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Original Article

The Effects of Plant Age and Harvesting Time on Chicoric and Caftaric Acids Content of *E. purpurea* (L.) Moench

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Abstract

Plants of Asteraceae are used in traditional medicine and phytotherapy. The two main caffeic acid derivatives, chicoric and caftaric acid which are found in many genus of Asteraceae including *Echinacea* exhibit important biological activities. The level of these acids in *E. purpurea* is affected by many factors such as growing situations, extraction methods, storage conditions and plant age. In this investigation, chicoric acid and caftaric acid content in aerial parts and roots of *E. purpurea* harvested in spring and summer from 1-, 2- and 3-years old plants cultivated in Iran were determined by using HPLC method. The results revealed that maximum level of chicoric acid achieved in aerial parts of 1- and 2-years old plants beside 2-years old roots collected in spring. Aerial parts of 1- and 2-years old plants harvested in spring had maximum content of caftaric acid as well. It is concluded that total parts of 2-years old *E. purpurea* harvested in spring can be a good source of caffeic acid derivatives and used for preparation of the plant products.

Keyword: Age of plant; Caftaric acid; Chicoric acid; *E. purpurea* (L.) Moench; Harvesting time.
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1. Introduction

The genus of *Echinacea* (Asteraceae) includes limited species. One of the three medicinal species is *E. purpurea* which is native to central and eastern parts of United States [1, 2]. It is cultivated in many other countries, including Iran and numerous pharmacological and phytochemical studies have been performed on this species [3].

In the past, *Echinacea* has been used against infectious diseases and snakebite [4, 5]. Nowadays, it is administered for prevention and treatment of colds and other respiratory tract infections to ameliorate the symptoms and

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shorten the duration of illness [2, 4, 6, 7]. The main constituents of *Echinacea* are alkamides, polysaccharides, caffeic acid derivatives, and glycoproteins [4]. These constituents are responsible for the plant effects such as immunomodulatory, antioxidant, anti-inflammatory, antifungal and antiviral activities [1, 3, 6, 8-18].

Chicoric acid and caftaric acid (Figure 1) are the main caffeic acid derivatives in *E. purpurea* which are responsible for some biological effects of the plant such as hyaluronidase inhibitory activity, antioxidant property and enhancement of insulin secretion [5, 8, 17].

The therapeutic effectiveness of different *Echinacea* products is depended on chemical composition of the plant. Many factors including species of *Echinacea* (E. purpurea, E. pallida or E. angustifolia), plant parts (leaves, flowers, stems or roots), plant age, the time of harvesting, grow¬ing, drying and storage conditions and method of extraction may influence the product quality [4, 10, 19-21]. So determination of the active ingredients level in different parts of the plant and best time of harvesting is very important for the industrial purposes. For this reason, we decided to measure chicoric acid and caftaric acid level in roots and aerial parts of *E. purpurea* harvested in spring and summer from 1- , 2- and 3-years old plants by using HPLC which is one of the most popular and precise method for analysis of herbal components [22-24].

2. Experimental

2.1. Plant material

Aerial parts and roots of *E. purpurea* were collected from 1-, 2- and 3-years old plants from their cultivating area in Gorgan, province of Golestan, Iran, by Dr. M.H. Soleimani, Giyah Essence Company and identified by Mrs. M. Khatamsaz, Botanist, from Research Institute of Forests and Rangelands (Tehran).

2.2. Chemicals

Acetonitrile (HPLC grade), ethanol (analytical grade), O-phosphoric acid (analytical grade) were purchased from Merck (Germany). The standard material of chlorogenic acid was prepared from Aldrich (Germany). The water used in HPLC and for sample preparation was produced with a Purelab UHQ (ELGA) with a resistivity over 18 MΩ-cm.

2.3. Instrumentation

HPLC experiment was performed using Waters Alliance 2695 system equipped with a vacuum degasser, quaternary solvent mixing, auto sampler and a waters 2996 photodiode array detector. UV spectra were collected across the range of 200-900 nm

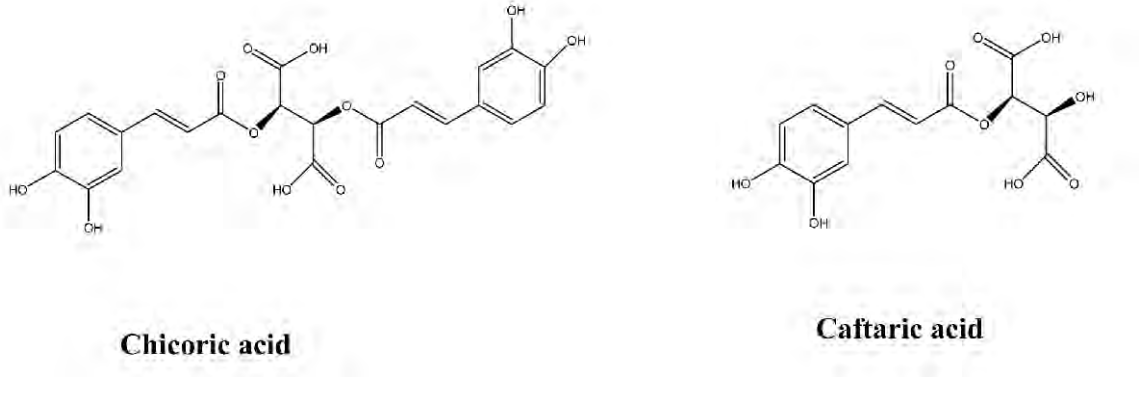

Figure 1. Structures of chicoric acid and caftaric acid.

extracting 330 nm for chromatograms. Empower™ chromatography data software was utilized for instrument control, data collection and processing. The column, an ACE C_{18} (4.6-mm 25cm, 5µm) was maintained at 35 ˚C. The mobile phase was a mixture of acetonitrile and phosphoric acid 0.085% in gradient mode (Table 1). The flow rate was 1.5 ml/minute. Injection volume for all test and standard solutions was 10 µl. Each test solution was injected 3 times.

2.4. Preparation of solutions

2.4.1. Standard solutions preparation

Stock standard solution was prepared accurately by weighing 10 mg of chlorogenic acid reference standard into 10 ml volumetric flask and dissolving in ethanol:water (7:3). Serial dilutions (10-500 µg/ml) were made from the stock solution.

2.4.2. Sample preparation

For the extraction of phenolic compounds of aerial parts and roots of *E. purpurea*, 125 mg of dried and finely powdered material from each plant sample was accurately weighed and transferred to a round bottom flask, and 25 ml of solvent (ethanol:water, 7:3) was added and heated under reflux system for 15 min. The resulting mixture was filtered and diluted to 25 ml with the solvent [25]. Three replicate of each sample were prepared as described.

2.5. Determination of chicoric and caftaric acid content in the plant samples

Quantitative determination of caftaric and chicoric acid was performed by using chlorogenic acid standard calibration curve. Since different compounds absorb various amounts of radiation at a fixed wavelength, it is necessary to use a correction factor to calculate caftaric and chicoric acid percentage by this method. This factor is determined by BP for each of the compounds, and they are equal to 0.695 and 0.881 for chicoric acid and caftaric acid, respectively [25]. At first, areas under the caftaric and chicoric acid curves were multiplied in the related correction factor to obtain area under the curve according to chlorogenic acid. Then concentration of the compounds in the test solution was calculated using the calibration curve and following equation:

$$\%w/w=(C\times V)/10M$$

C: concentration of phenolic compound (ppm) from linear regression analysis;
V: Extract solution volume (25 ml);
M: Sample weight (125 mg).

2.6. Statistical analysis

Comparisons between groups were made by SPSS software and ANOVA test.

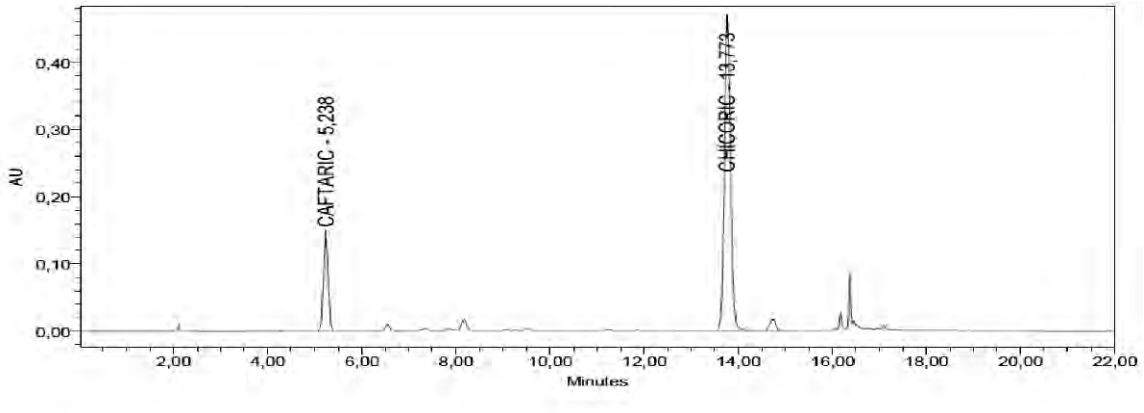

Figure 2. HPLC chromatogram of a sample from spring/2y *E. purpurea* aerial parts; the 5.2 min peak is related to caftaric acid, and 13.8 min peak is related to chicoric acid.

Differences with $p<0.05$ between groups were considered statistically significant.

3. Results and discussion

Main peaks were observed in retention times about 5.2 and 13.8 min in the HPLC chromatograms of the test solutions (Figure 2). Chlorogenic acid ST peaks appeared about 5.7 min in reference standard solutions chromatograms. Caftaric and chicoric acid were identified by comparison with authentic chromatogram in British Pharmacopeia [25]. Relative retention times with reference to chlorogenic acid ST were about 0.9 and 2.4 for two main peaks in test chromatograms and had good compatibility with BP relative retention times for caftaric and chicoric acid, respectively. So, main peaks have been recognized as caftaric (5.2 min) and chicoric acid (13.8 min).

According to chlorogenic acid standard calibration curve (y=2.0708x-10.361, r^2=0.9993), the content of both phenolic compounds in the plant samples were determined.

Caftaric and chicoric acid percentages in the aerial parts and roots of *E. purpurea* are shown in Figures 3 and 4, respectively.

The results of quantitative determination of chicoric acid and caftaric acid by HPLC demonstrated that 1- and 2-years old aerial parts, in addition 2-years old roots harvested in spring had maximum amount of chicoric acid and there was no statistical difference between these 3 sample groups ($p>0.05$). Maximum level of caftaric acid found in aerial parts of 1- and 2-years old plants harvested in spring. The minimum amounts of caftaric and chicoric acid were observed in the roots collected in summer from 2-years old plants.

The results also showed that in all samples, caftaric acid content of the roots was less than the aerial parts, but no distinct manner was found in chicoric acid content of roots and aerial parts. Chicoric acid amount was higher in the aerial parts than the roots in the plants harvested in spring (1 and 3 years) and summer (2 and 3 years), and it was higher in roots of summer (1 year) samples. Spring (2 years) roots and aerial parts had the same content of chicoric acid.

Chicoric acid is subsequently used as the indicator of caffeoyl phenols in the commercial products [26]. As it is obvious in Figures 2 and 3, chicoric acid content in the plant samples is higher than caftaric acid, so in general, it can be concluded that spring (2 years) plant has the best quality according to phenolic compounds and it is better to use total parts of the plant for preparation of the *Echinacea* products.

In a study, the effects of plantation age of *E. purpurea* on the flavonoids and phenolics

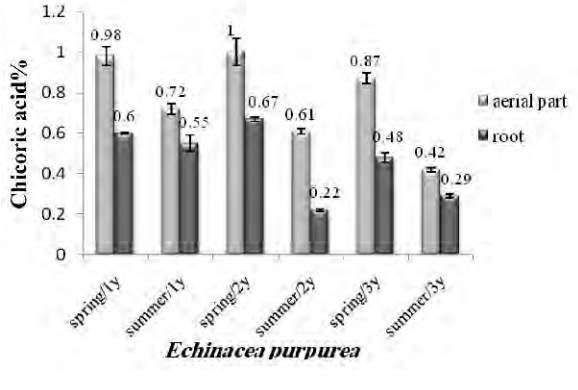

Figure 3. Caftaric acid content in aerial parts and roots of *E. purpurea*, harvested from 1-, 2- and 3-years old plants in spring and summer.

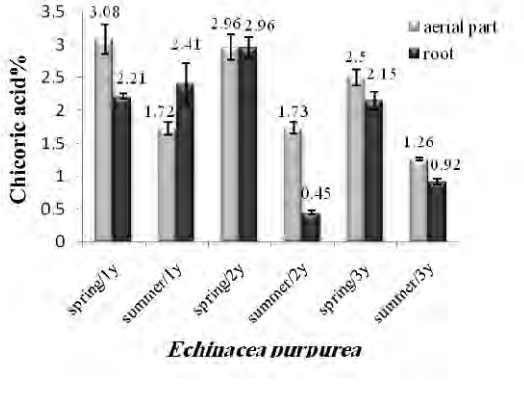

Figure 4. Chicoric acid content in aerial parts and roots of *E. purpurea*, harvested from 1-, 2- and 3-years old plants in spring and summer.

content has been investigated and no relationship was found between age of plants and flavonoid and phenolic content in flower heads. This study has proved that the phenolic acids content was slightly higher in the flower heads than in the rhizomes [27]. Another investigation indicated that *E. purpurea* flower heads from 2-years old cultivated plants contain about the same amount of chicoric acid as the young roots of that species. They demonstrated that caffeic acid derivatives in *Echinacea* contain compounds with closely structure that vary throughout the growth of the plants. In that study, the content of phenolic compounds of *E. purpurea* roots has been decreased with age [28]. According to references [25, 29] aerial parts of *E. purpurea* and roots of E. angustifolia are used as medicinal parts but this study showed that the cultivated plant in Iran is rich in phenolics in both aerial parts and roots, therefore, in order to produce a qualified product from *E. purpurea* it is necessary to consider the age and harvesting time of the plant.

4. Conclusion

Some of the most important factors which cause inconsistency in industrial products are plant parts and plant age in the time of harvesting. This study showed that 2- years old *E. purpurea* aerial parts and roots harvested in spring had the most content of chicoric and caftaric acids and it is better to use total plant for preparation of *E. purpurea* products.

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