L h^{-1}, 10 mL min^{-1} and 20 psi, respectively. Drying efficiency was assessed by determining the total powder content obtained in the process and comparing it with the theoretical powder content that can be obtained. The extraction processes and drying were carried out in triplicate. In parallel, the extracts obtained by reflux were dried in equality of conditions.

Statistical analysis

The results were subjected to an analysis of variance (ANOVA) followed by Duncan multiple comparisons post-test. The quality of the model equation was checked by *F*-test for a significance level of 0,05.

Results

Effect of high-frequency ultrasound-assisted extraction of coumarin

Table 1 shows the experimental conditions and the coumarin extraction results according to the factorial design by high-frequency ultrasound.

When the water is used as menstruum, a maximum at coumarin concentration (0,89 g 100 g^{-1} of drug vegetal) was obtained under the experimental conditions of ultrasonic frequency of 580 kHz and power of 1,7 W. Similar results at the same conditions were obtained when the hydroalcoholic solution was used as menstruum.

With the objective of evaluating the influence of ultrasonic frequency and power on coumarin extraction, a regression analysis was accomplished. The result, shown in the Table 2, indicated that the frequency, power, and the relationship between them, significantly affected the coumarin extraction when water is used as menstruum in the extraction process. The coumarin concentration was related with studied variables by the following equation: $Y = 0,745 – 0,0004F – 0,070P – 0,000007F \cdot P$.

On the other hand, the results showed that the frequency and the frequency squared significantly affected the coumarin extraction when hydroalcoholic solution is used as menstruum in the extraction process. The coumarin concentration was related with studied variables by the following equation: $Y = 0,645 – 0,0003F + 0,0000001F^2$.

Table 1. Experimental design for the coumarin extraction by high-frequency ultrasound.

			water			hydroalcoholic solution		
Run	**F (KHz)**	**P (W)**	**Coumarin (g 100g^{-1})**	**pH**	**Dry Matter (%)**	**Coumarin (g 100g^{-1})**	**pH**	**Dry Matter (%)**
1	580	1,7	0,89	4,6	1,22	0,89	6,7	2,34
2	862	1,4	0,78	5,0	1,21	0,82	6,8	2,55
3	1142	1,5	0,70	4,5	1,00	0,80	6,4	1,08
4	580	8,2	0,83	4,8	1,14	0,88	6,8	2,53
5	862	9,9	0,77 ± 0,01	4,7 ± 0,1	1,23 ± 0,05	0,84 ± 0,01	6,9 ± 0,1	2,14 ± 0,10
6	1142	6,4	0,76	5,1	1,21	0,81	6,6	1,16
7	580	17,7	0,80	4,4	1,25	0,87	6,8	2,34
8	862	29,0	0,73	4,9	1,09	0,81	6,6	1,23
9	1142	18,6	0,82	4,9	1,32	0,84	6,6	1,32

Table 2. Parameters of multiple regression analysis of the model for high-frequency ultrasound (F: Frequency (KHz), P: Power (W)).

Water as menstruum				
Source	**Estimation**	**Standard error**	**t-value**	**p-value**
X_0	0,7446	0,0781	9,4	0,0001
F	$-4\cdot10^{-4}$	$1\cdot10^{-4}$	-2,6	0,0380
P	-0,0070	$2{,}53\ 10^{-3}$	-2,7	0,0322
F^2	$2\cdot10^{-7}$	$1\cdot10^{-7}$	1,9	0,1060
P^2	$4\cdot10^{-5}$	$4\cdot10^{-7}$	2,1	0,0984
F·P	$3\cdot10^{-6}$	$2\cdot10^{-6}$	2,6	0,0424
Hydroalcoholic solution as menstruum				
Source	**Estimation**	**Standard error**	**t-value**	**p-value**
X_0	0,6448	0,0494	13,1	0,0000
F	-0,0003	$1\cdot10^{-4}$	-2,8	0,0301
P	0,0001	$1\cdot10^{-4}$	0,1	0,9465
F^2	$1\cdot10^{-7}$	$6\cdot10^{-8}$	2,6	0,0429
P^2	$1\cdot10^{-8}$	$7\cdot10^{-11}$	2,6	0,8896
F·P	$1\cdot10^{-6}$	$1\cdot10^{-6}$	0,8	0,4553

The lack of fit measures the failure of the model to represent the data in the experimental domain at point, which are not included in the regression. As showed in table 2, F-value and p-value of the lack of fit indicate that it was not significant relative to the pure error. This indicates that the model equation was adequate for predicting the concentration of extrated coumarin under any combination of variable values. The value of R^2 was 82,6% (water as menstruum) and 80,9% (hydroalcoholic solution as menstruum). However the low value of coefficient of variation (CV) showed a better precision and reliability of the carried out experiment. It confirmed that the model was highly significant and indicated a high degree of correlation between the observed and predicted data.

Table 3 show the analysis of variance (ANOVA) for the model of extraction of coumarin from *Justicia pectoralis*. The p-value lower that 0,05 indicating this model was significant in both studied designs.

An analysis of the obtained results in the determination of dry matter content showed that this parameter is in the accepted range (> 1,0% for coumarin extracts). However, when evaluating the behavior of the pH, a general decrease of this parameter is observed when the extraction is done with water (pH ≤ 5). On the other hand, a qualitative analysis by HPLC demonstrated that the coumarin was not oxidized at the pH-value (Figure 1). In the hydroalcoholic solution extraction, this pH variation was not observed.

Table 3. Analysis of variance for the model of coumarin extraction by high-frequency ultrasound.

Water as menstruum					
Source	**Sum of Squares**	**Degree freedom**	**Men square**	**F-value**	**p-value**
Model	0,0051	4	0,0013	7,11	0,0184
Residual	0,0011	6	0,0002		
Lack of fit	3,0470	3	1,0157	0,02	0,9951
Pure error	0,1028	2	0,0051		
Total Corr	0,0063	10			
R^2 = 82,6 % **CV** = 1,63 %					
Hydroalcoholic solution as menstruum					
Source	**Sum of Squares**	**Degree freedom**	**Men square**	**F-value**	**p-value**
Model	0,0018	4	0,0004	6,38	0,0237
Residual	0,0004	6	0,0001		
Lack of fit	3,8591	3	1,2863	0,53	0,8140
Pure error	0,0982	2	0,0491		
Total Corr	0,0023	10			
R^2 = 80,9 % **CV** = 1,05 %					

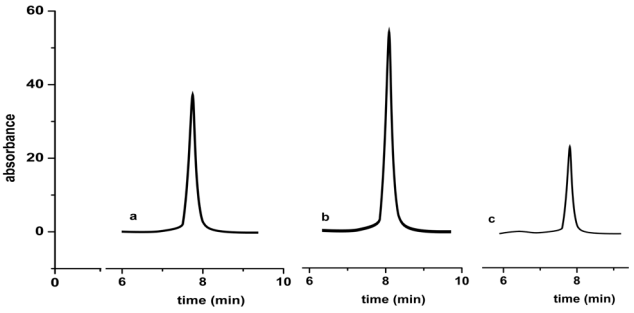

Figure 1. Chromatograms analysis by HPLC. (a): coumarin standard (tr = 7.70 min), (b): 7 hydroxycoumarin standard (tr = 8.08 min) and (c): extract of Justicia pectoralis (tr = 7.70 min).

Effect of low-frequency ultrasound-assisted extraction on coumarin

Table 4 shows the experimental conditions and the results coumarin extraction according to factorial design by low-frequency ultrasound. A maximum of coumarin concentration (1,46 g 100 g^{-1} of drug vegetal) was obtained for 50% hydroalcoholic solution at 120 min of sonication time.

Table 4. Experimental design for the coumarin extraction by low-frequency ultrasound.

Run	AC (%)	t (min)	Coumarin (g 100g^{-1})	pH	Dry Matter (%)
1	30	15	1,04	6,7	0,72
2	50	15	1,28	7,0	0,89
3	70	15	1,22	6,8	1,22
4	30	68	1,27	7,1	1,27
5	50	68	1,37 ± 0,04	7,2 ± 0,1	1,06 ± 0,09
6	70	68	1,44	7,0	1,44
7	30	120	1,32	7,2	1,32
8	50	120	1,46	7,5	1,46
9	70	120	1,39	7,0	1,39

The regression analysis demonstrates that the coumarin extraction was significantly affected by the sonication time ($p = 0,0028$) (Table 5). The coumarin concentration was related to ethanol concentration and sonication time by the following equation: $Y = 0,4093 + 0,004t$. The model was adequate for predicting the coumarin extract (Table 6) under any combination of values of the variable. The value of R^2 was 91,4% and the value of coefficient of variation (CV) was 1,38%.

Table 5. Parameters of the multiple regression analysis of the model of coumarin extraction by low-frequency ultrasound (AC Alcoholic concentration (%), t: Sonication time (min)).

Source	Estimation	Standard error	t-value	p-value
X_0	0,6097	0,0396	15,4	0,0023
AC	0,0008	0,0007	1,2	0,1352
t	0,0014	0,0003	5,5	0,0028
AC^2	$6,9 \cdot 10^{-8}$	$6,5 \cdot 10^{-8}$	2,0	0,3259
t^2	$2,04 \cdot 10^{-8}$	$1,53 \cdot 10^{-8}$	22,4	0,0767
AC·t	$1,19 \cdot 10^{-8}$	$9,5 \cdot 10^{-8}$	15,6	0,0773

Table 6. Analysis of variance for the model of coumarin extraction by low-frequency ultrasound.

Source	Sum of Squares	Degree freedom	Men square	F-value	p-value
Model	0,0354	2	0,0177	15,68	0,0010
Residual	0,0124	11	0,0011		
Lack of fit	0,0033	3	0,0011	6,91	0,0815
Pure error	0,0008	5	0,0002		
Total Corr	0,0478	13			
R^2 = 89,3 % **CV** = 1,38 %					

The pH and dry matter are in the accepted range.

Effects of sonication time on the release of coumarin

Coumarin content in the extract obtained by percolation was 1,45 ± 0,09 g 100 g^{-1} drug material, while the coumarin content in the extract obtained by reflux extraction was 1,20 ± 0,01 g 100 g^{-1} drug material. These results were similar to those obtained by repercolation method (4 extractions, drug/solvent ration 1:20, 30% hydroalcoholic solution as menstruum) and water decoction method (drug/solvent ration 1:20 and an extraction time of 15 minutes) reported by Rodriguez et al.[13]

Figure 2 shows the effects of sonication time on the release of coumarin. The maximum concentrations of coumarin extraction were achieved after 30 min of sonication in all experiments and starting from that time the concentration remains constant. It can be seen that the concentration of coumarin was higher when the low-frequency ultrasound was used (15,7% when water was used as menstruum and 30,8% when hydroalcoholic solution was used as menstruum).

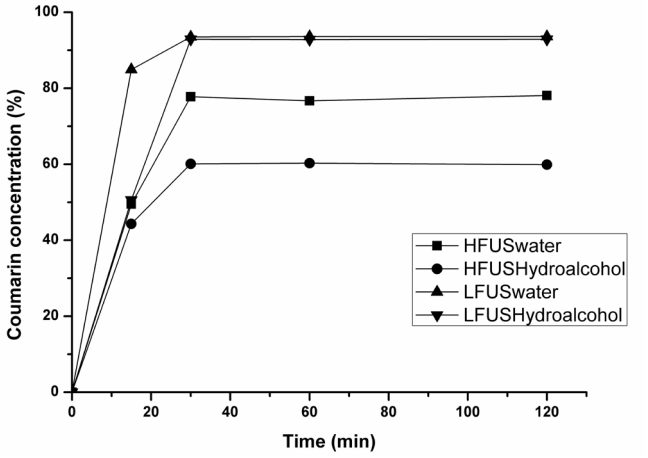

Figure 2. Effects of sonication time on the release of coumarin for best extraction conditions according to experimental design. (HFUS: high frequency ultrasound and LFUS: low frequency ultrasound)

After 30 min the concentration was higher at 92,5% independently to the used menstruum for low-frequency ultrasonic and lightly lower (about 7,5%) to the concentration obtained by the traditional methods evaluated in this study.

These results confirm that the employment of high-frequency causes a significant decrease of the concentration of coumarin extraction from *Justicia pectoralis*.

Effects of sonication on the spray dried of the extract

Table 7 shows the results of the spray drying of the obtained extracts. In all cases a fine and amorphous powder of brown

colour was obtained. Coumarin content was more than 2,85 mg g^{-1} dry extract. The yields went higher to 75%, considered appropriate for the working scale.

Table 7. Results spray drying by spray extracts obtained by different methods (Similar letter no significant for p < 0.05).

Extraction method	Yield (%)	Coumarin content (mg g^{-1} powder)
Reflux	76,2 ± 0,12 [a]	2,90 ± 0,09 [a]
Low-frequency ultrasound	75,0 ± 0,16 [a]	2,88 ± 0,10 [a]
High-frequency ultrasound	77,1 ± 0,09 [a]	2,85 ± 0,18 [a]

Discussion

Several studies about ultrasonically assisted extraction have reported that when high frequency ultrasound is employed, the extraction yield did not increase significantly, causing in many cases a degradation process of the main metabolites.[25]

In this study, the results of the application of high-frequency ultrasound showed that the increase of the frequency diminishes the coumarin extraction. It is also observed a decrease of the pH in the sample extracted with water. This can be motivated by the process of dissociation of water.

It is known that when high amounts of water (higher than 60%) is employed in the extraction process by high frequency ultrasound an increase of the free radicals production (highly reactive species such as hydroxyls, hydroxyperoxyl and hydrogen of peroxide) caused by the dissociation of water was observed. In presence of these high-energy species, oxidative reactions can cohabit with the extraction reactions, decreasing the amount of target compounds available in the matrix for extraction, consequently decreasing the extraction efficiency.[26,27] On the other hand, the presence of these radicals in the reaction medium can be the cause of the observed pH decrease. These results did not cause degradation of the molecule of interest, but affected the efficiency of the extraction process among a 15,7% and 30,8% depending on the used menstruum.

The physical effects that cause the cavitation process are dominants when working at low frequency. Due to this problem, the use of low-frequency ultrasound for the extraction of phytochemicals from plants is recommended. On the other hand, the effectiveness of the ultrasound-assisted extraction depends on the solvent's capacity for absorbing and transmitting the energy of the ultrasound.[26-30]

Polarity of the solvent is another important characteristic in the extraction efficiency. Water is the most widely used solvent due to its ecological and low cost, but often the water may not be able to extract the full content. In many cases, the presence of organic solvents such as ethanol is needed.[26-30]

In this study the best results were obtained when low frequency ultrasound was used. Differences among the process of water low frequency ultrasonic extraction and hydroalcoholic solution low frequency ultrasonic extraction were not observed. Also, the obtained yields are similar to the yields obtained by the traditional methods studied in this work. Finally the ultrasonic extraction does not affect the yield of the drying, as well as the physicochemical characteristics of the powder obtained.

Conclusion

In conclusion, the best results were obtained when low frequency ultrasound equipment was used. Under these conditions, differences among the coumarin yields extracted with water or with hydroalcoholic solution were not observed. The ultrasonic extraction doesn't affect the yield and the characteristics of the powders obtained by spray dry.

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