RESEARCH REGARDING *TARAXACUM OFFICINALE* (L.) WEBER WITH THE INTENTION OF THERAPEUTIC EXPLORING.

NOTE I. STUDIES OF PHENOLCARBOXYLIC ACIDS

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Abstract

The current study was intended to evaluate the accumulation of the cichoric acid (dicaffeoyltartaric acid) in *Taraxacum officinale* (L.) Weber (dandelion) in relation to the harvesting time and determination of an adequate method of extraction, required to obtain a pharmacological-active extract, standardized in cichoric acid, necessary for elaboration of a pharmaceutical product used in the therapy of the metabolic diseases.

The plant material was harvested from wild flora (from Limassol – Cyprus) in all vegetation phases. The extraction method (Ultrasound Assisted Extraction = UAE/maceration) and the solvent (ethanol 70%) were selected based on determination of the cichoric acid by high pressure liquid chromatography coupled with UV (HPLC/UV), the method selected from the scientific literature and optimized by the authors.

The highest amount of cichoric acid was found in leaves before the flowering phase, in March (51.77 mg/g) and in the roots after the flowering period, in December (4.89 mg/g) using the ultrasound. For flowers, fruits and stems the values were between 3.19 mg/g – 5.06 mg/g. The optimized method was adequate for the proposed objective

The quantitative determination revealed a considerable quantity of cichoric acid in the plant, for that reason the dandelion can be considered as a potential source of cichoric acid.

Rezumat

Cercetările constau în stabilirea dinamicii de acumulare a acidului cicoric (= dicaffeoyltartaric acid) în *Taraxacum officinale* (L.) Weber (păpădie) şi a metodei de extragere a lui pentru obținerea unui extract farmacologic activ, standardizat în acid cicoric, necesar elaborării unui produs farmaceutic util terapiei bolilor metabolice. Păpădia a fost colectată din flora spontană (Limassol - Cipru), în toate fazele de vegetație. Alegerea metodei de extracție (ultrasunete = UAE/macerare) şi a solventului (etanol 70 %) s-a bazat pe determinarea conținutului în acid cicoric prin cromatografie de lichide de înaltă

performanţă cuplată cu UV (HPLC/UV), metodă preluată din literatura de specialitate şi optimizată de autori. Cantitatea cea mai mare de acid cicoric a fost determinată în frunzele colectate înainte de înflorire, în luna martie (51,767 mg/g) şi din rădăcinile colectate în decembrie (4,89 mg/g). Inflorescenţele, fructele şi tijele florale conţin 3,19 mg/g – 5,06 mg/g acid cicoric. Extractul cu cel mai mare conţinut de acid cicoric s-a obţinut în etanol 70 % prin ultrasonicare. Metoda optimizată a fost adecvată scopului propus. Cantitatea semnificativă de acid cicoric din păpădie justifică utilizarea sa ca sursă potențială de acid cicoric.

Keywords: *Taraxacum officinale*, cichoric acid, ultrasound assisted extraction, HPLC/UV.

Introduction

Taraxacum officinale (L.) Weber (Dandelion) is a well - known traditional herbal remedy with a long history. Several health-promoting benefits including diuretic, choleretic, antiinflammatory, antioxidant, anticarcinogenic, antihyperglycemic, hypolipidemic and anticoagulatory have been attributed to the use of the plant and until recently only limited scientific data was available to justify the reputed use. These effects are ascribed to the presence of phenolic acids, flavonoids, coumarins, sesquiterpene lactones, various triterpenes, phytosterols and inulin [1, 12, 15]. Phenolic compounds are mainly responsible for the antihyperglycemic activity due to antioxidant, α-glucosidase and α-amylase inhibition proprieties which were suggested and demonstrated by several studies [4, 5, 13].

Cichoric acid (caffeic diester of tartaric acid), itself has been reported to inhibit HIV integrase [2], exhibit antioxidant [4], antidepressant [9], and antihyperglycemic proprieties [17].

The objective of this work was to determine the optimal harvesting time of *Taraxacum officinale* aerial part (leaves, flowers, fruits and stems) and roots, to find an adequate method of extraction and to optimize the chromatographic conditions, in order to obtain and analyse (qualitative by and quantitative by) extracts with the highest content of the cichoric acid and other phenolcarboxylic acids from the plant. The application of ultrasound as a laboratory technique for the extraction from plant material is a method of interest (increased yield of extracted components, reduction in extraction time and use of lower temperature to avoid degradation of pharmacological active compounds) [8, 18]. The Ultrasound Assisted Extraction (UAE) was used in comparison to maceration (M) to prove the advantage of the method.

Materials and Methods

The plant material of *Taraxacum officinale* originated from Limassol - Cyprus was harvested in March before the flowering stage (samples from leaves/roots: Lm_1/Lu_1 and Rm_1/Ru_1), in April during flowering stage (samples from leaves/roots/flowers and stems: Lm_2/Lu_2, Rm_2/Ru_2, FL and ST) and May, after flowering stage (samples leaves/roots/fruits: Lm_3/Lu_3, Rm_3/Ru_3 and FR) in 2009. Also, the plant material was collected in December (samples from leaves/roots: Lm_4/Lu_4 and Rm_4/Ru_4) of the same year. The raw material was naturally dried, stored in appropriate conditions in well closed containers. Samples from each lot have been reserved.

The total content of phenolcarboxylic acids (PCA) was verified using the spectrophotometric method according to Makkar [11] and European Pharmacopoeia [19].

All solvents and the standard substances used were of appropriate grade, purchased from Sigma Aldrich, Germany. The chromatographic conditions were optimized with the aim to obtain a chromatogram with a good resolution of adjacent peaks and shorter retention time of the cichoric acid. The optimum chromatographic conditions for separation of cichoric acid were found by comparing the chromatographic behaviour of the compound from the plant materials using different gradient elution, composition of mobile phase, volume of sample and flow rate.

In our experiment, various mobile phase combinations containing water and acetonitrile / or methanol with phosphoric acid as modifier were compared. To optimize the mobile phase for binary gradient profile different percentage of mobile phase B in mobile phase A were used [3, 6, 19]. The flow rate between 1.0 - 1.5 mL/min and volume of sample between 10 - 25 μL were studied.

The analysis were performed using a Waters HPLC series system, equipped with binary gradient pump, column thermostat, autosampler, degasser, dual λ UV detector and Empower software (Waters Corporation, Milford, Ireland) for data collection. For the separation, a reversed – phase analytical column (Sherisorb®, phase ODS 2, particle 5 μm, l x i.d 15 cm x 4.6 mm) and ambient temperature as working temperature were used. The mobile phase was a binary gradient prepared from phosphoric acid in water 99.9/0.1, v/v (solvent A) and acetonitrile (solvent B). The elution started with 5 - 25% B in 0- 20 min, 25 –50% B in 20-40 min, 50-80% B in 40-50 min ending with 80-95% B in 50- 60 min. [6]. The flow rate was 1 mL/ min and injection volume was 10 μL. The detection was performed at dual λ of 254 nm and 330 nm.

The standard stock solution of cichoric acid was prepared in the concentration of 0.1 mg/mL. The calibration standard samples were prepared by appropriate dilutions from the stock solution and filtered through a 0.45 μm syringe filter before injection. 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 ($R^2 > 0.998$, n = 5).

The extraction of cichoric acid was carried out using ethanol 70 % as an extraction solvent. It was selected based on studies claiming that this solvent is a proper one for extraction of phenolcarboxylic acids [7]. For preparation of samples, 1 g of dried plant material in 100 mL of solvent was used under the below conditions:

- maceration - extraction at room temperature, in a place protected from light for 12 hours;
- UAE - the samples were placed in the ultrasonic cleaner (type Raypa) at 35 kHz, 40° C for 30 minutes.

All samples were filtered using a 0.45 μm syringe filter before injection.

The identity of cichoric acid was achieved by comparing the Retention time (r_t) of peaks from the chromatograms obtained with the standard substance and samples [figures 1- 3]. Quantification was performed by the calibration procedure. All results are expressed as Mean values ± Standard Deviation of three successive determinations.

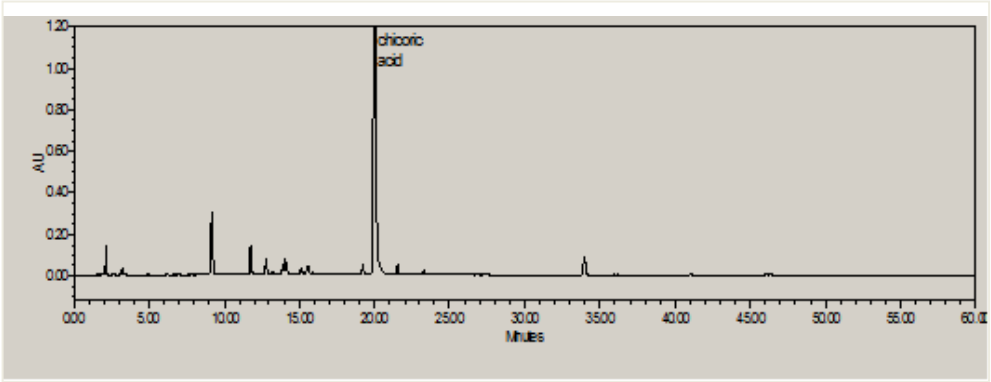

Figure 1.
Chromatographic profile of sample from leaves

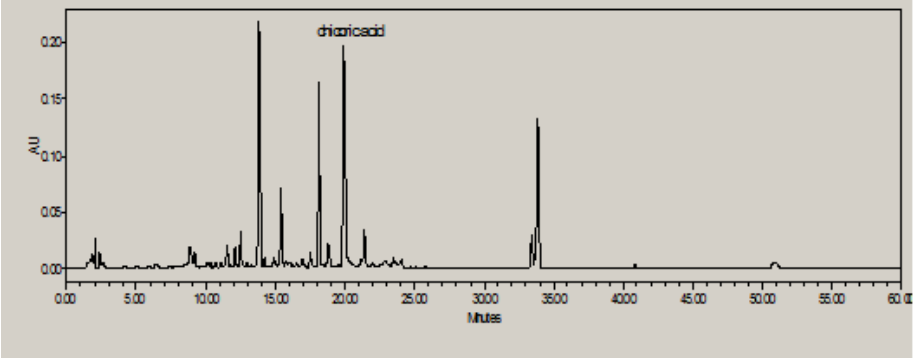

Figure 2.
Chromatographic profile of sample from root

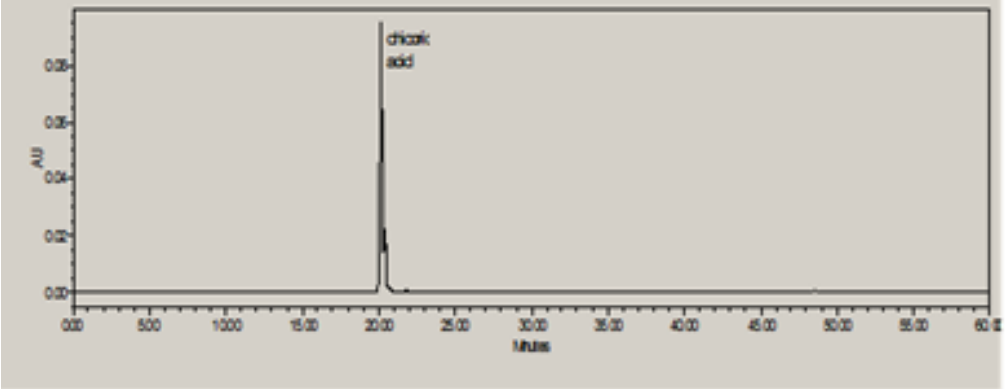

Figure 3.
Chromatographic profile of standard solution
UV detection at 254 and 330 nm

Results and Discussion

The highest content of cichoric acid was found in the leaves, collected in March, decreasing until December. The quantity of cichoric acid in the roots is much lower than in the leaves, with the same tendency to decrease, except that the roots collected in December contain the highest quantity of compound [Table I - II]. The values for the flowers, fruits and stems are between 3.19 mg/g – 5.06 mg/g. Also, these quantities are higher than the ones mentioned in the scientific literature for *Taraxaci herba* (34.08 mg/g), *Taraxaci formosani herba* (10.87 mg/g), *Cichorii herba* (38.67 mg/g), *Echinaceae purpureae radix* (38.55 mg/g) and *Lactucae serriolae herba* (28.0 mg/g) [3, 4, 10, 14, 16].

Table I

Quantity of phenolic acids by maceration

№	Sample	Total phenolic content (mg/g gallic acid) (Makkar)	Total PCA derivatives (mg/g chloro-genic acid) (E. Ph.)	Cichoric acid (mg/g) (HPLC/UV)
1	Lm_1	52.29 ± 0.0178	37.68 ± 0.0981	31.70 ± 0.1836
2	Lm_2	43.72 ± 0.0208	35.02 ± 0.1364	26.80 ± 0.8977
3	Lm_3	42.83 ± 0.0152	30.86 ± 0.1303	24.14 ± 0.0496
4	Lm_4	26.98 ± 0.0576	19.29 ± 0.0905	21.15 ± 0.1928
5	Rm_1	5.04 ± 0.0100	5.14 ± 0.0424	2.84 ± 0.0740
6	Rm_2	4.60 ± 0.2453	4.32 ± 0.0074	1.80 ± 0.0565
7	Rm_3	4.19 ± 0.0665	3.84 ± 0.0132	1.20 ± 0.0612
8	Rm_4	9.61 ± 0.1397	5.55 ± 0.0551	3.09 ± 0.1360
9	FL	10.41 ± 0.0787	3.08 ± 0.0087	-
10	FR	9.89 ± 0.0212	2.35 ± 0.0246	-
11	ST	14.91 ± 0.0971	3.78 ± 0.0025	-

"-" - not analysed

Table II

Quantity of phenolic acids by UAE

№	Sample	Total phenolic content (mg/g gallic acid) (Makkar)	Total PCA derivatives (mg/g chloro-genic acid) (E. Ph.)	Cichoric acid (mg/g) (HPLC/UV)
1	Lm_1	59.19 ± 0.4147	48.83 ± 0.0499	51.77 ± 0.3312
2	Lm_2	52.51 ± 0.2813	45.55 ± 0.0520	43.03 ± 0.0777
3	Lm_3	50.54 ± 0.0850	38.25 ± 0.0535	36.43 ± 0.0729
4	Lm_4	32.22 ± 0.1657	25.68 ± 0.0141	35.48 ± 0.1798
5	Rm_1	9.70 ± 0.0435	9.73 ± 0.0203	3.93 ± 0.0161
6	Rm_2	9.42 ± 0.0517	8.93 ± 0.0103	3.25 ± 0.0268
7	Rm_3	9.15 ± 0.0206	7.34 ± 0.0125	1.30 ± 0.0064
8	Rm_4	15.00 ± 0.0434	8.00 ± 0.0094	4.89 ± 0.0040
9	FL	14.87 ± 0.0402	5.42 ± 0.0017	3.19 ± 0.0070
10	FR	12.94 ± 0.0186	4.18 ± 0.0063	4.20 ± 0.1307
11	ST	16.25 ± 0.0645	6.06 ± 0.0031	5.06 ± 0.0030

Other phenolic acids are not representative in the roots. In the leaves they are found in small quantities (mg/g) and the predominant phenolic acids are: caftaric acid (3.17 – 4.07), chlorogenic acid (0.72 – 2.02) and gallic acid (0.30 – 1.01). Also, a small quantity of cynarin (0.35 - 0.51) was found in the leaves. The amount of mentioned phenolic acids is higher in March, with a tendency to decrease, except that the quantity of caftaric acid and cynarin are slightly increased in the leaves collected in December [Table III].

Table III

Quantity of phenolic acids by UAE

№	Sample	Caftaric acid (mg/g)	Chlorogenic acid (mg/g)	Gallic acid (mg/g)	Cynarin (mg/g)
1	Lu_1	4.07 ± 0.0456	2.02 ± 0.0064	1.01 ± 0.0017	0.51 ± 0.0124
2	Lu_2	3.46 ± 0.0208	1.51 ± 0.0160	0.86 ± 0.0039	0.40 ± 0.0075
3	Lu_3	3.17 ± 0.0017	1.41 ± 0.0121	0.30 ± 0.0005	0.35 ± 0.0144
4	Lu_4	3.84 ± 0.0781	0.72 ± 0.0011	0.77 ± 0.0023	0.41 ± 0.0007
5	Ru_1	0.41 ± 0.0135	0.31 ± 0.0000	0.09 ± 0.0066	0.23 ± 0.0073
6	Ru_2	0.36 ± 0.0282	0.28 ± 0.0020	0.08 ± 0.0014	0.22 ± 0.0098
7	Ru_3	0.27 ± 0.0025	0.17 ± 0.0125	nd	0.14 ± 0.0002
8	Ru_4	0.45 ± 0.0017	0.18 ± 0.0034	0.06 ± 0.0042	0.23 ± 0.0027

"nd" – not detected

Optimizing the chromatographic parameters, we achieved a good separation and a short retention time of the cichoric acid (rt ≈ 20 min).

Based on the results, it is evident that UAE is a more efficient method: less time-consuming, higher extraction yields and lower temperatures which protect the compounds from degradation.

Conclusions

The leaves of *Taraxacum officinale* have a higher content of cichoric acid before the flowering stage (in March), but for the roots it is higher after the flowering stage, in early winter (in December).

The extraction using ultrasounds is an efficient method and the quantities of cichoric acid obtained are higher compared to maceration.

For this reason, the extraction method and solvent used in the experiment can be considered as a proper one for the elaboration of a technological process to obtain a pharmacological active extract, standardized in cichoric acid from *Taraxaci folium* and *Taraxaci radix*.

The chromatographic method (proposed by us) is a proper method for qualitative and quantitative determination of the compound. Also, it is suitable for separation and analysis of hydroxybenzoic and hydroxycinnamic acids derivatives.

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