Determination of Cichoric Acid as a Biomarker in *Echinacea Purpurea* Cultivated in Iran Using High Performance Liquid Chromatography

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Chinese Medicine, 2010, 1, 23-27
doi:10.4236/cm.2010.11004 Published Online June 2010 (http://www.SciRP.org/journal/cm)

Determination of Cichoric Acid as a Biomarker in Echinacea Purpurea Cultivated in Iran Using High Performance Liquid Chromatography

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Received March 4, 2010; *revised April* 29, 2010; *accepted May* 10, 2010

Abstract

Echinacea purpurea (Purple coneflower) is an immunostimulating drug, containing multiple substances. The most important substance in activity is polysaccharide, caffeic acid derivatives (cichoric acid), alkamides and glycoproteins. It is not clear yet, which substances are responsible for activity. Cichoric acid is an appropriate marker of the quality of Echinacea purpurea containing product, because it has immune stimulatory effects and it is susceptible to degradation. In this study a TLC scanner system and HPLC method has been used for identification and determination of cichoric acid in aerial parts of Echinacea purpurea. The results showed that the cichoric acid content of Echinacea purpurea cultivated in Iran is about 1.50 ± 0.65% (w/w) which is comparable with cichoric acid content in native plants. The local conditions have no significant effect on cichoric acid content as a biomarker of Echinacea purpurea quality.

Keywords: Echinacea Purpurea, Cichoric Acid, TLC, HPLC, Iran

Aims

Effect of cichoric acid on *Echinacea Purpurea* which work as immune stimulating drugs

Determine effect of cichoric acid by using TLC and HPLC method

The cichoric acid content of *Echinacea purpurea* is appropriate for drugs in comparison with native plants Europe and America

Why herbal medicines are best in pharmacological use?!

because the **Herbal medicine** has lower side effects in comparison with chemical drugs.

So, the attention and stimulation for using are increasing in recent years.

Echinacea purpurea (purple coneflower) is an immune stimulating drug , originated in the United States of America and was brought to Europe in the late 19th century.

Echinacea purpurea has been cultivated in some parts of Iran (Isfahan) and used in some herbal drugs.

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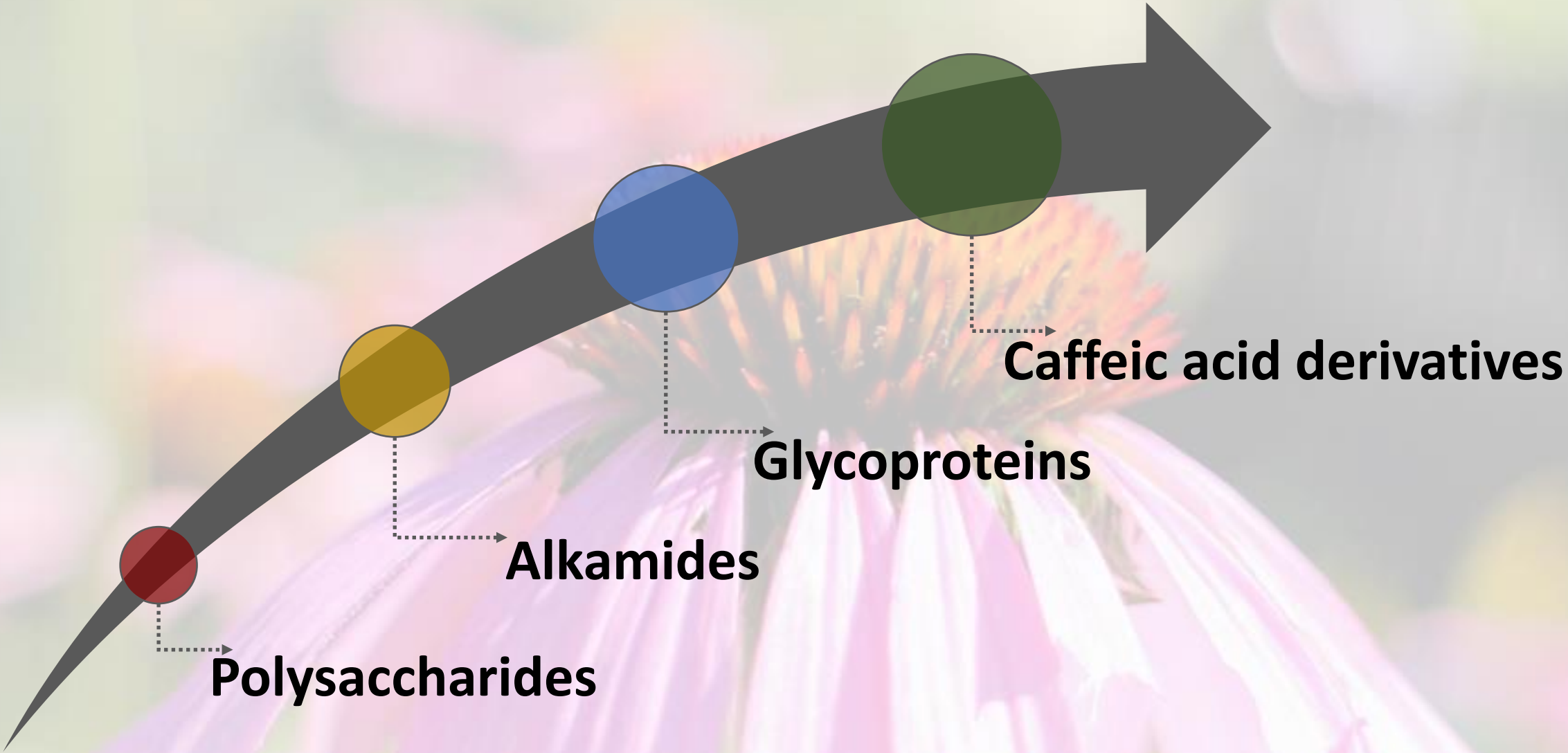
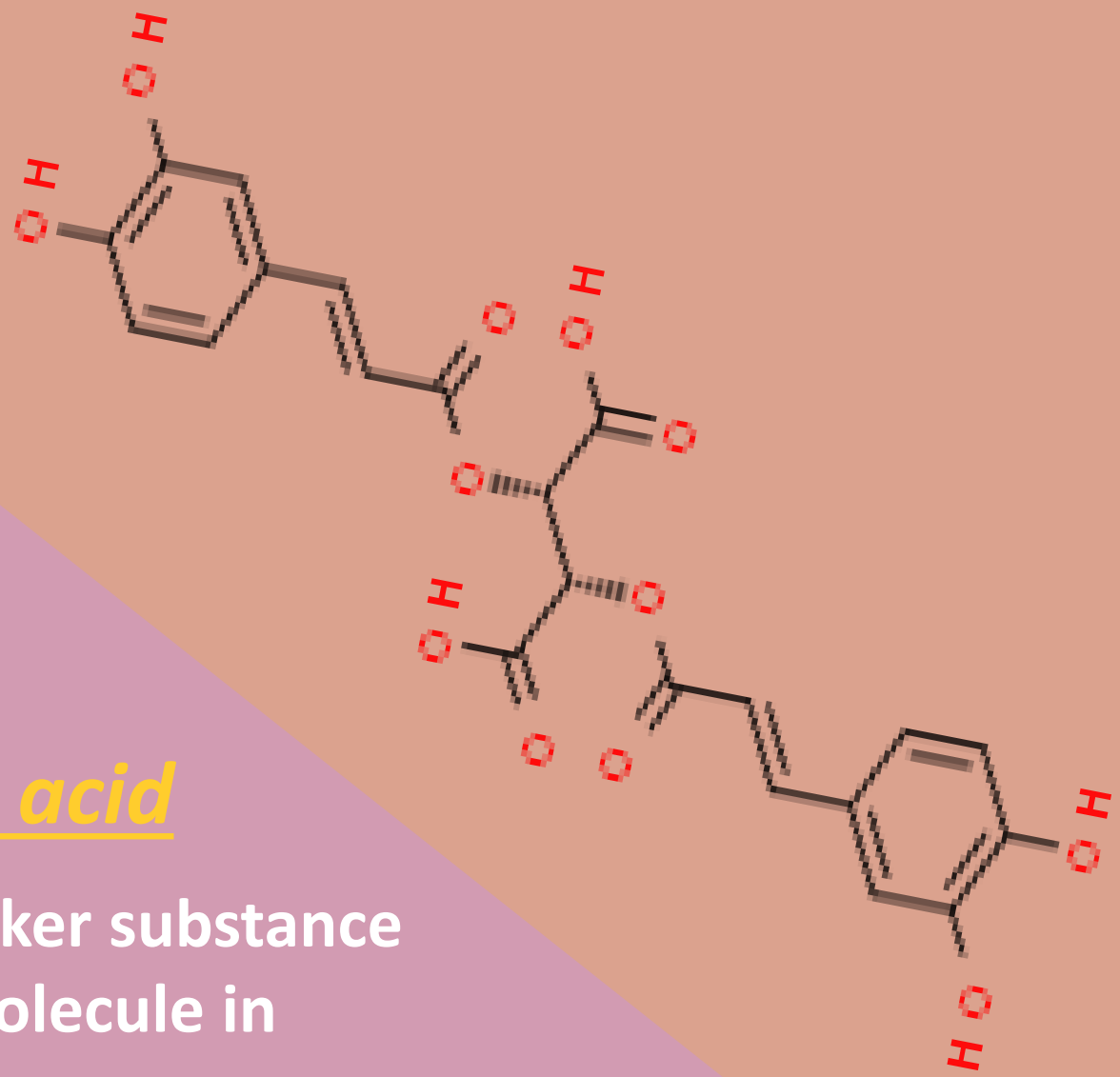
The active compounds in purple coneflower





Especially cichoric acid

Cichoric Acid the marker substance and most effective molecule in *Echinacea purpurea.*

In this study, a TLC scanner system and an HPLC method was used for identification and quantification of cichoric acid content in aerial parts of *Echinacea purpurea*.

TLC scanner system

HPLC

Experimental

Chemicals

- 2-aminoethyl diphenyl borinate (Sigma)

- caffeic acid

- chlorogenic acid

Solvent

- Methanol
- Ethyl acetate
- Formic acid

All solvents used for running HPLC, were HPLC grade

Deionized

- Double distilled water was used

Samples

- Several different batches of aerial parts of aerated and dried *Echinacea purpurea* were collected and ground with a 40 mm mesh to a fine powder.

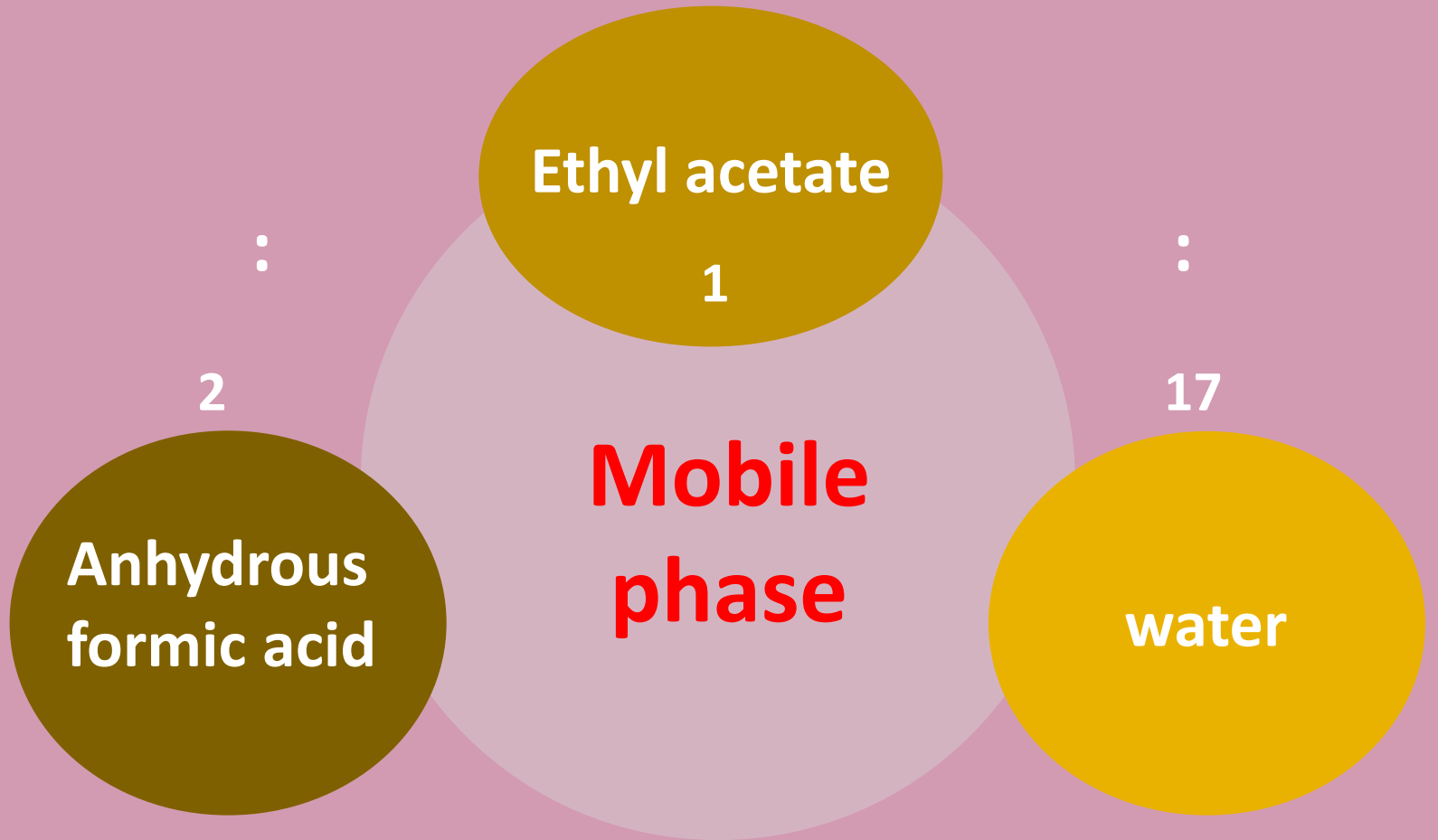
- Samples were prepared by solving the powder in methanol/water (70:30) with the ratio of 1 g in 10 ml using an ultrasonic bath for approximately 5 min and then filtered.

- Chlorogenic acid and caffeic acid were dissolved in alcohol/water.

TLC and HPLC

-The CAMAG TLC system with auto sampler and scanner which is controlled by Wincats software was used for identification

-The plates were prepared by TLC silica gel HF254 (5 µm).



Sample solution and standard solution were prepared respectively by:

dissolving 0.5 g powder in 5 ml solvent

AND

0.05 mg of caffeic acid and 0.05 mg of chlorogenic acid in 10 ml solvent

=

25 µl of sample solution and 10 µl of standard solutions were applied as TLC bands

For detection of stains under UV366 lamp add reagent

2-amino diphenyl borinate in methanol (10 mg/ml)

UV366 lamp

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High Performance Liquid Chromatography (HPLC)

The Cecil 1100 HPLC system was used for quantitative assay
This system equipped
a 20 µl injection loop
a C18 Lichrosorb column with 0.25 m length, and 4.6 mm

mobile phases is acetonitrile and phosphate buffer (phosphoric acid/water (1:99 V/V) in a gradient elution of solvents with 1.5 ml/min flow rate according to set programs in EP and USP .

Sample solution and standard solution were prepared by dissolving 0.25 mg of powder of sample and 0.47 mg chlorogenic acid in 10 ml solvent respectively.

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High Performance Liquid Chromatography (HPLC)

The percentage of cichoric acid content was calculated by the following equation:

$$Cu\% = 100\ (Au \times Cs \times 0.695)/(As \times Ct)$$

Cu is the concentration of cichoric acid in sample peak
Au, the peak area for cichoric acid
Cs, the concentration of standard chlorogenic acid solution (mg/ml)
Ct, amount of powder in sample solution (mg/ml) 0.0695, the response factor of cichoric acid relative to that of chlorogenic acid

The detector response factor was planned as the ratio of slope of the two calibration curves prepared with standard solutions.

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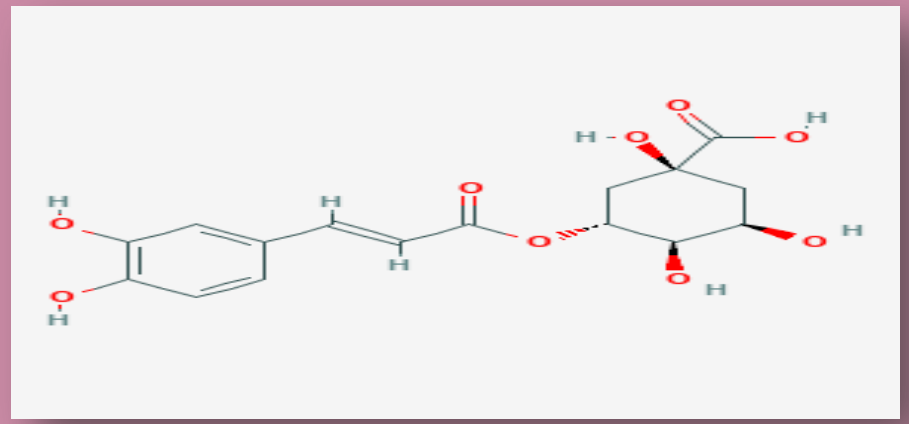
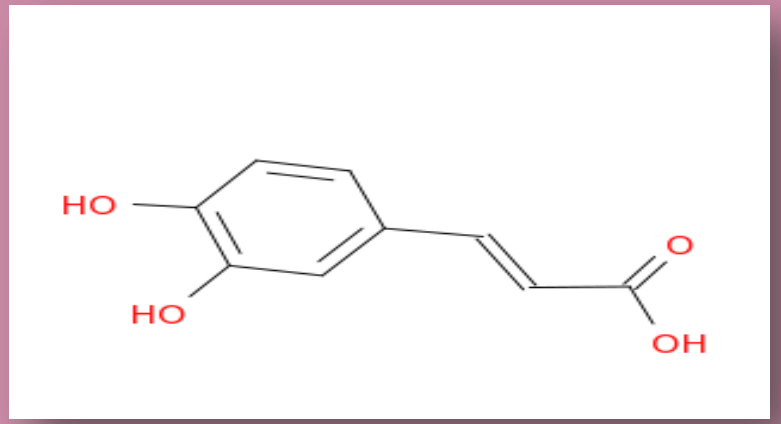
Results and Discussion

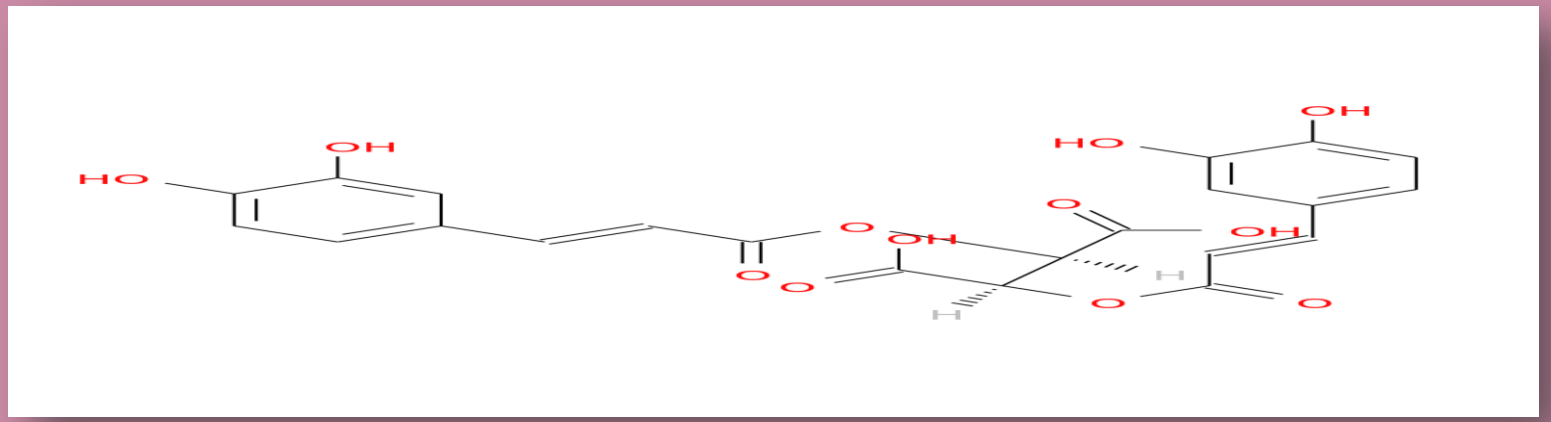
Chemical structure



Chlorogenic acid



Caffeic acid



Cichoric acid

Image of TLC plate of sample and standard solutions.

Caffeic acid

Chlorogenic acid

Cichoric acid

Caftaric acid

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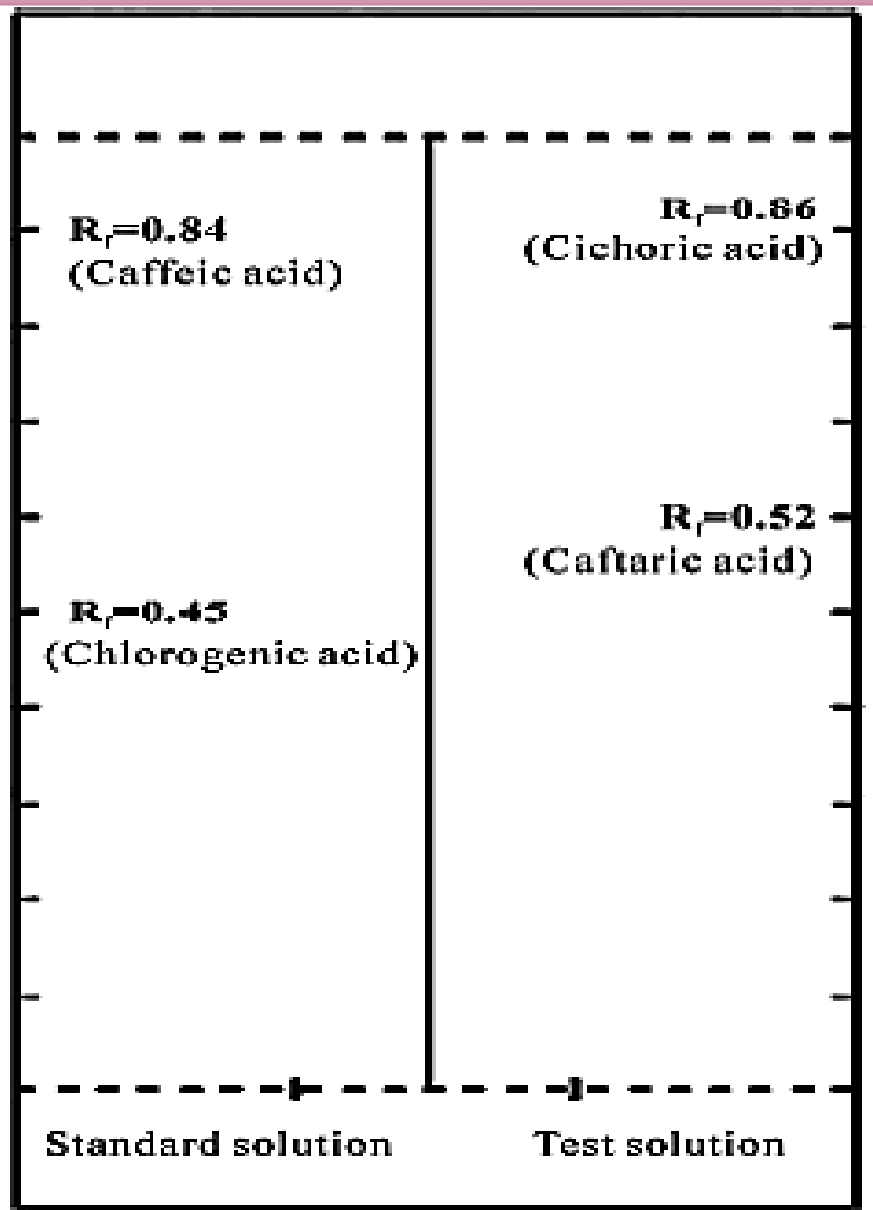
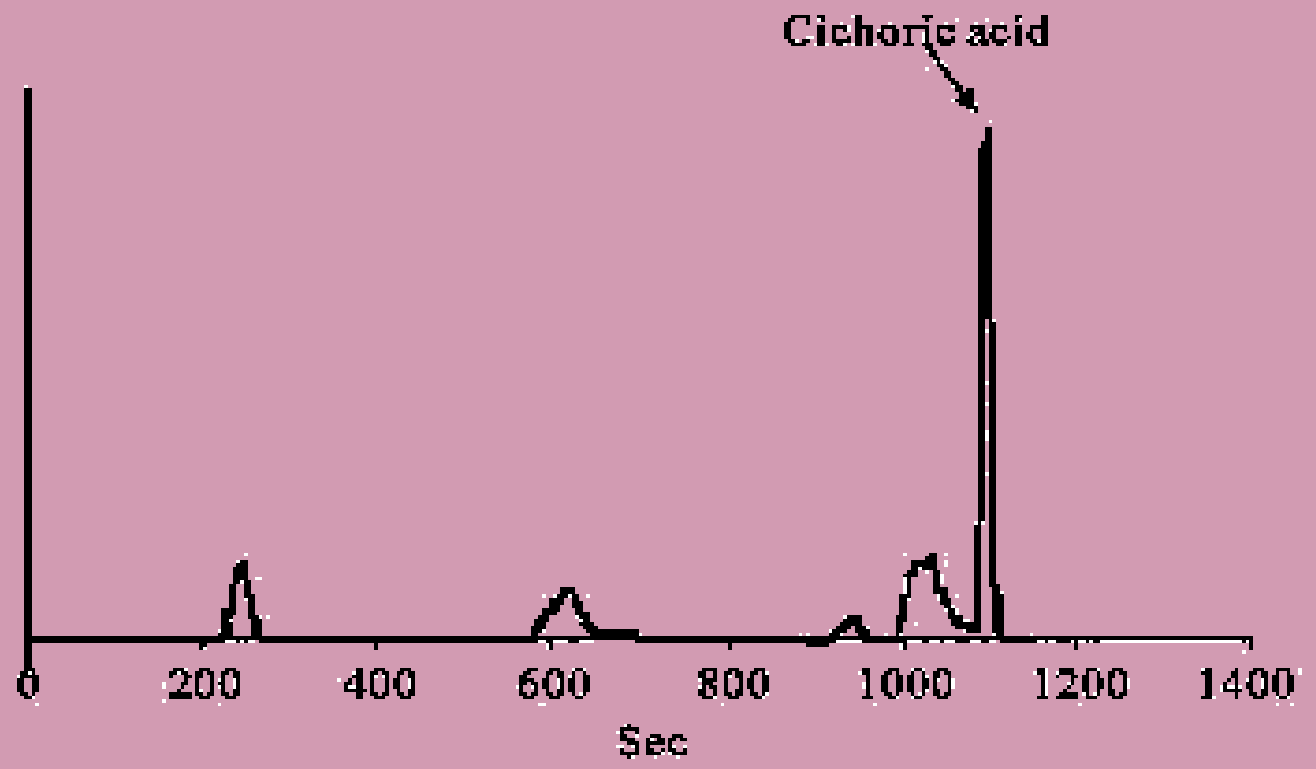
TLC schematic of sample and standard solutions



The chromatograms related to standard and sample



HPLC chromatogram of 2.5 mg/10 ml sample solution.

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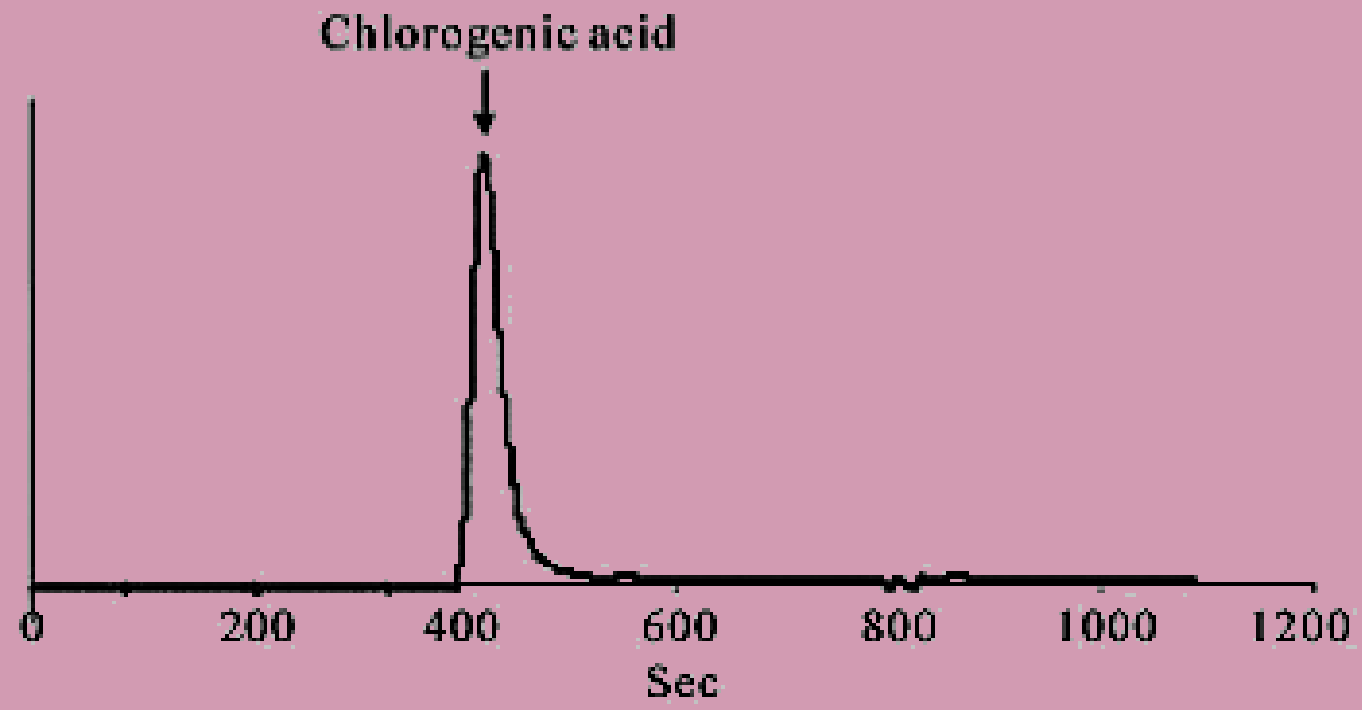
The chromatograms related to standard and sample



HPLC chromatogram of 0.25 mg/5 ml standard solution (chlorogenic acid).

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-The HPLC experimental data obtained is shown in Table 1

-Calculation results shows that cichoric acid concentration is about (1.5 ± 0.65)% (w/w) in the aerial parts of *Echinacea purpurea* cultivated in Iran.

Table 1. The HPLC experimental data.

C_s	0.047 mg/mL
C_t	2.5 mg/mL
A_s	4934823
A_u	5977445
C_u	%1.58
S_D	%0.65±

Cs: concentration of standard solution (chlorogenic acid).
Ct: concentration of sample solution containing cichoric acid.
Cu: concentration of cichoric acid in sample solution.

As: the peak area for chlorogenic acid.
Au: the peak area for cichoric acid.
SD: Standard deviation (n = 3).

This means that the local conditions of cultivation of this herbal drug have no significant effects on its medicine marker.

Conclusion

The qualitative and quantitative analysis of cichoric acid content in *Echinacea purpurea* which is cultivated in Iran has been studied.

Cichoric acid is probably one of the most important markers for controlling the quality of drugs containing *Echinacea purpurea*.

The results showed that the cichoric acid content of *Echinacea purpurea* is appropriate for drugs in comparison with native plants.

Reference

Zolgharnein, J., Niazi, A., Afiuni-zadeh, S. & Zamani, K. (2010). Determination of Cichoric Acid as a Biomarker in *Echinacea Purpurea* Cultivated in Iran Using High Performance Liquid Chromatography. *Chinese Medicine.* 1. pp: 23-27.

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