

## RESEARCH REGARDING *TARAXACUM OFFICINALE* (L.) WEBER

### II. OPTIMUM EXTRACTION PARAMETRES OF CAFFEIC ACID DERIVATIVES USING ULTRASOUND - ASSISTED EXTRACTION

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#### Abstract

Pharmacological studies confirmed the therapeutic properties of the phenolic acids from plants, and their efficiency in treatment of type 2 diabetes mellitus. Based on this and also, on the facts that *Taraxacum officinale* L. contains a considerable amount of caffeic acid derivatives (48.8 mg/g in the leaves) and it does not exhibit a cytotoxic activity, we aimed to obtain a pharmacological active dry extract, titrated in cichoric acid, which can be developed in a pharmaceutical product used in type 2 diabetes mellitus therapy.

The aim of this work was to optimize and evaluate the extraction parameters of caffeic acid derivatives from the plant material using Ultrasound - Assisted Extraction (UAE). The effects of different parameters of caffeic acid derivatives yields, in plants extracts were investigated using an experimental design - Taguchi L<sub>16</sub> (4<sup>5</sup>) orthogonal array design. An HPLC/UV method was employed for the qualitative and quantitative determination of caffeic acid derivatives from the liquid extracts and from the lyophilized extract. The amount of caffeic acid derivatives and cichoric acid in the dry extract was 127.0 mg/g and 107.50 mg/g.

#### Rezumat

Cercetările privind proprietățile farmacologice ale acizilor fenol-carboxilici, au demonstrat eficiența lor în tratamentul diabetului zaharat de tip II (DZ II). Plecând de la aceste informații, cunoscând conținutul speciei *Taraxacum officinale* L. în derivați cafeici (48,80 mg/g în frunze) precum și absența citotoxicității, ne-am propus să obținem un extract uscat farmacologic activ, titrat în acid cicoric, pe care să-l valorificăm într-un produs farmaceutic util terapiei diabetului zaharat de tip II (DZ II). Scopul acestor cercetări constă în determinarea condițiilor optime de extracție a derivaților acidului cafeic din plantă folosind ultrasunetele (UAE). De aceea am investigat rolul parametrilor de extracție asupra conținutului în principii active și a cantității de extract uscat obținute folosind planul

factorial ortogonal Taguchi  $L_{16} (4^5)$ . Identificarea și dozarea derivaților acidului cafeic din soluțiile extractive și extractul uscat prin liofilizare au fost efectuate prin metoda HPLC/UV. Cantitatea de derivați ai acidului cafeic determinată în extractul uscat a fost de 127,0 mg/g și cea de acid cichoric este de 107,50 mg/g.

**Keywords:** *Taraxacum officinale*, cichoric acid, HPLC/UV

### Introduction

Caffeic acid and its derivatives (cichoric, caftaric, chlorogenic, ferulic acids and cynarin), as well as its precursor *p*-cumaric acid are the most widely phenolic acids distributed in plant tissues. It is well known that these phenolic compounds are mainly responsible for the antihyperglycemic activity due to antioxidant,  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibition properties. Also, they act on hepatic glucogenesis, have insulin stimulating properties [2-6] and do not exhibit cytotoxic activity [8].

The aim of this work was to find and evaluate the optimum parameters for extraction of caffeic acid derivatives from leaves using the Ultrasound - Assisted Extraction (UAE), to obtain a dry extract titrated in cichoric acid and to find an appropriate HPLC method in order to have a reliable and non-time consuming method for qualitative and quantitative analysis of the above mentioned compounds in liquid and dry extracts.

### Materials and Methods

The dry plant material of *Taraxacum officinale* (originated from Limassol - Cyprus) was collected before the flowering stage (leaves) in March 2009 [7]. Purity and quality of the plant material were determined using the relevant methods described in European Pharmacopoeia, 7<sup>th</sup> Edition [9]. To optimize the UAE conditions, we considered five factors and four levels that could potentially affect the extraction efficiency. The evaluated factors were: A- temperature  $^{\circ}\text{C}$  [35, 45, 55, 65], B- extraction time - min. [15, 30, 45, 60], C- solvent type/polarity [ethanol %: 50, 60, 70, 80]; D - solid-to-solvent ratio, g/mL [ 1/25, 2/25, 3/25, 4/25]; E- number of extractions [1 - 4] see Table I. Taguchi  $L_{16} (4^5)$  orthogonal array design, consisting of 16 experimental runs was used to determine the optimum extraction conditions. The extraction conditions of every run were based on the combination of different factors and levels. All extractions (experimental runs) were carried out using the Raypa Model ultrasonic cleaning bath with programmable temperatures, ranging from 0-90 $^{\circ}\text{C}$ , time up to 15 minutes, with a frequency of 35 kHz. Ethanol of different concentrations was used as a solvent in the experiment, because it is a

proper solvent for extraction of phenolic acids and also, it is safe for consumption [7, 9]. All solvents and standard substances used in the experiment were of appropriate grade, purchased from Sigma Aldrich, Germany.

The samples were analysed using a Waters HPLC series system, equipped with binary gradient pump, column thermostat, autosampler, degasser, UV detector and Empower software (Waters Corporation, Milford, Ireland) for data collection. Separation was performed on a reversed – phase analytical column (Sherisorb<sup>®</sup>, phase ODS 2, particle 5  $\mu\text{m}$ , 1 x i.d 15 cm x 4.6 mm). The detection and working temperature were set at  $\lambda = 320 \text{ nm}$  /  $30^{\circ}\text{C}$  and the mobile phase used was the 0.01% phosphoric acid and acetonitrile with a flow rate 1 mL/min. The parameters of the method were optimized in order to obtain a better resolution between peaks [7, 9]. Quantification of cichoric, caftaric, chlorogenic, p-cumalic, ferulic acids and cynarin was performed using an external standard method. The calibration curves were obtained by plotting the peak area of the standards *versus* their concentration, with a good linearity in the range of 0.01 mg/mL – 0.1 mg/mL ( $R^2 > 0.999$ ,  $n = 5$ ). All standards and samples were filtered through a 0.45  $\mu\text{m}$  syringe filter before injection. The identity of compounds was achieved by comparing the Retention time (Rt) of peaks from the chromatograms obtained with the standard substance and sample [Figures 1 - 2].

After analysing the liquid extracts from each run, a decision was made regarding the extraction conditions to be used for the preparation of the dry extract. The milled plant material (2.0 g of leaves) was extracted with 25 mL of ethanol 50%. The sample was placed in the ultrasonic cleaner (type Raypa) at 35 kHz, at  $55^{\circ}\text{C}$  for 45 minutes and four consecutive extractions were performed.

The freeze drying process was employed for the drying of the liquid extract, using the laboratory scale freeze dryer Christ Alpha 1-4LD plus. The Aerosil<sup>®</sup> (colloidal silicon dioxide) was used as an auxiliary substance due to its high specific surface and absorbent power. The qualitative and quantitative analysis of the dry extract was performed using the same analytical method as it was mentioned above. The Minitab 16 statistical software was used for the experimental design and data analysis. The arrangement of importance of the five factors to the extracted quantity of the caffeic acid derivatives and to the quantity of cichoric acid was evaluated according to the effectiveness of each factor through the calculation of rank. The rank was assigned based on delta statistics (the highest minus the lowest average for each factor); to the highest delta it was assigned the lowest rank. The average of each response characteristic (Signal to Noise

ratios and means) for each level of each factor were calculated (Table I). The influence of the factors was determined after the evaluation of results and analysing the design. The quality of the plant material was verified and all results were within the prescribed limits [9]: foreign matter 0.4%, total ash 11.0%, ash insoluble in hydrochloric acid 3.3%, dry residue 0.17%, and loss on drying 9.0%. Sixteen experimental runs were performed in order to optimize the five individual parameters that determined caffeic acid derivatives and cichoric acid yields. All experiments were conducted in triplicate and average results were reported.

### Results and Discussion

The obtained data from the experiments are summarized in Table I. The results have been evaluated in order to distinguish the appropriate extraction parameters. The highest amount of compounds was found in the liquid extract which was obtained using the extraction conditions from run 11.

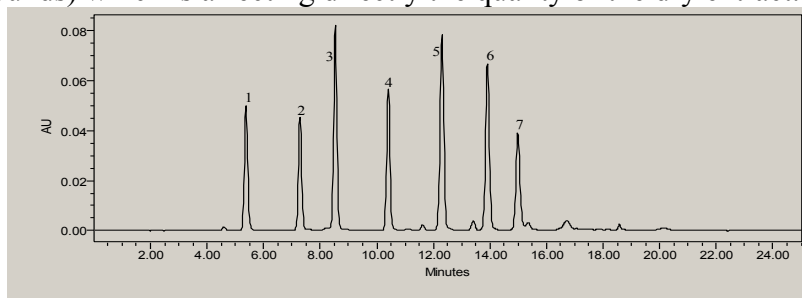
**Table I.**

Matrix and results of orthogonal design (extraction efficiency of caffeic acid derivatives and cichoric acid)

| Trial No  | A  | B  | C   | D    | E | Caffeic acid deriv., mg/g | Cichoric acid, mg/g |
|-----------|----|----|-----|------|---|---------------------------|---------------------|
| 1         | 35 | 15 | 50% | 1/25 | 1 | 54.39                     | 46.90               |
| 2         | 35 | 30 | 60% | 2/25 | 2 | 59.63                     | 51.30               |
| 3         | 35 | 45 | 70% | 3/25 | 3 | 60.07                     | 52.15               |
| 4         | 35 | 60 | 80% | 4/25 | 4 | 49.98                     | 43.40               |
| 5         | 45 | 15 | 60% | 3/35 | 4 | 67.73                     | 58.80               |
| 6         | 45 | 30 | 50% | 4/25 | 3 | 62.03                     | 52.90               |
| 7         | 45 | 45 | 80% | 1/25 | 2 | 60.42                     | 52.90               |
| 8         | 45 | 60 | 70% | 2/25 | 1 | 49.44                     | 41.70               |
| 9         | 55 | 15 | 70% | 4/25 | 2 | 48.19                     | 40.60               |
| 10        | 55 | 30 | 80% | 3/25 | 1 | 19.72                     | 16.80               |
| <b>11</b> | 55 | 45 | 50% | 2/25 | 4 | <b>68.24</b>              | <b>59.20</b>        |
| 12        | 55 | 60 | 60% | 1/25 | 3 | 64.62                     | 55.80               |
| 13        | 65 | 15 | 80% | 2/25 | 3 | 54.30                     | 47.30               |
| 14        | 65 | 30 | 70% | 1/25 | 4 | 62.17                     | 53.50               |
| 15        | 65 | 45 | 60% | 4/25 | 1 | 30.58                     | 25.20               |
| 16        | 65 | 60 | 50% | 3/25 | 2 | 58.70                     | 49.70               |

The quantities of caffeic acid derivatives in the liquid extracts are 19.72 - 68.24 mg/g, and the cichoric acid was the main phenolic acid (16.80-59.20 mg/g) (Figures 1, 2).

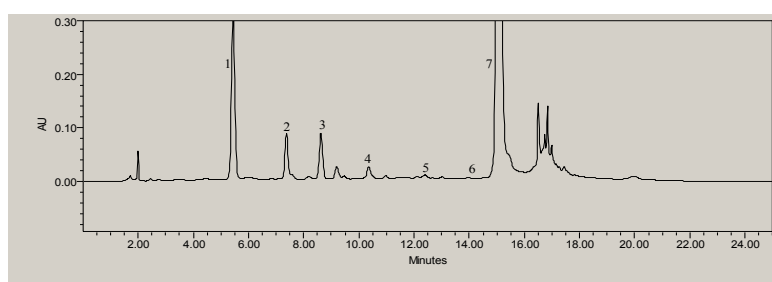
This experiment confirmed that the extraction parameters have an essential effect on the quality of the liquid extract (yield of active compounds) which is affecting directly the quality of the dry extract.



**Figure 1.**

Chromatographic profile of standard solution UV detection at 320 nm

Legend: 1-caftaric acid, 2-chlorogenic acid, 3-caffeic acid, 4-cynarin, 5- p-cumaric acid, 6-ferulic acid, 7-cichoric acid



**Figure 2.**

Chromatographic profile of the liquid extract from leaves, at 320 nm

The influence of the extraction parameters on the response (extracted quantities of compounds) was stated based on the obtained results and also based on the statistical evaluation of the data: number of extractions > solvent type > solid-to-solvent ratio > temperature > extraction time (Table II).

**Table II.**

Analysis of orthogonal design (extraction of caffeic acid derivatives and cichoric acid)

|                | Cichoric acid, mg/g |             |             |             |             | Caffeic acid derivatives, mg/g |             |             |             |             |
|----------------|---------------------|-------------|-------------|-------------|-------------|--------------------------------|-------------|-------------|-------------|-------------|
|                | A                   | B           | C           | D           | E           | A                              | B           | C           | D           | E           |
| K <sub>1</sub> | 33.68               | 33.62       | 34.32       | 34.35       | 29.59       | 34.94                          | 34.92       | 35.65       | 35.60       | 31.05       |
| K <sub>2</sub> | 34.18               | 31.94       | 33.14       | 33.89       | 33.69       | 35.49                          | 33.28       | 34.51       | 35.19       | 35.04       |
| K <sub>3</sub> | 31.76               | 33.07       | 33.37       | 32.04       | 34.31       | 33.11                          | 34.40       | 34.75       | 33.36       | 35.58       |
| K <sub>4</sub> | 32.50               | 33.50       | 31.31       | 31.85       | 34.54       | 33.91                          | 34.86       | 32.55       | 33.30       | 35.79       |
| <b>Delta</b>   | <b>2.42</b>         | <b>1.68</b> | <b>3.01</b> | <b>2.49</b> | <b>4.95</b> | <b>2.38</b>                    | <b>1.64</b> | <b>3.11</b> | <b>2.30</b> | <b>4.74</b> |
| <b>Rank</b>    | <b>4</b>            | <b>5</b>    | <b>2</b>    | <b>3</b>    | <b>1</b>    | <b>3</b>                       | <b>5</b>    | <b>2</b>    | <b>4</b>    | <b>1</b>    |
| k <sub>1</sub> | 48.44               | 48.40       | 52.17       | 52.27       | 32.65       | 56.02                          | 56.15       | 60.84       | 60.40       | 38.53       |
| k <sub>2</sub> | 51.58               | 43.63       | 47.77       | 49.88       | 48.63       | 59.91                          | 50.89       | 55.64       | 57.90       | 56.73       |
| k <sub>3</sub> | 43.10               | 47.36       | 46.99       | 44.36       | 52.04       | 50.19                          | 54.83       | 54.97       | 51.56       | 60.25       |
| k <sub>4</sub> | 43.92               | 47.65       | 40.10       | 40.52       | 53.72       | 51.44                          | 55.69       | 46.11       | 47.69       | 62.03       |

|              |             |             |              |              |              |             |             |              |              |              |
|--------------|-------------|-------------|--------------|--------------|--------------|-------------|-------------|--------------|--------------|--------------|
| <b>Delta</b> | <b>8.48</b> | <b>4.77</b> | <b>12.08</b> | <b>11.75</b> | <b>21.07</b> | <b>9.71</b> | <b>5.27</b> | <b>14.73</b> | <b>12.71</b> | <b>23.50</b> |
| <b>Rank</b>  | <b>4</b>    | <b>5</b>    | <b>2</b>     | <b>3</b>     | <b>1</b>     | <b>4</b>    | <b>5</b>    | <b>2</b>     | <b>3</b>     | <b>1</b>     |

K = average of Signal to Noise ratios for each level of each factor; k = average of means for each level of each factor; delta = the highest minus the lowest average for each factor.

The impact of the extraction factors: number of extractions, solid-to-solvent ratio and solvent type is statistically significant ( $p < 0.1$ ) (Tables III and IV).

**Table III.**  
Parameters of the statistical analysis for the extraction of cichoric acid

| Source                 | DF | SS      | MS     | F     | P            |
|------------------------|----|---------|--------|-------|--------------|
| Temperature            | 3  | 189.72  | 63.25  | 3.47  | 0.167        |
| Extraction time        | 3  | 54.69   | 18.23  | 0.29  | 0.833        |
| Solvent type           | 3  | 299.04  | 99.68  | 5.47  | <b>0.098</b> |
| Solid-to-solvent ratio | 3  | 338.97  | 112.99 | 6.20  | <b>0.084</b> |
| Number of extractions  | 3  | 1115.73 | 371.91 | 20.40 | <b>0.017</b> |
| Total                  | 15 | 1998.15 |        |       |              |

**Table IV.**  
Parameters of the statistical analysis for the extraction of caffeic acid derivatives

| Source                 | DF | SS      | MS     | F     | P            |
|------------------------|----|---------|--------|-------|--------------|
| Temperature            | 3  | 237.60  | 79.20  | 3.45  | 0.168        |
| Extraction time        | 3  | 68.97   | 22.99  | 0.29  | 0.832        |
| Solvent type           | 3  | 448.56  | 149.52 | 6.50  | <b>0.079</b> |
| Solid-to-solvent ratio | 3  | 405.27  | 135.09 | 5.88  | <b>0.090</b> |
| Number of extractions  | 3  | 1398.91 | 466.30 | 20.28 | <b>0.017</b> |
| Total                  | 15 | 2559.31 |        |       |              |

\* All the factors and the interaction terms are significant at a  $\alpha$ -level of 0.10.

Using the freeze drying method, we obtained a dry extract with a high amount of caffeic acid derivatives (127.0 mg/g). The quantity of cichoric acid was also considerable (107.50 mg/g).

### Conclusions

The assessed optimized conditions can be applied in the preparation of an extract (pilot batches). The manufacturing process of the dry extract (laboratory scale) and the HPLC/UV method of analysis are the proper ones. Based on our data, we may conclude that *Taraxacum officinale* is a potential source of cichoric acid. The pharmacological profile of the obtained dry extract will be evaluated in further studies.

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