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Short communication

Quality Assesment of *Urtica dioica* L. Samples Collected from Different Locations of Turkey

Sanem HOŞBAŞ, Mustafa ASLAN*, Ekrem SEZİK

Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, 06330 Ankara, TURKEY

Urtica dioica L. (Urticacea) is widely distributed throughout the temperate regions of the world. Leaves of the plant recommended not only for the complaints associated with rheumatoid arthritis, cardiovascular diseases and diabetes but also have antiviral, antioxidant, anti-inflammatory activities. It is also used as traditional medicine as panacea in Turkey. Despite, *U. dioica* leaf is monographed in European Pharmacopeia as "Nettle Leaf – Urtica folium" and standardized by liquid chromatography for its chlorogenic acid content; aerial parts of plants are sold instead of leaves at the herbal shops for medical purposes in Turkey. The objective of our study is to determine quality of samples and compare the chlorogenic acid amount in aerial parts and leaves collected from different localities of Anatolia. Extraction procedure was employed according to European Pharmacopeia 6.0. *p*-Coumaric acid was used as internal standart. RP-HPLC was performed in order to determine chlorogenic acid amounts.

The leaf samples collected from Düzce, Maraş, Işık Mountain and Palandöken Mountain has possesed highest chlorogenic acid amounts 1.91±0.0003%, 1.156±0.0019%, 0.67±0.00068%, 0.63±0.0024% respectively. However amount of chlorogenic acid in aerial parts of same samples has been found 1.047±0.0007%, 0.751±0.0033%, 0.31±0.0085%, 0.488±0.0051% respectively. Results indicate that in all samples, chlorogenic acid content has been found higher in leaves than aerial parts. Present study revealed that usage of the aerial parts instead of leaves for medical purposes isn't appropriate.
Key words: *Urtica dioica*, Nettle, HPLC, Chlorogenic acid.

Türkiye'nin Farklı Bölgelerinden Toplanmış *Urtica dioica* L. Örneklerinin Kalite Değerlendirmesi

Urtica dioica L. dünyanın ılıman bölgelerinde geniş yayılış gösteren bir bitkidir. Bitkinin yaprakları başta romatoit artrit olmak üzere, kardiyovasküler hastalıklarda ve diyabette kullanılmaktadır. Ayrıca antiviral, antioksidan ve anti-enflamatuvar aktivitelere sahiptir. Bitki Türkiye'de halk arasında her derde deva olarak tavsiye edilmektedir. Avrupa Farmakopesi'nde Isırgan yaprakları "Nettle Leaf – Urtica folium" olarak kayıtlıdı. Monografta yaprakların standardizasyonu Yüksek Basınçlı Sıvı Kromatografisi (YBSK) kullanılarak klorojenik asit üzerinden yapılıyor olmasına rağmen, Türkiye'de aktarlarda medikal amaçlarla yaprak yerine toprak üstü kısımları satıldığı bilinmektedir. Çalışmamızın amacı Türkiye'nin farklı bölgelerinden toplanan örneklerin içerdiği klorojenik asit miktarlarını birbirleriyle ve Avrupa Farmakopesi standardı ile karşılaştırarak örneklerin farmakopeye uygun olup olmadıklarını tespit etmektir. Ekstraksiyon prosedürü Avrupa Farmakopesi 6.0.'a göre gerçekleştirilmiştir. İnternal standart olarak *p*-kumarik asit kullanılarak klorojenik asit miktarları hesaplanmıştır.

Düzce, Maraş, Işık Dağı ve Palandöken Dağı'ndan toplanan yaprak numunelerindeki klorojenik asit miktarları sırasıyla %1.91±0.0003, %1.156±0.0019, %0.67±0.00068, %0.63±0.0024 oranlarında tespit edilmiş ve Farmakope standartlarına (≥ %0.3) uygun bulunmuştur. Toprak üstü kısımlarından hesaplanan klorojenik asit miktarları ise sırasıyla %1.047±0.0007, %0.751±0.0033, %0.31±0.0085, %0.488±0.0051 olarak tayin edilmiştir. Tüm numunelerin yapraklarında bulunan klorojenik asit miktarı toprak üstü kısımlarındakinden fazladır. Bu çalışma göstermiştir ki yapraklar yerine toprak üstü kısımların kullanılması uygun değildir.
Anahtar kelimeler: *Urtica dioica*, Isırgan,YPSK, Klorojenik asit.

***Correspondence:** E-mail: maslan1969@gmail.com; Tel:+903122023184; Fax Number: +903122235018.

INTRODUCTION

Urtica dioica L., known as Nettle, is a dioecious plant with opposite, sharply toothed leaves, persistent stipules, and stinging trichomes. It is widely distributed throughout the temperate regions of the world (1). The plant is very rich in terms of chemical constituents and has been used as herbal medicine, food, fiber, colour agent and cosmetic for many centuries. The aqueous methanolic extract of nettle roots has been used in clinics in Europe for the treatment of prostatic hyperplasia (2); *U. dioica* leaves are recommended for the complaints associated with rheumatoid arthritis (3), cardiovascular diseases and diabetes (4) but also have antiviral, antioxidant, anti-inflammatory activities. Furthermore, nettle leaves have demonstrated antiplatelet action, useful in the treatment and/or prevention of cardiovascular diseases (5).

In Turkey, *U. dioica* growing in border of field, road and forest naturally and is known with local names such as "Dızlağan, Ağdalak, Dalagan, Isırgan". Aerial parts and leaves of plant are used as folk remedy in different region of Anatolia. Moreover aqueous extract prepared from aerial parts have been occasionally used as an herbal medicine by cancer patients (6-10).

So called "Food Supplements" containing *Urtica dioica* produced in Turkey are sold mostly online and in akthars (herb shops) and these products are used as traditional medicine and claimed as panacea.

The number of scientific studies on stinging nettle and market values for different kind of pharmacological product derived from this plant has increased recently. *U. dioica* leaf is monographed in European Pharmacopeia as "Nettle Leaf - *Urtica folium*" and standardized by liquid chromatography for its chlorogenic acid content (11). Despite leaf is monographed in European Pharmacopeia, generally aerial parts are sold instead of leaves in herb shops in Turkey. However, any comprehensive and comparative study has not been performed on the quality assessment of the aerial parts and leaves of *U. dioica* growing in Turkey so far. The aim of present study is to determine quality of samples and compare the chlorogenic acid amount in aerial parts and leaves collected from different localities of Anatolia. For this purpose samples were extracted and analysed to compare the chlorogenic acid amount of leaves and aerial parts collected from different localities of Anatolia by RP-HPLC.

EXPERIMENTAL

Chemicals and standards

Chromatographic grade double distilled nanopure water, HPLC grade methanol, analytical grade phosphoric acid were used for analysis. Chlorogenic acid (C3878) and *p*-coumaric acid (C9008) were purchased from Sigma.

Plant materials

Plant materials were collected from different locations of Anatolia in 2010 (Table 1). Taxonomic identities of the samples were confirmed by Prof. Dr. Ekrem Sezik, a pharmacognost in the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara. Authenticated voucher specimens were deposited in the Herbarium of Faculty of Pharmacy, Gazi University.

Preparation of extracts

Extraction procedure was employed according to European Pharmacopeia 6.0. (11)

Internal standard solution

20.0 mg of *p*-coumaric acid R was dissolved in a 40 per cent V/V solution of methanol R and diluted to 200.0 mL with the same solution.

Test solution

25.0 mL internal standard solution was added to 0.2 g each of the powdered drug samples and extracted for 30 min in ultrasonic bath at 40 °C and filtered.

Reference solution

10.0 mg of chlorogenic acid CRS was dissolved in 100.0 ml of the internal standard solution.

Table 1. Location, collection periods and herbarium numbers of *Urtica dioica* L. samples

Sample Code	Locations	Collection Periods (2010)	Herbarium Numbers
UD1	Işık Mountain (Ankara)	July	2928
UD2	Palandöken Mountain (Erzurum)	August	3218
UD3	Giresun	October	3219
UD4	Güvem Village (Ankara)	September	3217
UD5	Ağva (İstanbul)	October	3216
UD6	Antalya	July	3220
UD7	Düzce	July	3221
UD8	Selçuk University (Konya)	June	2927
UD9	Yalova	October	3222
UD10	Kahramanmaraş	August	3223

High Pressure Liquid Chromatography Analysis

Instrument: Thermo Finnigan HPLC
Dedector: Thermo Finnigan: Surveyor PDA Dedector
Auto Sampler: Thermo Finnigan: Surveyor Autosampler
LC Pump: Thermo Finnigan: Surveyor LC Pump
Column: RP-18 Schim-pack 4.6x250 mm packed with 5µm
Time table: Described below

Method

Mobile phase A: mixture of 15 volumes of methanol R and 85 volume of water R adjusted to pH 2.0 with dilute phosphoric acid *R*
Mobile phase B: methanol *R*
Flow rate: 1 mL/min.
Detection: Spectrophotometer at 330 nm.
Injection: 20 µL of the reference solution and the test solution.
Column temperature: 25 °C

Time	(per cent V/V)	(per cent V/V)
0-1	100	0
1-25	100 → 85	0 → 15
25-35	85	15
35-36	85 → 0	15 → 100
36-37	0 → 100	100 → 0
37-41	100	0

Relative retention with reference to *p*-coumaric acid: chlorogenic acid = about 0.53.
Calculate the percentage content of chlorogenic acid (C_A) from the following expression:

$$\frac{A_1 \times A_4 \times C_1 \times 2500}{A_2 \times A_3 \times m_1}$$

A_1 = Area of the peak due to chlorogenic acid in the chromatogram obtained with the test solution;

A_2 = Area of the peak due to chlorogenic acid in the chromatogram obtained with the reference solution;

A_3 = Area of the peak due to *p*-coumaric acid in the chromatogram obtained with the test solution;

A_4 = Area of the peak due to *p*-coumaric acid in the chromatogram obtained with the reference solution;

m_1 = Mass of the drug to be examined, in milligrams;

C_1 = Content of chlorogenic acid in the reference solution, in milligrams per milliliter

Limits of detection and quantification

Limits of detection (LOD) were established at a signal to noise ratio (S/N) of 3. Limits of qualification (LOQ) were established at a signal to noise ratio (S/N) of 10. LOD and LOQ were experimentally verified by three injections of reference solution at the LOD and LOQ concentrations.

The LOD was 0.124 µg/mL and LOQ was 0.372 µg/mL for chlorogenic acid with RSD% less than 0.36% for triplicate injections.

Precision

The precision of the method was checked by injection three times of the reference solution at the LOQ level.

RP-HPLC analysis

Volumes of 20 µL of each prepared solutions of samples were injected into the column and chromatograms were recorded from 200 to 400 nm. Standard solutions were analyzed and three-dimensional chromatograms (wavelength; time; absorbance) were obtained to select the optimum wavelength for detection of chlorogenic acid with maximum sensitivity. Quantification was performed by setting the detection wavelength as 330 nm for chlorogenic acid using photodiode array detector. The results were obtained as a mean value of three separate injections by using internal standart method. The peaks in the chromatogram were identified by comparing the retention times and relative times according to the method described in European Pharmacopea 6.0.

RESULTS AND DISCUSSION

A number of study have been conducted on the chemical composition of plants and its biological activity so far by different research groups (12-15). Results obtained from previous studies indicated that presence of phenolic compounds in nettle extracts, prepared from stalks, leaves, and fibers, too, is an important result for the biological property (antioxidants and antiradicals) of these metabolites and, thus, for their possible application in various industrial activities, such as food/feed, cosmetic, phytomedicine, and textiles. Since *U. dioica* is a very important plant economically, its wild and cultivated forms have been used to prepare some officinal extract and and many investigations have been done to evaluate their quality. Thus, the nettle extracts obtained from leaves, both cultivated and wild, differ in phenolic composition from a quantitative point of view. In the fresh tissues of nettle three classes of phenolics were characterized: hydroxycinnamic acid derivatives (main compounds being chlorogenic acid and 2-O-caffeoyl-malic acid); flavonoids (rutin, quercetin p-coumaroyl-glucoside, kaempferol 3-O-glucoside, kaempferol 3-O-rutinoside, isorhamnetin 3-O-rutinoside); and anthocyanins [peonidin 3-O-rutinoside, rosinidin 3-Orutinoside, peonidin 3-O-(6″-O-p-coumaroylglucoside)] (12). Moreover the leaf samples contain large amounts of caffeic acid derivatives, in particular, chlorogenic and 2-O-caffeoylmalic acid. The flavonoids are represented by glycosides, in particular, quercetin 3-O-glucoside, and the 3-O-rutinosides of quercetin, kaempferol and isorhamnetin (13).

Many phenolic compounds are characterized in the leaves of *U. dioica*, European Pharmacopea 6.0 indicated that leaves must contain not less than 0.3% chlorogenic acid or caffeoylmalic acid.

In the view these data we determine and compare chlorogenic acid content of leaves and aerial parts of *U. dioica* samples. Chromatogram of chlorogenic acid and *p*-coumaric acid standards is given in Figure 1. Two examples of HPLC chromatograms are shown in Figures 2 and 3. Results are given in Table 2.

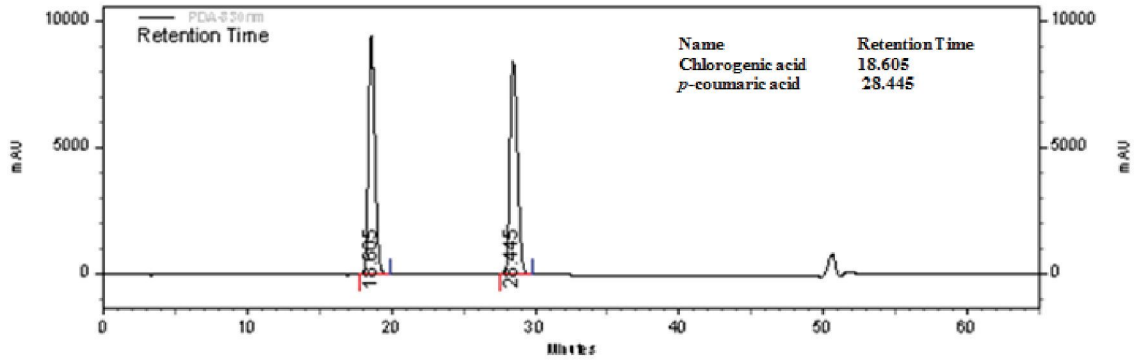

Figure 1. HPLC chromatogram of chlorogenic acid and *p*-coumaric acid.

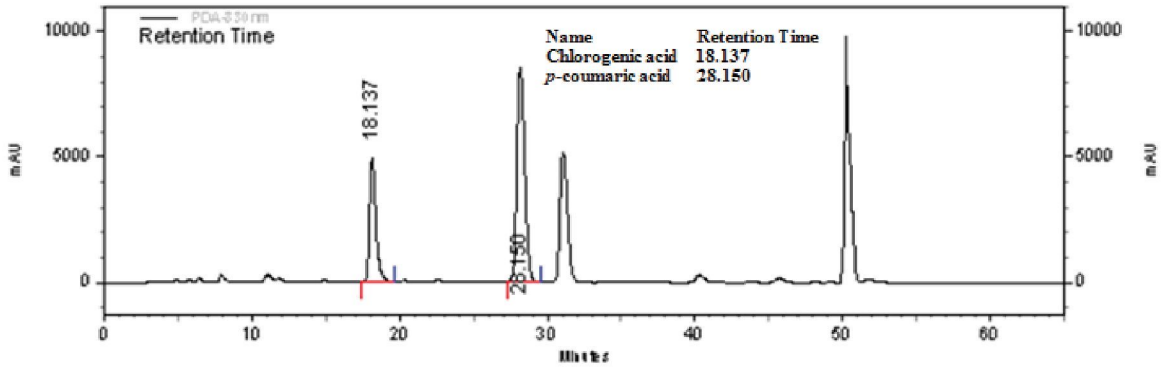

Figure 2. HPLC chromatogram of *Urtica dioica* leaves collected from Işık Mountain.

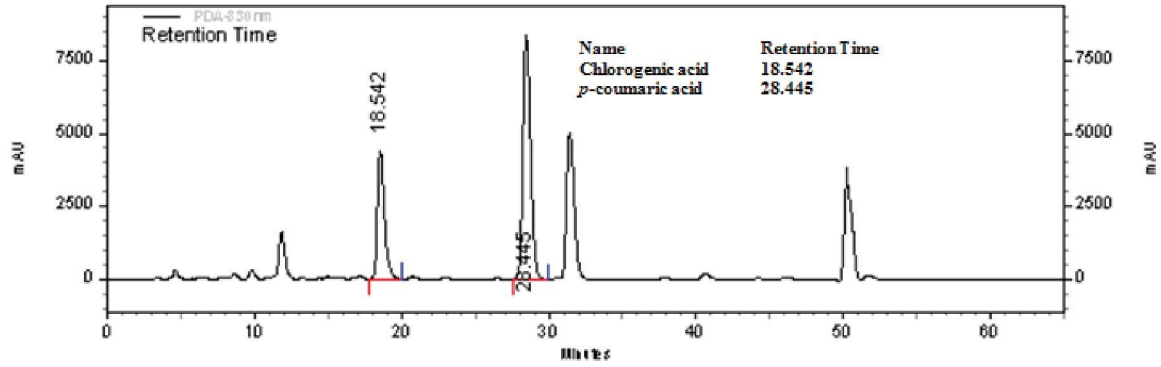

Figure 3. HPLC chromatogram of *Urtica dioica* leaves collected from Palandöken Mountain.

Table 2. Chlorogenic acid amount in the different samples of *U. dioica* leaves and aerial parts.

Sample	Chlorogenic acid (%)	
	leaves	aerial parts
UD1	0.67±0.00068	0.488±0.0051
UD2	0.63±0.0024	0.31±0.0085
UD3	0.07±0.0009	0.07±0.005
UD4	0.13±0.0013	0.12±0.0045
UD5	0.23±0.0073	0.14±0.0072
UD6	0.31±0.0017	0.24±0.0035
UD7	1.91±0.0003	1.047±0.0007
UD8	0.08±0.0010	0.05±0.0040
UD9	0.18±0.0021	0.17±.00028
UD10	1.156±0.0019	0.751±0.0033

As demonstrated in Table 2 chlorogenic acid contens of different *U. dioica* samples were analysed by RP-HPLC and the results indicated that the highest chlorogenic acid amount was found in the leaf samples collected from Düzce in July. According to European Pharmacopea 6.0 officinal Nettle leaves must contain not less than 0.3% chlorogenic acid. Chlorogenic acid contents of five samples were satisfactory based on European Pharmacopeia. All of these samples were collected in July and beginning of August. Samples collected in June and October contain less than 0.3% chlorogenic acid. On the other hand leaves and aerial parts of same samples were compared according to their chlorogenic acid amounts. In all of the samples, chlorogenic acid content was found higher in the leaves than in the aerial parts. For instance while UD6 leaves, collected from Antalya contain 0.31% chlorogenic acid, aerial parts of same sample contain 0.24% chlorogenic acid.

As seen in Table 2, samples collected in the beginning (June), and at the end (October) of flowering period (UD3, UD4, UD5, UD8, UD9) did not contain adequate chlorogenic acid.

Present study revealed that usage of the aerial parts instead of leaves isn't suitable for medical purposes.

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