The effects of extraction method on recovery rutin from *Calendula officinalis* L. (Asteraceae)

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ABSTRACT

Background: *Calendula officinalis* L. (Asteraceae) is a Mediterranean specie, but in Europe and America it is cultivated for ornamental or medicinal purposes. This species is widely used for presenting activities, antiinflammatory antibacterial and antioxidant. However the therapeutic action is linked to the amount of assets of the extracted raw material. The extraction method of bioactive compounds is an important step in the manufacturing of herbal medicines, because secondary metabolites with therapeutic potential are usually found in small quantities in plant materials. **Objective:** Due the medical and commercial importance of *C. officinalis*, this study aimed to evaluate the impact of the extraction method on the quality of herbal extract and optimize the extraction of rutin from *C. officinalis.* **Materials and Methods**: The extraction of rutin was performed by ultrasound and shaker and the optimized conditions were determined by response surface methodology. **Results:** The results of ultrasound extraction assisted (UEA) and maceration dynamic (MD) showed that rutin yield ranged from 0.218 to 2.28% (w/w) when extract by ultrasound and 0.1-1.44% by MD. The optimal extraction condition for rutin (2.48% to UEA or 1.46% to MD) from *C. officinalis* by UEA or MD were a 19-22 min extraction, ethanol: water ratio of 35-40% and 0.05-0.056 mg/mL to raw material: solvent ratio. **Conclusion:** The UEA is more efficient to extraction rutin.

Key words: Factorial design, optimization of extraction, rutin

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INTRODUCTION

Calendula officinalis L. (Asteraceae) is a Mediterranean specie, but in Europe and America it is cultivated for ornamental or medicinal purposes.[1] This species is widely used for presenting activities, antiinflammatory,[2] antibacterial[3] and antioxidant.[1] Phytopharmacological studies of *C. officinalis* extracts have shown antitumoral activities.[4] In clinical studies, the extract of *C. officinalis* was efficacious in the prevention of acute dermatitis caused in patients treated with irradiation ultraviolet (UV).[4] Phytocosmetic from *C. officinalis* is indicated for the treatment of acne, eczema, abscesses and impetigo, and prevention of diaper rash in children and as protector against UV A and UV B.[1] The therapeutic action of *C. officinalis* that is explained by the presence of flavonoids and especially by the presence of rutin.[1-4]

However the therapeutic action is linked to the amount of assets of the extracted raw material. The extraction method of bioactive compounds is an important step in the manufacturing of herbal medicines, because secondary metabolites with therapeutic potential are usually found in small quantities in plant materials. For such industries use different methods of extraction as: (1) Heating maceration, (2) refluxing, (3) soxhlet extraction, (4) supercritical fluids, (5) ultrasonic baths and (6) percolation.[5-7]

In conventional extraction methods the extraction of rutin is realized by heating, boiling or refluxing. These methods have any disadvantage as loss rutin due ionization, hydrolysis and oxidation during extraction.[8] The ultrasound assisted extraction (UAE) is a widely used method for the extraction of chemical markers from raw materials,[7,9,10] because of their advantages over other extraction technologies, including operational flexibility, low cost, reducing

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extraction time, increasing maximum extraction yields and applicability for heat-sensitive materials.[7,11,12]

Despite the medicinal importance of rutin, the associated processing factors and extraction methods have received little attention. Accordingly, a study to elucidate the effects of processing factors on extract properties is fully justified. In this sense, the present study aimed to evaluate the influence of the extraction method over the quality of extracts of *C. officinalis*.

MATERIALS AND METHODS

Herbal material

The flowers *C. officinalis* L. were acquired from BioTae Extratos Vegetais (Batch: 12.860).

Reagents and chemicals

Rutin (>99%) and ethanol (95% v/v) ware purchased from Sigma-Aldrich® (Sigma-Aldrich Co., Steinheim, Germany). The acetonitrile high performance liquid chromatography (HPLC) grade was purchased from Merck (Merck KGaA, Darmstadt, Germany).

Characterization of herbal material

The raw material was characterizes in accordance the parameters of 5th Brazilian Pharmacopeia.

High performance liquid chromatography-photodiode array detector rutin analysis

High performance liquid chromatography analyses of herbal extracts and powered roots were performed using a waters HPLC system (Alience), e2695 separation module, e2998 photodiode array detector, and Empower 3 data processing system (Waters®).

The following analysis conditions were used: A C18 reverse phase column X-Bridge 250 × 4.6 mm Waters®, an acetonitrile: metanol: water (30:2:68) mobile phase, a flow rate of 0.5 mL/min, and detection wavelengths of 254 nm. The analytical method was validated according International Conference on Harmonisation to guideline Q2 (R1).[13]

Evaluation of degradation of rutin by ultrasound

A previous study of stability was done with rutin solution (1 mg/mL), it was kept for 25 min in ultrasound bath (37°C) (USC 1400, Unique®). A control solution in the same concentration was made and the areas of chemical marker were compared by HPLC.

Experimental design extraction by ultrasound-assisted extraction and by maceration dynamics

The ultrasound assisted extraction (UEA) was performed in an ultrasonic bath (USC 1400, Unique® - 50/60 Hz) and was used flask volumetric (25 mL) with 50 mg of powered flowers and the 25 mL of hydroethanolic mixture. The flask volumetric was partially immersed in the ultrasonic bath and submitted to ultrasound energy for specific time. The extracts obtained were filtered and then analyzed by HPLC. Maceration dynamics (MDs) was performed in shaker (45 rpm) (Eppendorf®) and was used flask volumetric (25 mL) with 50 mg of powered flowers and the 25 mL of hydroethanolic mixture.

The influence of extraction method on rutin yield (*Ry*) was evaluated using a factorial drawing 3^3 (Box-Behnken) with 15 experimental runs, including three replicates at the center point. The factorial design matrix contained extraction time (min, *Et*), ethanol: water ratio (v/v%, EW_r) and drug solvent ratio (mg/mL, *DSr*) are shown in Table 1. Experimental data were fitted to a polynomial model and regression coefficients obtained [Equation 1].

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum\sum \beta_{ij} x_i x_j \quad [1]$$

Where *y* is the dependent variable; β_0 is the constant term; *k* number of variables; β_i represents the coefficients of linear parameters; β_{ii} represents the coefficients of quadratic terms; β_{ij} represents the coefficients of interaction parameters. The Design expert 7.0 Stat-Ease, Inc. software was used to generate response surfaces. In order to verify the predictive capability of the model, optimum conditions were established by response surface methodology (RSM) and comparisons between the predicted results and the practical values were done by experimental rechecking using those presumed optimal conditions.

Optimization of extraction parameters

The optimized conditions were determined by RSM and the criterion of desirability was the maximum extraction of rutin.

RESULTS AND DISCUSSION

It can be observed that all the system suitability parameters were in accordance with the literature specifications [Table 2]. Thus, the HPLC system and procedure showed to be

Table 1: Coded factors and respective levels in the factorial design

Factors	Level		
	−1	0	+1
Et (min)	5	20	35
EW_r (% v/v)	35	65	95
RS_r (mg/mL)	0.02	0.04	0.06

Et: Extraction time; EW_r: Ethanol: water ratio; RS_r: Raw material: Solvent ratio

capable of providing data of acceptable quality. Performing the selectivity test, it was found, for all samples, that there was no compound interfering with the retention time of rutin (25 min). Table 3 resumes the parameters values obtained from method validation, the calibration curves showed a linear response obtaining correlation coefficients (*r*) 0.998. Limit of detection (0.02 μg/mL) and limit of quantitation (0.18 μg/mL) showed that the present method has adequate sensitivity to detect and quantification of rutin in *C. officinalis*.

The stability study show that content rutin was not altered by the action of ultrasound, there was a range of <0.5% between the sample content and the control. The results of UEA and MD experiments are summarized in Table 4. Under the established conditions, the *Ry* ranged from 0.218% to 2.28% (w/w) when extract by ultrasound and 0.1-1.44% by MD. The higher extraction yields obtained by the ultrasound-assisted method may be attributed to the effects of acoustic cavitation produced in the solvent. The ultrasonic wave also exerts a mechanical effect, allowing greater penetration of the solvent into the herbal matrix, which increases the contact surface between the solid and liquid phases and encourages the solute to diffuse from the solid phase into the solvent.[12,14,15] Several authors have reported high efficiencies for the ultrasound-assisted extraction of foods and bioactive compounds.[12,14,15]

The tables with complete ANOVA for each dependent variable and RSM analysis are listed in Table 5.

The model's lack of fit F = 1.73 (UEA) and 1.21 (MD) implied the model's lack of fit was not significant relative to pure error, as there was a 38.2% chance that a lack of fit F-value this large could occur due to noise. The nonsignificance of the lack of fit F-value indicated the validity of the regression model. The adjusted R^2 for the equation was close to unity (R^2 = 0.94 to UEA) and (R^2 = 0.83 to MD), indicating a high correlation between the observed and predicted values.

Three-dimensional response surface plots are presented in Figure 1a showed that *Et and* DS_r had a positive influence on *Ry* UEA, *Et* and EW_r had a positive influence *on Ry* MD. An R^2 value (multiple correlation coefficient) closer to one denotes better correlation between the observed and predicted values. In this case, the high values of *r* indicate good correlation between the experimental and predicted values [Equations 2 and 3].

$$Ry_{(UEA)} = +0.97 + 0.35xEt - 0.52xEW_r \quad [2]$$

$$Ry_{(UEA)} = +0.55 + 0.0.38xEt - 0.012xEWr + 0.035xRSr \quad [3]$$

Table 2: System suitability parameters values to rutin from *C. officinalis*

Parameter	Rutin	Recommendations
Repeatabilitya	<0.1%	RSD≤1% to *n*≥5
Tailing factor (*T*)	1.6	<2.0
Theoretical plates (*N*)	16614	>2000
Resolution	2.3	>2.0

[a]Data expressed as mean±SD. SD: Standard deviation; RSD: Relative standard deviation, *C. officinalis: Calendula officinalis*

Table 3: Validation parameters values obtained from HPLC-PDA method for the determination of rutin from *C. officinalis*

Parameter	Rutin
Linearity (μg/mL)	
Linearity range	1-20
Sensitivity (μg/mL)	
LOD	0.02
LOQ	0.18
Precision (%)	
RSD	<0.1
Accuracy (%)	
Recovery 80	100.11%±0.07[a]
Recovery 100	100.35%±0.81[a]
Recovery 120	99,8%±0.51[a]
Robustness (%)	
Changing column mark/RSD	<0.1
Temperature of column/RSD	
Ratio of solvent/RSD	

[a]Data expressed as mean±SD. RSD: Relative standard deviation, SD: Standard deviation, LOD: Limit of detection, *C. officinalis: Calendula officinalis*, HPLC: High performance liquid chromatography, PDA: Photodiode array, LOQ: Limit of quantification

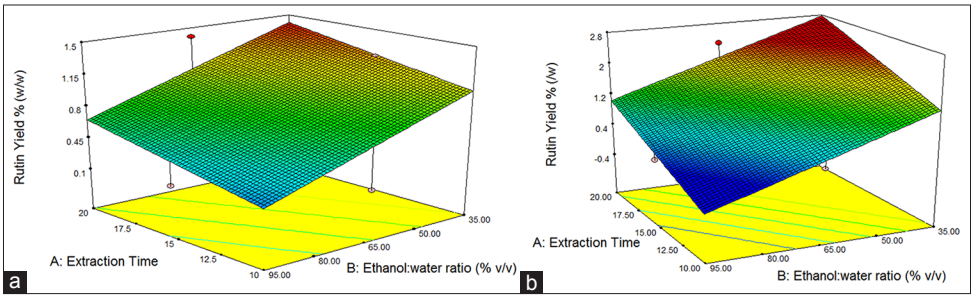

Figure 1: (a) Surface response of extraction of rutin by ultrasound extraction assisted and (b) maceration dynamic

Table 4: Box-Behnken factorial design matrices and result of UAE

Et	EW_r	DS_r	Yield (%)	
			UAE	MD
15	65	0.04	1.37	0.9
15	65	0.04	1.16	0.85
20	95	0.04	0.5	0.3
20	65	0.06	2.28	1.4
20	35	0.04	1.61	0.92
20	65	0.02	0.83	0.65
15	95	0.02	0.62	0.32
10	65	0.06	0.41	0.19
10	65	0.02	0.17	0.1
10	95	0.04	0.34	0.11
15	95	0.06	0.21	0.2
15	35	0.02	0.86	0.39
15	35	0.06	1.88	1.2
15	65	0.04	0.78	0.81
10	35	0.04	1.46	1.44

UAE: Ultrasound assisted extraction, MD: Maceration dynamic, *Et*: Extraction time, EW_r: Ethanol: water ratio, DS_r: Drug solvent ratio

Table 5: Summary of factor effects and significances (*P*) ANOVA

Factors	UEA				MD			
	SQ	df	*F* value	*P* value *P>F*	SQ	df	*F* value	*P* value *P>F*
Intercept	1.27	3	7.31	0.0057	0.56	3	5.14	0.0183
Et	1.01	1	5.78	0.0349	0.29	1	2.67	0.13
EWr	2.14	1	12.31	0.0049	1.14	1	10.42	0.008
RSr	0.67	1	3.84	0.0757	0.26	1	2.34	0.154
Residual	1.91	11						
Lack of fit	1.73	9	1.62	0.43	1.21		1.28	0.35

*Significant *P*<0.005. UAE: Ultrasound assisted extraction, MD: Maceration dynamic; *Et*: Extraction time; EW_r: Ethanol: water ratio; RS_r: Raw material: Solvent ratio

The optimal theoretical extraction parameters for rutin (2.48% to UEA or 1.46% to MD) from *C. officinalis by* UEA or MD were a 19-22 min extraction, ethanol: water ratio of 35-40% and 0.05-0.056 mg/mL to raw material: solvent ratio. The verification test showed that the *Ry* contents obtained from extraction under optimal conditions were 2.37 ± 0.09% w/w (n = 3) to UEA and 1.31 ± 0.06% w/w (n = 3) to MD. The good correlation between the theoretical results and the rechecked values confirmed that the response model represented the expected optimization well.

As seen in Figure 1, the efficiency of extraction could be increased at times of extractions >35 min. However when conducting experiments with times of 40 and 50 min, the extraction of rutin (2.52 ± 0.03% to UEA and 1.49 ± 0.02% to MD) did not increasing. Previous studies by Paniwnyk *et al*.[16] reported the use of ultrasonic bath, reduced the extraction time of rutin from *Sophora japonica* L. (Fabaceae), but In according Virot *et al*., long time of extraction by ultrasound decrease the rutin content, because the there are formation of hydroxyl radical species that can oxidize rutin extracted.[10]

CONCLUSION

In the extraction processes there are multiple independent variables interacting with responding factors. Optimization studies are important for cost reduction, process time, energy, raw materials and therefore environmental impacts. The method of extraction is another determining factor, the ultrasonic extraction is efficient, however with prolonged periods may degrade rutin.

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