

PRELIMINARY RESEARCH REGARDING THE THERAPEUTIC USES OF *URTICA DIOICA* L NOTE II. THE DYNAMICS OF ACCUMULATION OF TOTAL PHENOLIC COMPOUNDS AND ASCORBIC ACID

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Abstract

Urtica dioica (nettle) is one of the most popular and cosmopolitan plants (present in Europe, Africa, Asia, America), whose dietary and therapeutic benefits have been known since ancient times. The therapeutic activity of nettle extracts is related to the presence of phenolic compounds (mainly cafeoil-malic acid, chlorogenic acid, ferulic acid, rutin, isoquercitrin and astragalin) and their antioxidant activity. Another compound with certain antioxidant activity is the ascorbic acid. Plants maturation leads to changes of their chemical composition. Therefore, in order to evaluate the optimal moment of plant harvesting, dynamics of accumulation of polyphenols and ascorbic acid were undertaken. The results show that young nettle leaves have the highest content of polyphenols and ascorbic acid.

Rezumat

Urtica dioica (urzica) este una dintre cele mai cunoscute și cosmopolite plante (prezentă în Europa, Africa, Asia, America), ale cărei beneficii dietetice și terapeutice sunt cunoscute din cele mai vechi timpuri. Efectele terapeutice pot fi atribuite derivaților fenolici (în special acidului cafeoil-malic, acidului clorogenic, acidului ferulic, rutozidei, isoquercitrozidei și astragalinei) și a acțiunii lor antioxidante. Se știe că maturizarea plantei conduce la modificări ale compoziției chimice. De aceea, în scopul precizării perioadei optime de recoltare pentru obținerea materiei prime de cea mai bună calitate, s-a determinat dinamica de acumulare a polifenolilor și a acidului ascorbic. Rezultatele determinărilor indică faptul că produsul imatur prezintă cel mai mare conținut de polifenoli și de acid ascorbic.

Keywords: *Urtica dioica*, ascorbic acid, polyphenols

Introduction

Urtica dioica (nettle) is one of the most popular and cosmopolitan plants (present in Europe, Africa, Asia, America), whose dietary and therapeutic benefits have been known since ancient times [16]. The leaves are used as soup and mashed or can be dried during the winter [10]. Beside its nutritional importance, stinging nettle has therapeutical effects that are

recognized in old writings, as Avicenna's Journal [4]. Due to its traditional uses as a hemostatic, antirheumatic and remedy for urinary infections and stones, the scientists have developed a great interest in the chemical composition and pharmacological action of nettle leaves [26]. Chemical investigations revealed the presence of polyphenolcarboxylic acids [caffeoylmalic acid (1.6 g%), chlorogenic acid (0.5 g%), ferulic acid and neochlorogenic acid] of flavonoids [rutin (0.3 - 0.8%), isoquercitrin (0.14 - 0.31 g%), astragalin], pelargonidin, epigallocatechin - gallate (89 µg%), coumarins [scopoletin (0.1 - 1 mg%)], sterols, carotenoids (β-carotene, lycopene, lutein, neoxanthin, luteoxanthin) and lectins (isoelectine, *Urtica dioica* Agglutinin = UDA) [5, 8, 12, 14, 17, 19, 27, 28]. Concerning the pharmacological action of nettle, recent investigations have shown immunomodulatory actions [1], hypocholesterolemic [2], hypoglycemic [7], analgesic, antiinflammatory [23], inhibition of platelet aggregation for extracts from leaves of stinging nettle [3].

The conclusions of the pharmacological studies link the therapeutical activity of nettle extracts with the presence of phenolic compounds (mainly cafeoil-malic acid, chlorogenic acid, ferulic acid, rutin, isoquercitrin and astragalin), and theirs antioxidant activity [1-3, 7, 11, 23]. Another compound with certain antioxidant activity and that may contribute to the antioxidant activity of the nettle extracts is ascorbic acid [20]. The presence of reactive oxygen species is related to diabetes mellitus, cardiovascular, inflammation, cancer, osteoporosis and degenerative diseases. The free radicals mainly act by attacking the unsaturated fatty acid in the biological membrane which extend to membrane lipid peroxidation and finally to the cell inactivation or death. The antioxidants mechanism is mainly scavenging the free radicals [9, 15]. Thus emerges the need to obtain new sources of antioxidants. It is known that plants maturation leads to changes of their chemical composition. Therefore, in order to evaluate the optimal moment of plant harvesting, dynamics of accumulation of polyphenols and ascorbic acid were undertaken.

Materials and Methods

Leaves of *Urtica dioica* were collected from Ghergani village, Dambovită county, in March (batch U1), April (batch U2), June (batch U3), and July (batch U4) 2009. A voucher specimen of each batch was deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Carol Davila, Bucharest.

Reagents and solvents

Sodium carbonate, sodium tungstate, sodium molybdate, phosphoric acid, lithium sulfate, hydrochloric acid and bromine water were provided

from Fluka-Chemicals, Dorset, England. Tannic acid, ascorbic acid, oxalic acid, sodium dihydrogen phosphate were purchased from Merck, Darmstadt, Germany.

Ethanol 96% p.a was provided by Fluka-Chemicals, Dorset, England, and the solvents for HPLC analysis, water and methanol, by Merck, Darmstadt, Germany.

Preparation of extracts. For polyphenol determination: 0.3 g of powdered raw material was extracted for 30 minute with 50% ethanol, using a reflux condenser. The mixture was filtered through a cotton plug. For ascorbic acid determination: 10 g of fresh chopped leaves were mixed with 50 mL of 0.5% oxalic acid and placed in an ultrasonic bath, for 30 minutes. The mixture was filtered through a 0.45 μm filter. 20 μL of the filtrate were injected in the HPLC system.

Determination of total phenolic content. Determination of total phenolic compounds and tannins, expressed as tannic acid equivalent, was performed according to *Folin-Ciocalteu* method modified (Makkar method modified by Humadi 2009) [13, 21]. Our modification consists in the dilution of the extract sample with water up to 1 mL.

The chromophore development reaction is based on oxidation of polyphenols *via Folin-Ciocalteu* reagent, which is a mixture of phosphomolybdenic and phosphotungstic acids, in a basic medium. The blue complex thus formed, is assessed by absorbance at 750 nm, and is directly proportional to the total amount of polyphenols in the medium [6]. 0.1 to 0.9 mL extract were diluted with distilled water up to 1 mL, followed by the addition of 1 mL of Folin-Ciocalteu reagent (diluted 1:1), and 8 mL of 20% sodium carbonate. The blanks were prepared using the same chemical reagents excluding the extracts. The flasks were mixed well and left in the dark at room temperature for 40 minutes. The absorbance was read at 725 nm using a UV-Vis spectrophotometer Jasco V-530, Japan 2005. Tannic acid was used to prepare a calibration curve in the range 2 – 12 mg/mL (fig. 1A). The concentration of total phenols (A %) was expressed in percentage (gram of tannic acid equivalents per 100 g of dry plant material) (g %).

Determination of tannins content. The tannins content was determined using Folin-Ciocalteu method (Makkar method modified by Humadi 2009), with modification. The modification was using hide powder instead of PVP (polyvinyl-poli-pyrrolidone) [13]. 100 mg of hide powder was weighed, then 5 mL of the extract were added. The mixture was shaken for 60 minute, then filtered and the supernatant collected. The supernatant possesses non-tannins phenols because the tannins were precipitated with the hide powder. The phenolic content of the supernatant was measured following the

same procedure as described above, using the same standard calibration curve. The concentration of non-tannins phenols (B%) was expressed in the same manner as the total phenols (tannic acid equivalents/100 g of dry plant material) (g %). The content of tannins was calculated as follows: % T = A (%) – B (%), where A %: content of total phenolic compounds, B %: content of the non-tannins phenols.

Statistical analysis. The results of the spectrophotometric determination are expressed as Mean $\pm$ standard deviation upon three independent replicates.

Determination of ascorbic acid content. A Beckman HPLC system with solvents Module 126, Rheodyne injection system and UV Gold 166 detector was used. The separation of ascorbic acid was performed with the following experimental conditions: Lichrosorb RP-18 column (15 cm x 4,6 mm, 5 μ m p.d) from Merck (Darmstadt, Germany), Isocratic elution system A – B 95 : 5 (v/v), A = 0.05 M NaH₂PO₄ and B = methanol, flow rate 0.7 mL/min, the injection volume 20 μ L, UV detection at 242 nm. For the experimental parameters control, data acquisition and processing „Gold Software” was used. For the quantitative determination of ascorbic acid the direct calibration method using the calibration curve Area = f (conc.) in the range 12.5 – 125 ng/ μ L (fig. 1B) concentration range was performed. The results were expressed in percentage (mg of ascorbic acid/100 g of dry plant material).

Results and Discussion

The highest content of total phenolic compounds was found in young leaves of *Urtica dioica* (batch U1), and the lowest content was determined in mature leaves (batch U4).

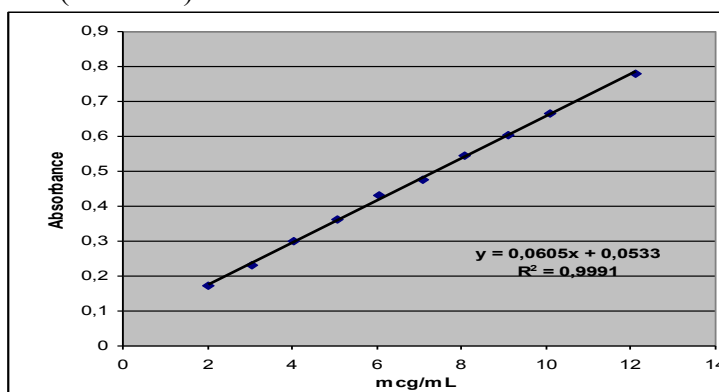
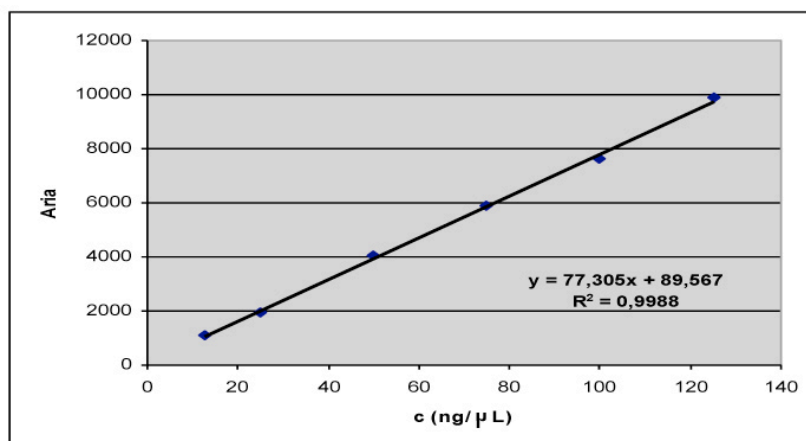


Figure 1A
Standard calibration curves for tannic acid

**Figure 1B**

Standard calibration curves for ascorbic acid

The results from table I reveal that the content of total phenolic compound decreases with the plant growth. The total phenolic derivatives decrease is due to the decrease of non-tannin phenols (phenolcarboxylic acids and flavonoids), which are the most important compounds from nettle leaves.

Table I
The phenolic compounds and the ascorbic acid contents

No.	Batch	Total phenolic compounds g% tannic acid	Non-tannins phenols g% tannic acid	Tannins g% tannic acid	Ascorbic acid mg/100 g
1	U1	7.0125 ± 0.2875	5.4825 ± 0.0957	1.1134 ± 0.1080	40.51 ± 1.19
2	U2	6.0279 ± 0.0379	5.2075 ± 0.1333	0.8270 ± 0.0675	14.1 ± 0.77
3	U3	1.8471 ± 0.0510	1.1027 ± 0.0223	0.7444 ± 0.0281	8.6 ± 0.37
4	U4	1.4446 ± 0.1023	0.3830 ± 0.0024	1.0616 ± 0.0034	-

The content of these compounds in nettle leaves shows a gradual decrease up to the stage of flowering plant. This hypothesis is supported by Rolson and colab. who reported a sudden drop of phenolcarboxylic acids in leaves harvested at plant flowering stage [24]. Regarding other studies, the reported phenolic content varies from 0.044 g to 1.41 g galic acid per 100 g of dry plant material, or from 24.1 mg to 36.78 mg acid galic per g of dry extract [4, 6, 11, 15, 22].

Our results indicated a higher content of polyphenol derivatives, in comparison to related results in literature [4, 15]. Unfortunately, in other studies the moment of plant harvesting was not specified, and therefore the comparison of our results with the ones found in literature, are not accurate.

Regarding the tannins content, its values show significant variations depending on plant development stage. As is shown in table I, a higher content of tannins is found both in young nettle leaves as in mature ones. Although the tannins content decreases with plant maturation (from march till june), after the flowering stage the content seems to increase again. So, it seems that the contents of tannins is little linked with the stage of plant development. Due to the fact that the highest content of total phenolic compounds was found in young nettle leaves, we consider that March is the optimum moment of plant harvesting.

The results of chromatografic determination are shown in figure 2 and table I.

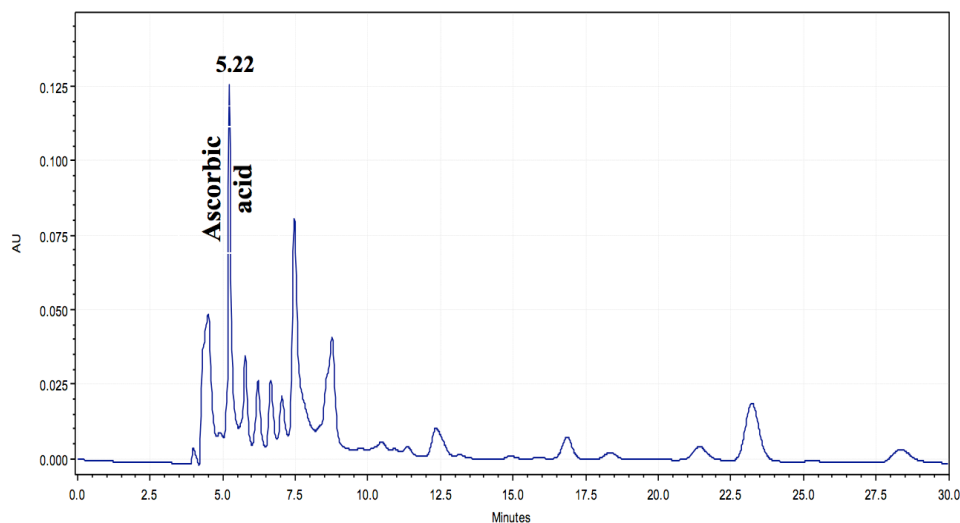


Figure 2
HPLC chromatogram of *Urtica dioica* extract

The retention time of ascorbic acid is short (5.22 min.), but the time required for all co-extracts to eluate is about 30 minutes. The highest content of ascorbic acid is found in the young nettle leaves. The results of the chromatographic determination indicate that the ascorbic acid content decreases with the plant development. As in the case of the total phenolic compounds, the raw material with the highest content of ascorbic acid comes from batch U1. The vitamin C content of nettle leaves reported by other authors varies from 36 to 269 mg % [20]. Our results are at the low limit of this range. The results of spectrophotometric and chromatografic determinations indicate that the plant growth is associated with a decrease in the content of phenolic compounds and ascorbic acid. Therefore, for further determination we will select the raw material from batch U1.

Conclusions

The total polyphenol content (mainly phenolic non-tannins compounds) and the ascorbic acid content decrease with plant development. The tannins content has little variations depending on the plant harvesting moment. The highest quantities of phenolic compounds and ascorbic acid were found in the young nettle leaves.

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