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

Frederico Severino Martins, Edemilson Cardoso da Conceição¹, Elane Sousa Bandeira², José Otávio Carréra Silva Junior², Roseane Maria Ribeiro Costa²

Departments of Pharmaceutical Sciences, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, ¹Faculty of Pharmacy Research, Federal University of Goiás, ²College of Pharmacy, Federal University of Para, Pará, Brazil

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**ABSTRACT**

**Background:** *Calendula officinalis* L. (Asteraceae) is a Mediterranean species, but in Europe and America it is cultivated for ornamental or medicinal purposes. This species is widely used for presenting activities, anti-inflammatory, 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

**INTRODUCTION**

*Calendula officinalis* L. (Asteraceae) is a Mediterranean species, but in Europe and America it is cultivated for ornamental or medicinal purposes.[1] This species is widely used for presenting activities, anti-inflammatory, antibacterial[2] and antioxidant.[1] Phyto-pharmacological studies of *C. officinalis* extracts have shown antimicrobial 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).[6] Phyto-cosmetic 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,[6,9,10] because of their advantages over other extraction technologies, including operational flexibility, low cost, reducing
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) were 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 characterized 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: methanol: 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 ultrasonic 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 3³ (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*), and drug solvent ratio (mg/mL, *RS*) are shown in Table 1. Experimental data were fitted to a polynomial model and regression coefficients obtained [Equation 1].

\[
y = \beta_0 + \sum_{i=1}^{k} \beta_{i} x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}^{k} \beta_{ij} x_i x_j
\]

Where *y* is the dependent variable; *β₀* is the constant term; *k* number of variables; *β* 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

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

| Factors | Level |
|---------|-------|
|         | −1    | 0    | +1    |
| *Et* (min) | 5     | 20   | 35    |
| *EW* (% v/v) | 35    | 65   | 95    |
| *RS* (mg/mL) | 0.02  | 0.04 | 0.06  |

*Et*: Extraction time; *EW*: Ethanol: water ratio; *RS*: 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 Calendula officinalis.

The stability study showed 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]

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

| Parameter                   | Rutin Recommendations |
|-----------------------------|-----------------------|
| Repeatabilitya              | <0.1%                 |
| Tailing factor (T)          | 1.6                   |
| Theoretical plates (N)      | 16614                 |
| Resolution                  | 2.3                   |

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

| Parameter       | Rutin |
|-----------------|-------|
| Linearity (µg/mL) | 1-20  |
| LOD             | 0.02  |
| LOQ             | 0.18  |
| Precision (%)   | <0.1  |
| RSD             |       |
| Accuracy (%)    |       |
| Recovery 80     | 100.11±0.07* |
| Recovery 100    | 100.35±0.81* |
| Recovery 120    | 99.8±0.51*  |
| Robustness (%)  |       |
| Changing column mark/RSD | <0.1  |
| Temperature of column/RSD |       |
| Ratio of solvent/RSD |       |

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, | DS, | Yield (%) |
|-----|-----|-----|-----------|
| 15  | 65  | 0.04| 1.37      |
| 15  | 65  | 0.04| 1.16      |
| 20  | 65  | 0.04| 0.5       |
| 20  | 65  | 0.06| 2.28      |
| 20  | 35  | 0.04| 1.61      |
| 20  | 65  | 0.02| 0.83      |
| 15  | 95  | 0.02| 0.62      |
| 15  | 65  | 0.02| 0.41      |
| 10  | 65  | 0.02| 0.17      |
| 10  | 95  | 0.04| 0.34      |
| 10  | 95  | 0.06| 0.21      |
| 15  | 95  | 0.06| 0.86      |
| 15  | 35  | 0.02| 1.88      |
| 15  | 65  | 0.04| 0.78      |
| 10  | 35  | 0.04| 1.46      |

UAE: Ultrasound assisted extraction, MD: Maceration dynamic, Et: Extraction time, EW: Ethanol:water ratio, DS: Drug solvent ratio

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

| Factors | UEA | MD |
|---------|-----|----|
| 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: Ethanol: water ratio; DS: Drug 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 Panwinsky et al[10] 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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