Stability of Bioactive Compounds and Antioxidant Activity of *Echinacea purpurea* Extract

P. Rattanadechsakul, J. Rattanadechsakul, S. Okonogi, J. Sirithunyalug, and S. Yotsawimonwat*
Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand (songwut_y@yahoo.com)

1. Introduction

Echinacea purpurea (L.) Moench (Asteraceae) is one of the most widely used medicinal plants in the world. Originally, it is a native perennial medicinal herb of North America and Europe, but it is nowadays cultivated in many parts of the globe, including Thailand. This plant has been commonly used for relieving symptoms of common cold. Recently, many researches revealed that it has many other pharmacological actions such as immunostimulating activity, antimicrobial and antiviral activity, anti-inflammatory activity, would healing activity and antioxidant activity (WHO, 1999). Major bioactive constituents of *E. purpurea* are alkamides, polysaccharides and phenolic compounds. Cichoric acid and caftaric acid are only two phenolic compounds found in *E. purpurea*. These chemical constituents can be used for the differentiation of *E. purpurea* from its close species, *E angustifolia* and *E. pallida* (Pellati, 2004). Both cichoric acid and caftaric acid are recognized as strong antioxidants. Therefore, the extract of *E. purpurea* can be used for various applications such as a natural antioxidant supplement and anti-aging topical preparations. However, the stability of the bioactive compounds should be thoroughly investigated before the dosage formulation is implemented. This study aims to evaluate the extract of *E. purpurea* grown in Thailand in terms of the stability of its bioactive compounds (cichoric acid and caftaric acid) and the stability of its antioxidant activity.

2. Material and Methods

2.1 Chemicals and reagents

Dried *E. purpurea* powder (aerial parts) was obtained from Rai-Tan-Ta-Wan-Daeng, Chiang Mai, Thailand. Caftaric acid and cichoric acid (Figure 1) were purchased from Phytolab (Germany). ABTS (2, 20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) diammonium salt was purchased from Fluka Chemie AG (Switzerland). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Aldrich (Germany). Methanol, and acetonitrile HPLC grade were from Merck (Germany). All other reagents were of analytical grade.

2.2 Preparation of *E. purpurea* extracts and stability protocol

2.2.1. Liquid extract

A weighed amount (0.5 g) of dried *E. purpurea* powder was extracted with 10 ml of 50:50 v/v ethanol-water at room temperature using a magnetic stirrer for 15 min. After centrifugation at 4000 rpm for 10 min, the supernatant solution was collected. The residue was re-extracted with the same process for another 2 times. All supernatants were pooled, divided into three equal portions, filled in close amber glass bottles, and separately stored in three different temperatures: 4°C, 30°C and 45°C for 2 months. The extracts were periodically analysed for cichoric acid and caftaric acid contents by HPLC for 60 days.

2.2.2 Dried extract

The dried powder of *E. purpurea* (100 g) was extracted with 50:50 v/v ethanol:water (500 ml) in an ultrasonic bath at room temperature for 7 min 3 times. The vaccum filtration was used to remove the solid. The filtrates were pooled and the solvent was removed under vacuum at 45^{o}C using a rotary evaporator followed by lyophilization to obtain the dried extract. The dried extract was divided into three parts and then separately stored at 4^{o}C, 30^{o}C and 45^{o}C for 2 months. The antioxidant activity was determined by ABTS assay occasionally for 60 days and the concentrations of cichoric acid and caftaric acid in the extract were determined by HPLC for 180 days, respectively.

2.3 Determination of cichoric acid and caftaric acid contents

A reversed phase high performance liquid chromatography (HPLC) was used to investigate the concentration of cichoric acid and caftaric acid in liquid and dried extracts. The HPLC condition was modified from Pellati *et al.* (2004). The analyses were carried out on a Zorbax RP-18 column (150 × 4.6 mm i.d., 5 μm) with a temperature controlled at 26 ˚C. A mobile phase was a gradient of acetonitrile and phosphoric acid solution (0.1%) at a flow rate of 1.5 ml/min. The UV detector was set at 330 nm**.** The sample injection volume was 5 μl. Three injections were performed for each sample.

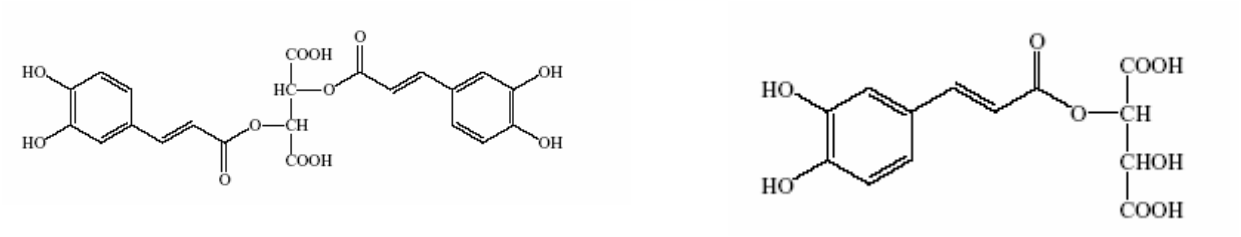
2.4 Determination of antioxidant activity by ABTS assay

The antioxidant activity of *E. purpurea* extracts were analysed using the ABTS free radical decolorization assay developed by Re *et al.* (1999) with some modifications. Briefly, the pre-formed radical monocation of ABTS was generated by reacting ABTS solution (7 mM) with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was allowed to stand for 15 h in the dark at room temperature. The solution was diluted with ethanol to obtain the absorbance of 0.70 ± 0.02 units at 750 nm. The plant extract were dissolved in ethanol to yield a concentration of 1 mg/ml. An aliquot of 20 μl of the sample solution was mixed with 180 μl of ABTS free radical cation solution. After left for 90 min, the absorbance was measured spectrophotometrically at 750 nm using a microtitre plate reader. All measurements were performed in triplicate. The free radical-scavenging activity of the sample was expressed as trolox equivalent antioxidant capacity (TEAC), which was obtained by comparing the absorbance change at 750 nm in a reaction mixture containing a sample of plant extract with that containing trolox. This index is defined as the millimolar concentration of a trolox solution whose antioxidant capacity is equivalent to 1.0 mg of the extract (Antolovich et al., 2002).

3. Results and discussion

The 50% ethanolic liquid extract of *E. purpurea* was pale brown in colour. It contained 11.18 ± 0.08 mg/ml of cichoric acid and 4.23 ± 0.03 mg/ml of caftaric acid. The dried *E. purpurea* extract was prepared by rotary evaporation followed by lyophilization of the liquid extract. The percentage yield of the dried extract was 18.14% of the amount of the *E. purpurea* powder used. The contents of cichoric acid and caftaric acid in the dried extract were 36.45 ± 0.37 and 13.47 ± 0.17 mg per gram of the extract, respectively. Cichoric acid was stable at 4 ^{o}C for at least 2 months in both liquid and dried extracts (Figure 2). However, its concentration decreased significantly during storage at 30^{o}C and 45 ^{o}C conditions, particularly in the liquid extract. Caftaric acid appeared to be much more stable. Its concentrations in both liquid and dried plant extracts remained relatively constant at every condition for at least 2 months (Figure 3). The TEAC value of dried plant extract from ABTS assay was 1.899 ± 0.033 micromole Trolox equivalent per mg sample. The antioxidant activity steadily decreased as the storage duration was increased in every condition (Figure 4). The dried

extract lost its antioxidant activity faster when it was stored at a higher temperature. The antioxidant activity data fitted very well with the first-order kinetics; the coefficients of determination (r^2) were above 0.9000 (Table 1). The tentative shelf-lives ($t_{90\%}$) of the antioxidant activity of the dried extract were 525, 210 and 175 days at 4 ^oC, 30^oC and 45 ^oC, respectively. It was suggested that the degradation of bioactive compounds, especially chicoric acid might contribute to the decrease in antioxidant activity upon storage of *E. purpurea* extract.



Cichoric acid Caftaric acid

Figure 1 Chemical structures of cichoric acid (left) and caftaric acid (right)

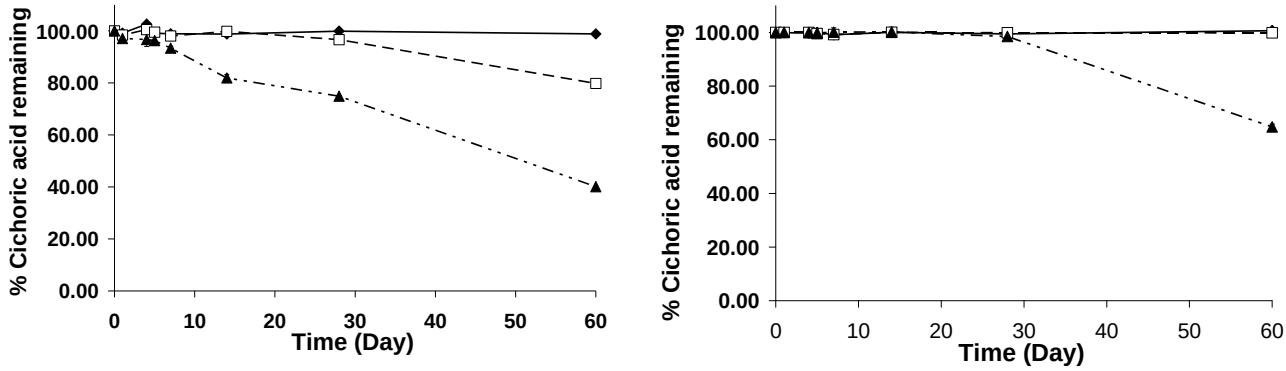


Figure 2 Stability of cichoric acid in the liquid *E. purpurea* extract (left) and the dried *E. purpurea* extract (right) at 4°C (♦), 30°C (□) and 45°C (▲)

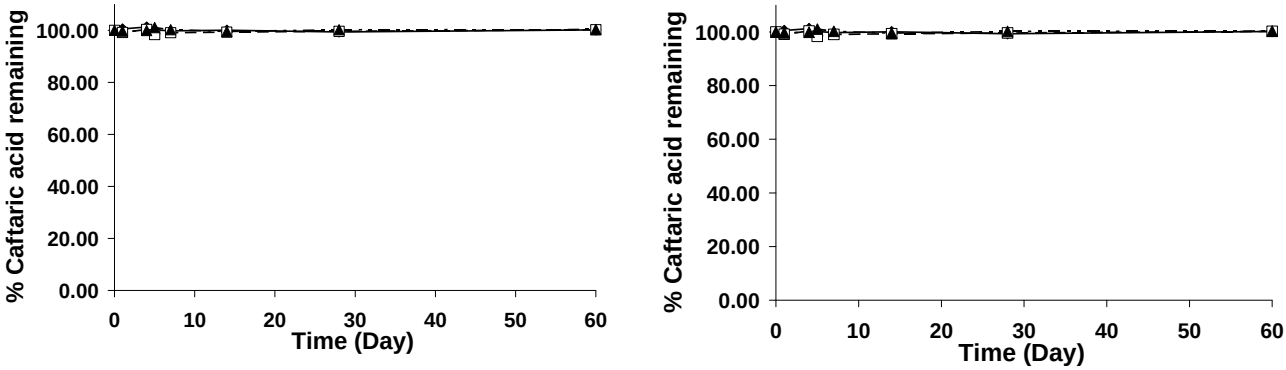


Figure 3 Stability of caftaric acid in the liquid *E. purpurea* extract (left) and the dried *E. purpurea* extract (right) at 4°C (♦), 30°C (□) and 45°C (▲)

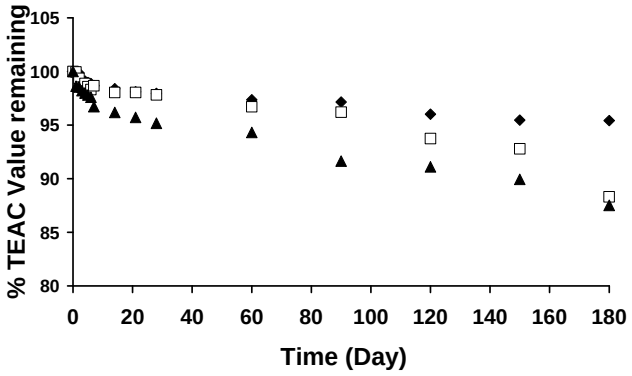


Figure 4 Stability of antioxidant activity of the dried *E. purpurea* extract at 4°C (♦), 30°C (□) and 45°C (▲)

Storage Condition	Slope	r^2	Shelf-lives ($t_{90\%}$) (days)
4°C	-0.0002	0.9054	525
30°C	-0.0005	0.9259	210
45°C	-0.0006	0.9485	175

Table 1 Slope, r^2, and shelf lives ($t_{90\%}$) obtained from the first-order kinetics analysis of antioxidant activity data of the dried *E. purpurea* extract stored at 4°C, 30°C and 45°C

4. Conclusions

The chemical composition of *E. purpurea* raw material and extract is of interest to both the herbal industry and regulatory agencies as a determinant of product quality and authenticity, with an end towards to protecting consumers from low quality or fraudulent products. The degradation of the chemical constituents as well as the lost in antioxidant activity of *E. purpurea* extracts both in liquid and solid states have been established in this study. It is highly recommended that the *E. purpurea* extract and its relating products should be kept in a refrigerator for prolonged shelf-life. In addition, appropriate formulation techniques, such as encapsulation, should be applied to increase its stability.

5. References

Antolovich M. et al. (2002) *Methods for testing antioxidant activity*. Analyst 127, 183–198.

Pellati F. et al. (2004) *Analysis of phenolic compounds and radical scavenging activity of Echinacea spp*. Pharmaceutical and Biomedical Analysis, 35, 289–301.

Re R. et al. (1999) *Antioxidant activity applying an improved ABTS radical cation decolorization assay.* Free Radicals in Biology and Medicine 26 (9/10) 1231–1237.

WHO (1999) *WHO Monographs on Selected Medicinal Plants*, Vol. 1. Geneva, WHO, 136–144.