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Effect of different drying methods on quantity and quality of active substances of Purple coneflower (*Echinacea purpureae* L.)

Taleb Ghabaei[*1], Somayeh Nazirzadeh[2], Hassan Nourafcan[3]

[1]*Young Researchers and Elite Club, Ardabil Branch, Islamic Azad University, Ardabil, Iran;*
Email:talebghabaei@yahoo.com*

[2]*Islamic Azad University, Saveh, Iran;*
[3]*Department of Horticulture, College of Agriculture, Miyaneh Branch, Islamic Azad University, Miyaneh, Iran;*

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ABSTRACT

Background & Aim: The genus Echinacea (family Asteraceae), commonly known as purple coneflower, is an important medicinal plant that has been used for centuries, customarily as a treatment for the common cold, coughs, bronchitis, upper respiratory infections, and some inflammatory conditions . The present study was carried out to evaluate the effect of six different methods of drying to obtain the highest of active substances from *Echinacea purpurea*.

Experimental: To determine the effect of drying methods on quantity and quality of active substances, the samples were dried with six drying methods (sun, shade, greenhouse and industrial-drying at 50° C, 60° C and 80 ° C). Active substances of aerial parts were extracted by ultrasonic method and the amounts caffeic acid derivatives, alkamides (8/9) were measured by using high performance liquid chromatography analysis, and also total phenolics determined with spectrophotometer.

Results: The results showed that different drying methods had significant effects on active substances. Samples dried in greenhouse had higher amounts of chlorogenic acid (3.18 mg/g.dw), echinacoside (4.29 mg/g.dw), cynarin (1.55 mg/g.dw) and total phenols contents (363 mg / g.dw). Also, samples dried in shade had higher amounts of caftaric acid (25.8 mg / g.dw), cichoric acid (36.57 mg / g.dw) and alkamides (361.1 µg / g.dw).

Recommended applications/ industries: Greenhouse drying and shade-drying were proper methods of drying to achieve more amounts of active compounds in *E. purpurea.*

1. Introduction

The genus *Echinacea*(family Asteraceae), commonly known as purple coneflower, is an important medicinal plant that has been used for centuries, customarily as a treatment for the common cold, coughs, bronchitis, upper respiratory infections, and some inflammatory conditions (Percival, 2000). Commercial farming of *Echinacea* is mainly located in the United States and Canada; nevertheless, Europe, Russia and Australia also have well-established cultivation (Letchamo *et al.*, 2002; Wills and Stuart, 1999).

Echinacea has nine species that are indigenous to North America and only three of them *Echinacea purpurea* L. Moench (*purple coneflower*), *E. pallida* (Nutt.) Nutt. (pale coneflower), and *E. angustifolia* DC

(prairie coneflower), are known for their economic importance (Mckeown, 1999).

Coneflower (*Echinacea purpurea*), a medicinal plant from asteraceae family, exhibits antioxidative, antibacterial, antiviral and antifungal activities, and affects various immune parameters of body. Because of its immuno-enhancing activity, it has been recently used in AIDS therapy (Zolgharnein *et al.*, 2010).

The most fundamental and common method for post-harvest preservation of medicinal plants is drying. The reduction of the water content of freshly harvested medicinal plants is imperative for handling and storage purpose and less susceptible to microorganisms (Tanko *et al.*, 2005; Ghasemi Pirbalouti *et al*., 2013)

Drying reduces the enzymatic activity inhibiting chemical reaction as hydrolysis, oxidation, and fermentation (Santana *et al.*, 2014). Drying of medicinal plants must be carefully performed in order to preserve the aroma, appearance, and nutritional values (Crivelli *et al.*, 2002). Drying is a fundamental requirement to achieve a high quality product. Because of high investment and energy costs, drying is also a large expense in medicinal plant production. In addition, the main purpose of drying is to extend product shelf life, minimize packaging requirements and reduce shipping weights (Ghasemi Pirbalouti *et al*., 2013).

Natural drying and hot weather drying are still popular methods used in obtaining dry herbal substances due to their lower costs and being economic. Natural drying (shade-drying and sun-drying) has many disadvantages, for example it is not possible to transport large amounts of herbal materials and achieve constant quality standards. In addition, high temperature and severe solar radiations negatively affect the quality of samples (Soysal and oztekin, 2001).

The present study tried to assess the effects of six drying methods (sun, shade, greenhouse and industrial-drying at 50° C, 60 °C and 80 °C) on the active substances of *E. purpurea* and determine the best drying methods.

2. Materials and Methods

2.1. Preparation of herbal substances

The aerial parts (up to ~5cm) of *E.purpurea* were collected at 1407 m. above sea level in Ardabil , northwest of Iran (38 09' N and 48 27' E), in July 2016. In order to preserve their original quality, the aerial parts were stored in a refrigerator at 4±1 ° C until drying experiments.

The initial samples were divided into six batches each containing 3 sets of 40 g. One was used for fresh analysis and the remaining batches were dried by using one of the following methods: (1) air drying at shade and ambient temperature (20±5 ° C); (2) sun drying; (3) drying in Greenhouse; (4) drying in industrial- dryer at 50° C; (5) drying in industrial- dryer at 60° C; (6) drying in industrial- dryer at 80° C.

2.2. Drying methods

Samples were randomly selected from six kinds of treatments:

- Shade drying was carried out under natural air flow and ambient temperature (20±5 °C).
- Sun drying aerial parts dried into trays under direct sunlight at temperatures from 25 to 35 °C.
- Greenhouse drying aerial parts dried with plastic cover greenhouse at 30 °C.
-Drying in industrial-dryer at three different temperatures (50 °C, 60 °C and 80 °C).

The time needed for reaching the final drying point in each of the assayed drying methods were as follows: 8 days for shade drying, 5 days for sun drying , 4 days for greenhouse drying, 10 h for industrial- dryer at 50 °C, 8 h for industrial- dryer at 60 °C and 7 h for industrial- dryer at 80 °C.

2.3. Sample preparation

The dried aerial parts were carried to the lab where each individual sample was completely powdered with blender machine.

2.4. Determining the amounts of Caffeic acid derivatives

Extraction was performed using ultrasonic method by ultrasound waves (Hu and Kitts, 2000). Powdered samples (5 g) were extracted with 8 ml of 70% methanol at room temperature using an ultrasonic bath with 40 KHz for 40 min, and then the volume was adjusted to 10 ml. The extracted sample was transferred to a centrifuge bottle and centrifuged at 6000 rpm for 10 min. Fifty microliter of filtered extract injected into a HPLC column by a special syringe. The HPLC system consisted of a Hitachi L-2130 pump, a Hitachi

L-2400 UV detector, and a Lichrospher 100 RP-18e column (250×4.6 mm×5 µm, Merck Co., Germany). The mobile phase was (A) acetonitrile/water containing 0.1٪ H_3PO_4 (10:90) and (B) acetonitrile/water containing 0.1٪ H_3PO_4 (25:75). A gradient elution profile was used with B increasing from 0٪ to 100٪ in 30 min and maintained at 100٪ for 10 min. Then a linear gradient of 100٪ B decreased to 0٪ B in 10 min. The flow rate was 1.5 ml/min, and the wavelength of the UV-visible detector was set at 330 nm. The sample injection volume was 20 µl. The contents of various caffeic acid derivatives were calculated on the basis of the calibration curve of each caftaric acid, chlorogenic acid, echinacoside, cichoric acid and cynarin (Chen *etal*., 2008). Each analysis was carried out in quadruplicate.

2.5. Determination of total phenolics

Total phenolics was measured based on chlorometric method, using spectrophotometry (Taga*et al*., 1984). One gram of powdered sample was extracted with 80 ml of 70٪ethanol using an ultrasonic bath (T 710DH, Elma Hans Schmid-bauer GmbH & Co.KG, Germany) with 40 KHz for 40 min, and then the cooled volume was adjusted to 100 ml.

After the extract had settled it was centrifuged prior to spectroscopic measurements. Folin-Denis reagent (5 ml) was added to either 0.5 ml of extract or 0.5 ml of standard solution. After 3 min 35٪ sodium carbonate solution (5 ml) was added and the test solution was made up to 50 ml with H_2O and mixed. After 45 min of incubation in room temperature, the tested solution was centrifuged at 6000 rpm for 10 min (3460g), and then the absorbance of clear solution was measured at 745 nm. Chlorogenic acid (10, 20, 30, 40, and 50 mg) was used as standard and was dissolved in 100 ml of 70٪ ethanol. The content of total phenolics in the extract was calculated using the linear regression from the standard. Each analysis was carried out in quadruplicate.

2.6. Identification of Alkamides

We used methods of Perry *et al*. (1997) for quantification of alkamide 8 and alkamide 9, dried ground tissue (50 mg) was extracted with 2.5 mL acetonitrile for 5 min and centrifuged at 18,000 g for 15 min. The supernant(20 µl) was filtered through a 0.2 µm syringe filter (Minisart RC 15, Sartorius) and then analyzed using a HPLC (Hitachi, Japan) consisting of pump (L-7100), column oven (655A-52) (35°C), UV-VIS detector (L-7420) (254 nm) and auto sampler (L-2200). The column used was Mightysil RP-18 GP 5 µm 250 × 4. 6 mm (Kanto, Tokyo, Japan). Two different eluents were used: A acetonitrile 100% and B: water. Various levels of alkamide 8 plus alkamide 9 (alkamide 8 and 9) were used in the construction of standard curves (Chung *et al*., 2009).

2.7. Statistical analysis

The experiment was arranged based on completely randomized factorial with three repetitions.Analysis of variance was performed using SAS statistical software (version 9.1). Mean were separated using Duncan's multiple test at 5% significance.

3. Results and discussion

The content of Caftaric acid of *E. purpurea*dried in different drying methods showed significant difference at 1% probability level. As figure 1 shows the highest amounts of Caftaric acid were observed in shade dying, also industrial- dryer at 80° C have the lowest amounts of Caftaric acid content. These results are in agreement with finding of Hevia*et al*. (2002).

Cichoric acid and caftaric acid 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 (Thygesen*et al*., 2007).

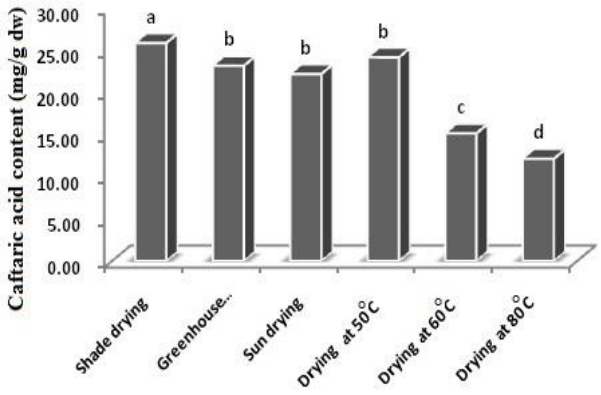

Fig 1. Caftaric acid content of *Echinacea purpurea* dried with different drying methods ($p<0.05$).

Chlrogenic acid content of *E. purpurea* dried in different drying methods was statistically significant at 1% probability level. The amount of chlrogenic acid varied from 1.18 mg/g in industrial- dryer at 80° C to 3.18 mg/g in greenhouse drying methods (Fig 2). The highest amounts of Chlrogenic acid were observed in shade dying, also industrial- dryer at 80° C have the lowest amounts of Chlrogenic acid content.

These finding are in agreement with previous study by Lin *et al.* (2011) on *E. purpurea* in Taiwan.

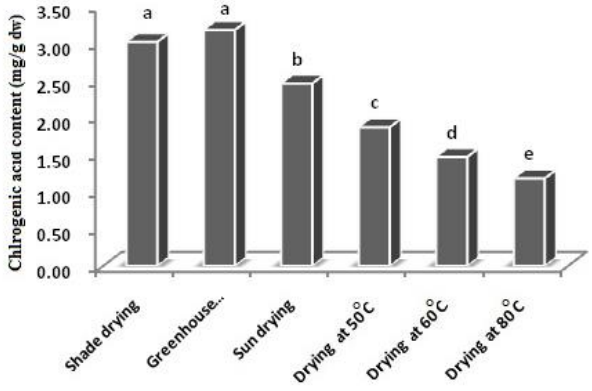

Fig 2. Chlrogenic acid content of *Echinaceapurpurea* dried with different drying methods.

Echinacoside acid content of *E. purpurea* dried in different drying methods was significantly different at 1% probability level. Drying in an oven with a temperature of 40 ° C is the best method of drying (Coksari*et al.*, 2011). In this study of Echinacoside acid content the highest amounts was measured by using shade drying (3.94 mg/g) and the lowest measured by industrial dryer at 80 ºC (1.15 mg/g) respectively.

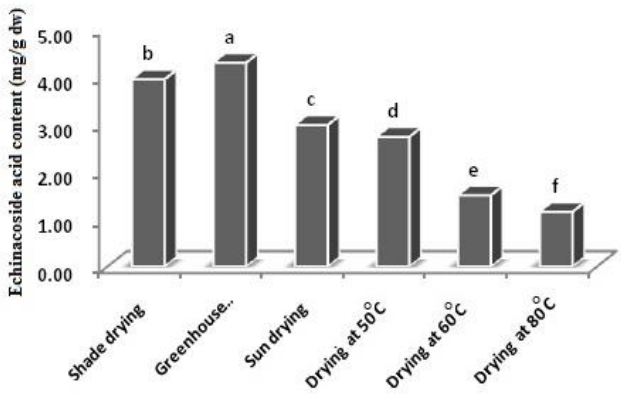

Fig 3. Echinacoside acid content of *Echinacea purpurea* dried with different drying methods.

Cichoric acid content of *E. purpurea* dried in different drying methods was significant difference at 1% probability level. In terms of cichoric acid content, the highest and lowest amounts were measured by using shade drying (36.57 mg/g) and industrial- dryer at 80 ºC (13.7 mg/g) respectively (Fig 4). The cichoric acid content gradually decreased with the increasing of temperature.

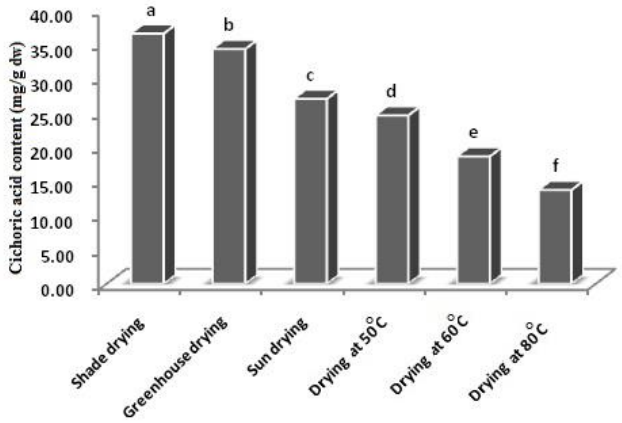

Fig 4. Cichoric acid content of *Echinacea purpurea* dried with different drying methods.

Different drying methods have significant effects on cynnarin content of *E. purpurea*. As the Fig 5 shows with increasing of temperature, the cynnarin content decreased. The highest amounts of cynnarin were observed in shade dying andindustrial-dryer at 80° C has the lowest amounts of cynnarin content. These results are in agreement with finding of Lin *et al.* (2011).

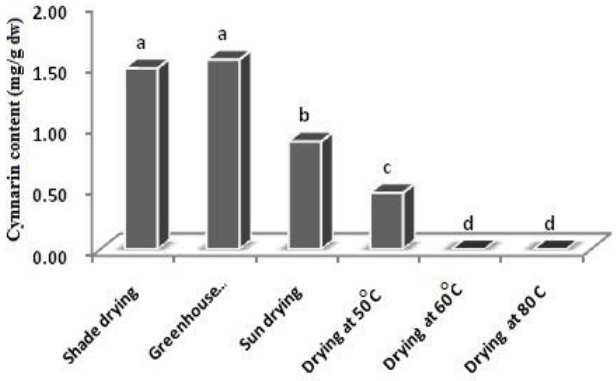

Fig 5. Cynnarin content of *Echinacea purpurea* dried with different drying methods.

Alkamides content of *E. purpurea* dried in different drying methods was significant difference at 1% probability level. As Fig 6 displays, the highest amounts of Alkamides content were observed in shade drying and industrial- dryer at 80° C have the lowest amounts of Alkamides content. These results are in agreement with finding of Thygesen *et al.* (2007).

Various alkamides have been isolated and identified from the *E. purpurea* (Binns*et al*., 2002), with dodeca- 2E, 4E, 8Z, 10 Etetraenoic acid isobutylamide (alkamide 8) and dodeca- 2E, 4E, 8Z, 10Z-tetraenoic acid isobutylamide (alkamide 9) being predominant (Kim *et al.*, 2000). Only alkamide 8 and alkamide 9 were determined in the present study.

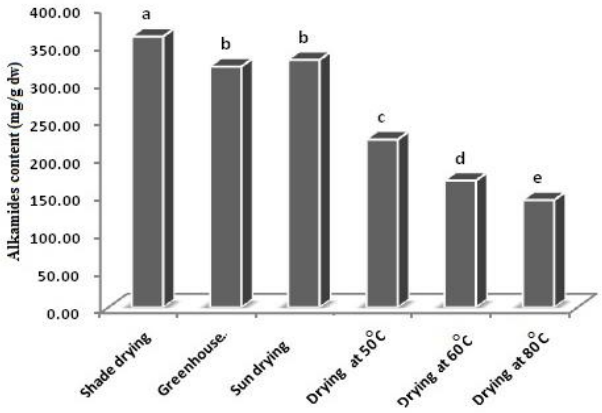

Fig 6. Alkamides content of *Echinacea purpurea* dried in different drying methods.

Total phenols content of *E.purpurea* dried with different drying methods was significantly different at 1% probability level. The highest amounts of phenols were observed in shade drying and greenhouse drying. The industrial- dryer at 80° C has the lowest amounts of phenols. These results are in agreement with flinging of Lin *et al*. (2011).

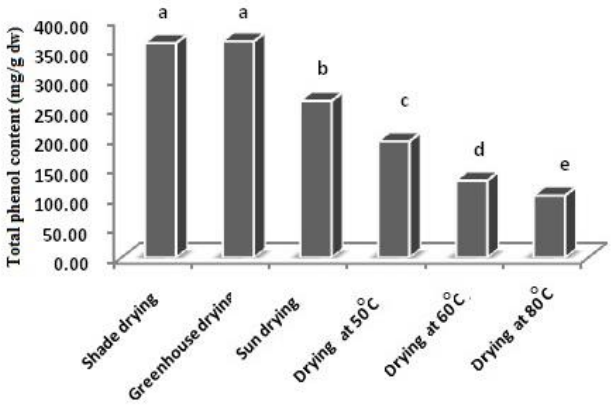

Fig 7. Total phenols content of *Echinaceapurpurea* dried in different drying methods.

Medicinal herbs are often dried and stored for a long time before use in manufacturing various types of product, and the quality of dried medicinal samples is strongly affected by the drying process. Caffeic acid derivatives and total phenolic are sensitive to heat, and increasing temperature will result in a significant loss of caffeic acid derivatives and total phenolics in *E. purpurea* (Lin *et al.*, 2011).

4. Conclusion

Drying is one of the basic process of medicinal plants that is affected on quantitative and qualitative characteristics. As the bioactive components of medicinal plants may be very sensitive to drying procedures, for obtaining the highest quality of medicinal plants, it is necessary to investigate the influence of drying methods on quality parameters. According to our results, shade drying and drying with greenhouse method would be proper methods to obtain highest amounts of active substance of *E.purpurea* also the amounts of active substance reduces with increasing of temperature in different methods of drying.

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