Journal of Chemical Health Risks

Journal of Chemical Health Risks (2015) 5(1), 15–19

ORIGINAL ARTICLE

Evaluation of Cichoric Acid of *Echinacea purpurea* Extract under Different Ecological Conditions in Semnan Province Iran

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(Received: 16 September 2014 Accepted: 17 November 2014)

KEYWORDS

Echinacea purpurea
Extract
Cichoric acid
HPLC

ABSTRACT: *Echinacea purpurea* is an herbaceous perennial plant as members of the Asteraceae family. It is one the important medicinal plant in pharmacy industrial. Active substances of *Echinaceae* are amplifier of body's immune system and antivirus. *Echinacea* is not native to Iran. The phytochemical traits of medicinal plants depend on ecological conditions involving growing areas, climate conditions; various grow stages and genetic modifications. The aim of this study was the evaluation of cichoric acid of *E. purpurea* extract in different ecological conditions in Semnan, Iran. Dormancy in seeds was broken by treating them with strafication in 4 °C for 48 h, was grown in nursery beds for autumn and early winter and transfered to four areas with different ecological conditions in late winter. Chashm, Ahuvan, Semnan and Foladmahale were chosen for this experiment. The spacing of plantlet was about 25 × 45 cm in the field. After 2- 3 months, aerial parts of the plants collected in all areas and extract samples were prepared and characterized using high- performance liquid chromatography (HPLC). The components of a basic HPLC system were shown significant values of the cichoric acid in plants under four habitats. The level of cichoric acid in one condition was more than standards level that reported in another references. In addition, ecological diversities have significant impacts the quantity of cichoric acid in *E. purpurea*.

INTRODUCTION

Herbal medicine has lower side effects in comparison with chemical drugs. Attention and stimulation for using herbal drugs are increasing in recent years. Systematic study about characteristics properties of herbal drug has been increased. *Echinacea purpurea* Monech (purple coneflower), originated in the United States of America and was brought to Europe in the late 19th century [1]. *Echinacea purpurea* has been cultivated in some parts of Iran (e.g. Isfahan) and used in some herbal drugs. It exhibits antioxidative,

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antibacterial, antiviral and antifungal activities, but most of it effect on various immune parameters [2]. Because of its immuno-enhancing activity, it has been recently used in AIDS therapy [3]. It is used for treating influenza and cold, against which symptomatic treatment is an established procedure.

Most important potential active compounds in purple coneflower are polysaccharides, caffeic acid derivatives (especially cichoric acid), alkamides and glycoproteins [4]. Cichoric acid is an appropriate marker since it has immune stimulatory effects and is probably the most effective substance in *E. purpurea* (Figure 1).

Various extraction techniques have been developed to obtain phytochemicals from plant materials [5]. Both the classical extraction methods including solvent extraction with or without mechanical agitation (or shaking) and some novel techniques such as maceration and microwave-assisted extraction are fast and efficient for extracting phytochemicals from plant materials [6]. Various methods for the determination of cichoric acid such as HPLC have been used [7].

Climate and cultivation conditions could affect cichoric acid content in *E. purpurea.* Recently, deficit or lack of studies on the adaptability of the culture, the productivity and the quality of the product was apparent.

The goals of this research were to find the best region for *E. purpurea* cultivation with higher levels of cichoric acid.

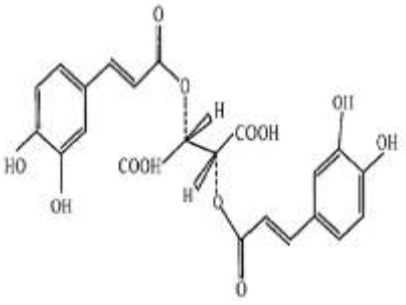

Figure 1. Structure of Cichoric acid [8]

MATERIALS AND METHODS

Station select

The natural habitat of south Alborz in Semnan region, Iran is a unique area with special climate and ecology, so that many different plants can grow in this condition. This region has different environment with much temperature changes. In order to evaluate cichoric acid from *E. purpurea* extract, four regions with different ecological characteristic as Chashm, Ahuvan, Semnan and Fouladmahale were chosen for this experiment. Table 1 and 2 respectively show the ecological characteristics and soil for all areas.

Planting

Overall, 250 g-Seeds of *E. purpurea* for implementing this experiment**,** prepared from Pakan Bazr Company in Esfahan**.** Dormancy in seeds was broken by treating them with strafication in 4°C for 48h. Plants were grown in nursery beds for autumn and early winter and transferred to four areas with different ecological conditions in late winter (Chashm, Ahuvan, Semnan and Foladmahale). The spacing of plantlet was about 25 × 45 cm in the field. During experiment, any fertilizer did not add to soil in 4 regions. After 2-3 months, aerial parts of the plants collected in all areas.

Extraction

After 2-3 months, aerial parts of the plants were collected in all areas and dried in shadow. Dried aerial parts of plants were powdered with mill. The extract was produced my maceration method. For extraction 2 gm of dried powder was solved with 20 ml methanol: water (70:30, v/v) and placed for 24 h in 4 ^{o}C in refrigerator. After that, samples were filtered with filter papers. The methanolic extract was centrifuged for 5 min (3000 rpm)**.** Extract of each sample was filtered with filter papers. Finally the extracts in glass tubes, were transferred to phytochemical laboratory.

Table 1. Ecological characteristics in four regions

Number	Location	Geographic Coordinate	Altitude (m)	Average annual temperature (C°)	Average annual rainfall (mm)	Maximum absolute temperature (C°)	Minimum absolute temperature (C°)
1	Chashm	$53^{0}15^{\prime}29^{\prime\prime}$E $35^{0}53^{\prime}46^{\prime\prime}$N	2153	6	497	29	-17.2
2	Semnan	$53^{0}23^{\prime}10^{\prime\prime}$E $35^{0}34^{\prime}32^{\prime\prime}$N	1130	17.6	140	44.5	-4.6
3	Foladmahale	$53^{0}42^{\prime}20^{\prime\prime}$E $36^{0}3^{\prime}40^{\prime\prime}$N	1860	10	390	32	-9
4	Ahuvan	$53^{0}41^{\prime}17^{\prime\prime}$E $35^{0}45^{\prime}14^{\prime\prime}$N	1720	14	220	35	-6

Table 2. Soil characteristics in four regions

Number	Location	EC (ds/m)	pH	Organic materials (%)	Organic carbon (%)	Carbonate (mg/ Lit)	Bicarbonate (mg/Lit)	N (%)	P (ppm)	K (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
1	Chashm	1.07	7.42	1.34	0.019	0	1.6	0.02	7.5	370	40	24	36	Silty loamy
2	Semnan	0.96	6.78	1.65	0.245	0	1.8	0.04	8.2	541	30	34	36	Silty loamy
3	Foladmahale	1.86	7.66	0.65	0.014	1	2.45	0.01	6.5	263	50	40	10	Sandy loamy
4	Ahuvan	3.1	7.92	1.05	0.035	1	3.53	0.04	14.5	437	42	25	33	Silty loamy

Chromatography

The general characteristics of HPLC for recognition of chicoric acid in this experiment are represented in Table 3.

Table 3. Characteristic of HPLC for recognition of chicoric acid

Column	**3.9*300mm**
Stationary phase	Octadecylsilyl silica gel for chromatography R(C18) (5µm)
Temperature	35^{0}C
Reference solution	Standard 1: Chicoric acids solution in ethanol 70%: 53ppm Standard 2: Chicoric acids solution in ethanol 70%:80ppm
Mobile phase	Mobile phase A: phosphoric acids R, double distilled water (1: 999 v/v) Mobile phase B:acetonitrile R

Time (min)	Mobile phase A (percent v/v)	Mobile phase B (percent v/v)
0	90	10
0-13	90 → 78	10 → 22
13-14	78 → 60	22 →40
14-14.5	60	40

Flow-rate	1.5 mL/min
Detection	Spectrophotometer (UV)_ at λ=330 nm
Injection volume	10 µL

RESULTS AND DISCUSSION

Injection summary report

Reports of injection to HPLC is shown in Figure 2.

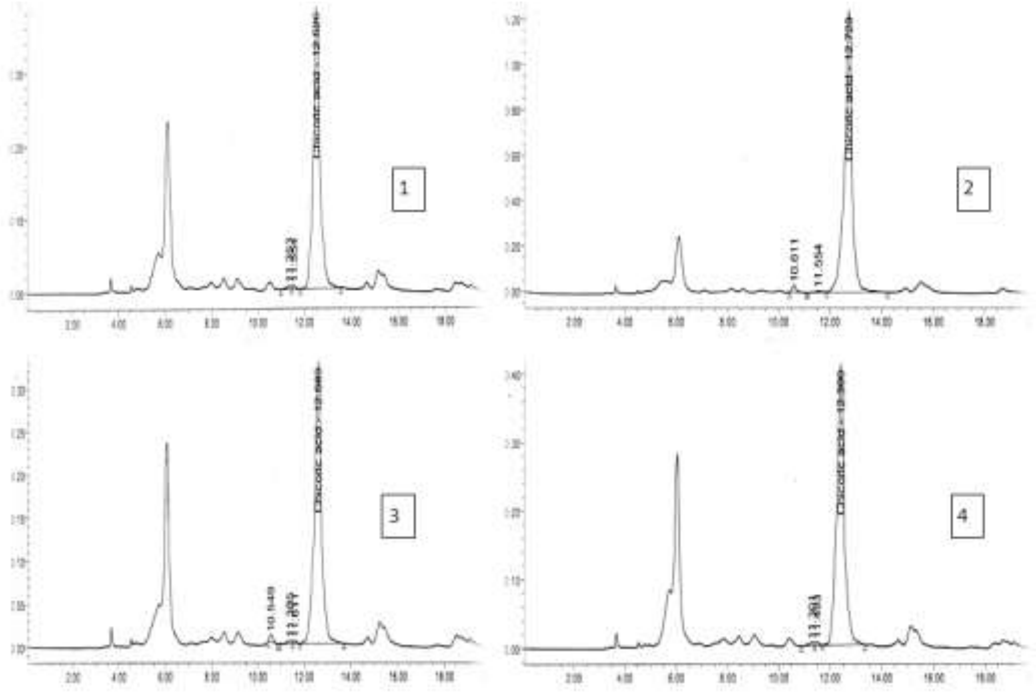

Figure 2. Graphs report of injection to HPLC (1: Ahuvan 2: Chashm 3: Fouladmahale 4: Semnan)

Quantity of cichoric acid was different in extract of 4 different ecological conditions in *E. purpurea* (Table 4). The highest level of chchoric acid in areal parts of *E. purpurea* was about 2.5% in another study [10] but in this research quantity of cichoric acid in Chashm plants extract was 2.83%, which was more than it.

Table 4. Quantity of Cichoric acid in 4 regions of Semnan, Iran

Region	Quantity of Cichoric acid (%)
Ahuvan	1.25
Chashm	2.83
Fouladmahale	2.11
Semnan	0.98

Effect of different ecological conditions on active compounds in other medicinal plants has been proved [9, 10]. However, altitude and temperature of Ahuvan region is similar to Chashm region but due to rainfall difference, quantity of cichoric acid in Ahuvan is very lower than Chashm.

According the result of this research by increasing altitude and rainfall and decreasing temperature, quantity of cichoric acid is increased.

Research on evaluation adaptability *of E. purpurea* in Iran implemented in Zardband (North of Tehran) showed that these regions are suitable for *E. purpurea* planting [11]. These regions have similar ecological conditions to Chashm in Semnan.

In addition, regions with ecological conditions similar to Chashm are suitable region for planting and extraction cichoric acid of *E. purpurea*.

CONCLUSIONS

The evaluation of cichoric acid of *E. purpurea* extract in different ecological conditions in Semnan Province, Iran show that different ecological condition are effective on quantity of cichoric acid. In addition, region Chashm has the best conditions of the tree other regions for cultivate *E. purpurea*.

ACKNOWLEDGMENTS

The authors declare that there is no conflict of interests.

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