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Research Article

Phytochemical quality analysis of commercial preparations containing *Echinacea purpurea*

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ABSTRACT

Echinacea species have been medicinally important plants from the past to the present. *Echinacea* is used in many diseases, especially cold and wound treatment. The root, flower, and leaf parts of the plant, especially the flower, contain medicinally important components such as chlorogenic acid, caffeic acid, echinacoside and cichoric acid. Today, the medicinal use of the *Echinacea* can be achieved with its flowers and herbal teas sold in herbal markets. In addition, *Echinacea* preparations available in pharmacies are used especially for immune boosting and to prevent colds and flu. In the present study, it was aimed to analyze 5 different *Echinacea* preparations by the HPLC method. According to the HPLC results of *Echinacea* preparations in different forms (syrup, capsule, etc.), none of the 4 expected standards could be detected in the 2 samples stated to contain *Echinacea*, while 0.1% caffeic acid was detected in EP-1 and 0.4% cichoric acid, 0.6% chlorogenic acid, 0.2% caffeic acid were detected in EP-3. In the sample purchased directly as a dried herb, all four of the required standards were determined to be following the ranges specified in the European Pharmacopoeia.

Keywords: *Echinacea*, HPLC, echinacoside, caffeic acid, market preparation

1. INTRODUCTION

Echinacea sp. grows and originates in West America, three of them are valuable as medicinal plants and used for a long time. These species are *Echinacea purpurea* L. Moench. (roots and aerial parts), *Echinacea angustifolia* D.C. (roots) and *Echinacea pallida* (Nutt.) Nutt. (roots) [1]. *E. purpurea* is a valuable medicinal plant that belongs to Asteraceae (Compositae) family and is known as 'purple coneflower', 'red sunflower' and 'rudbeckia' [2]. All three species contain polysaccharides, alkamides, flavonoids and caffeoyl conjugates such as cichoric acid, echinacoside and caffeic acid in different concentrations [3]. There are 4 different *Echinacea* monographs in the European Pharmacopoeia: *Echinacea purpurea* root, *Echinacea purpurea* aerial part, *Echinacea angustifolia* root, *Echinacea pallida* root. The materials used in this study are mostly samples containing *E purpurea*. According to European Pharmacopoeia 8.0, *E. purpurea* roots should contain a minimum of 0.5%, aerial parts should contain a minimum of 0.1% caftaric acid and cichoric acid, total [4].

Recently, natural alternatives for prevention from colds and flu have become very popular. There are more than one plant species that may be used for prophylactic treatment of cold, such as *Echinacea* species [5]. Since 1600, Americans traditionally used these species for many reasons such as cough, dyspepsia, sore throat, toothache, tonsillitis and

snake bite [6]. There are lots of beneficial effects of *Echinacea* sp. supported by *in vitro* trials such as immunomodulatory, antianxiety, cytotoxicity, anti-inflammatory, antioxidant, antiviral, antifungal, antiosteoporotic and antimicrobial effects [7-11]. There are recent studies that demonstrates the antiviral effect of *Echinacea purpurea* on SARS-CoV-2 variants [12]. The mechanism of action of *Echinacea* sp. on cold and influenza is in vogue and still unknown. About that, a study demonstrated that endotoxin-free *E. purpurea* extract activates the production of interleukin-6, interleukin-12, tumor necrosis factor and nitric oxide which also means it stimulates the immune system, *in vitro* [13]. Moreover, a meta-analysis of 14 studies supported that *Echinacea* sp. has beneficial effects on increasing the incidence and duration of common cold [3]. Caffeic acid derivatives, polysaccharides, alkamides and glycoproteins are believed to be the responsible for the immunostimulatory effect of *Echinacea* sp [8]. Another meta-analysis investigated the capacity of *Echinacea* sp. on reducing the antibiotic usage by preventing respiratory infections. The study resulted that especially alcoholic extract of the leaves of *Echinacea purpurea* lowered the risk of recurrent infections, complications from respiratory tract infections and the necessity for antibiotic treatment, resulting in a 80% reduction in total antibiotic therapy days. It is a safe opportunity for preventing recurrent infections [14]. A study stated that water is the optimal solvent for extracting a polysaccharide-containing complex (PSC) which may be a responsible compound for its immunostimulant activity [15]. Ethnobotanical usage of *Echinacea* species for cold is reported [16], hence obviously there is missing knowledge about the mechanism of action. Prophylaxis is an important step for influenza, because of that reason, commercial products (supplements) that contain *Echinacea* sp. are on the market and herbalists, recently. They promise to contain a significant amount of *Echinacea* extract or marker compounds for preventing colds, however, the results may not meet the promises for any commercial product. The most important factor that determines the quality of plants is that they meet certain phytochemical standards. The quality of a commercial health product directly affects public health [1].

The aim of this study is to analyze the phytochemical properties of samples from five different commercial products that contain *E. purpurea* in the herbalists and compare the phytochemical profiles with marker compounds by using the High Performance Liquid Chromatography (HPLC) method. Thus, quality control assessment of five different supplements from market and herbalists was studied in Türkiye.

2. MATERIALS AND METHODS

2.1. Material

Market preparations containing *E. purpurea* roots and aerial parts were purchased commercially from different pharmacies (Istanbul, Türkiye). Five different samples, three of which were capsules, one was syrup, and the other was directly dried *Echinacea* root, are coded from EP-1 to EP-5.

2.2. Extraction

100 g of ground *Echinacea* roots were weighed and macerated with ethanol for 3 days. Market preparations in powder and liquid form were directly treated with the same solvent. At the end of three days, the extracts were filtered through Whatman no:1 filter paper and concentrated with a rotary evaporator. Filter papers were soaked with ethanol before the process. The process was repeated three times in total [17].

2.3. HPLC Analyses

The prepared extracts were studied at the concentration of 10 mg/mL and were analyzed by filtering through a 0.22-micron membrane filter after dissolving in ethanol. HPLC analyses were carried out with the UV detector connected to the Agilent 1100 HPLC system. While the C18 column (100 x 4.6mm, 5 µm) was used as the stationary phase, A: Water: Formic acid (100:0.1, *v/v*), B: Acetonitrile was used as the mobile phase. It was studied as a linear gradient flow from 10% B to 78% B concentration between 0-18 minutes. Between 18-21 minutes in the flow, it returned from 90% B to 10% B (initial conditions). The flow rate was set at 1.2 mL/min. Retention times (tR) of caffeic acid, chlorogenic acid, cichoric acid and echinacoside were identified

by matching those of the standard analyzed under the same conditions. The peaks were analyzed at 330 nm and the calibration curve was obtained by working with 5 different standard concentrations (0.1-0.5 mg/mL). The injection volume was set to 5 µL and the column temperature was set to 26°C [18].

3. RESULTS AND DISCUSSION

The amount (*w/w*) of caffeic acid, chlorogenic acid, echinacoside and cichoric acid in the EP-1 sample was calculated by the HPLC method. HPLC chromatograms of caffeic acid, chlorogenic acid, echinacoside, cichoric acid and EP-1 are shown in Figures 1-5. In EP-1 HPLC analysis, it was confirmed that the peak at tR: 6.942 belonged to caffeic acid. Based on the calibration graphs, the amount of caffeic acid in EP-1 was calculated quantitatively as 0.1% (*w/w*).

EP-2 content was also analyzed by the HPLC method. The HPLC chromatogram of EP-2 is shown in Figure 6. However, no peaks belonging to the required standard substances could be detected in the HPLC chromatogram (Figure 6).

The content of EP-3 was studied with the HPLC method. The HPLC chromatogram of EP-3 is shown in Figure 7. In the HPLC analysis performed on the sample, it was confirmed that the peak at tR: 6.72 belonged to caffeic acid, the peak at tR: 4.497 belonged to chlorogenic acid, and the peak at tR: 13.579 belonged to cichoric acid. Based on the standard calibration graphs, the amount of caffeic acid in EP-3 was calculated quantitatively as 0.2% (*w/w*), the amount of chlorogenic acid as 0.6%, and the amount of cichoric acid as 0.4%, respectively.

EP-4 content was analyzed by the HPLC method. The HPLC chromatogram of EP-4 is shown in Figure 8. However, no peaks belonging to the required standard substances could be detected in the HPLC chromatogram (Figure 8).

The HPLC chromatogram of EP-5 is shown in Figure 9. In the HPLC analysis performed on the samples, it was confirmed that the peak at tR: 6.72 belonged to caffeic acid, the peak at tR: 4.800 belonged to chlorogenic acid, the peak at tR: 9.5492 belonged to echinacoside, and the peak at tR: 14.967 belonged to cichoric acid, respectively. Based on the standard calibration charts, the amount of caffeic acid in EP-5 was calculated quantitatively as 0.3% (*w/w*), the amount of chlorogenic acid as 0.6%, the amount of cichoric acid as 0.1% and the amount of echinacoside as 0.2% (Figure 9). A comparative table of contents of all samples is included in Table 1.

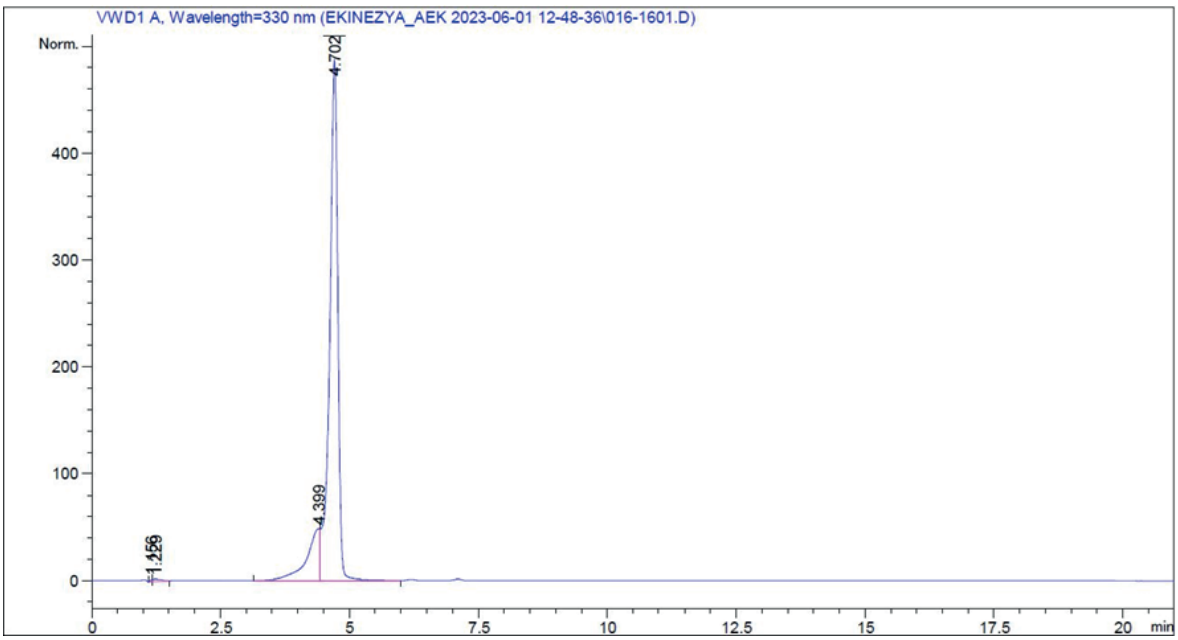

Figure 1. HPLC chromatogram of chlorogenic acid (tR: 4.702)

Table 1. Phytochemical analysis results of market preparations (*w/w*)

	Cichoric acid	Chlorogenic acid	Echinacoside	Caffeic acid
EP-1	-	-	-	%0.1
EP-2	-	-	-	-
EP-3	%0.4	%0.6	-	%0.2
EP-4	-	-	-	-
EP-5	%0.1	%0.6	%0.2	%0.3

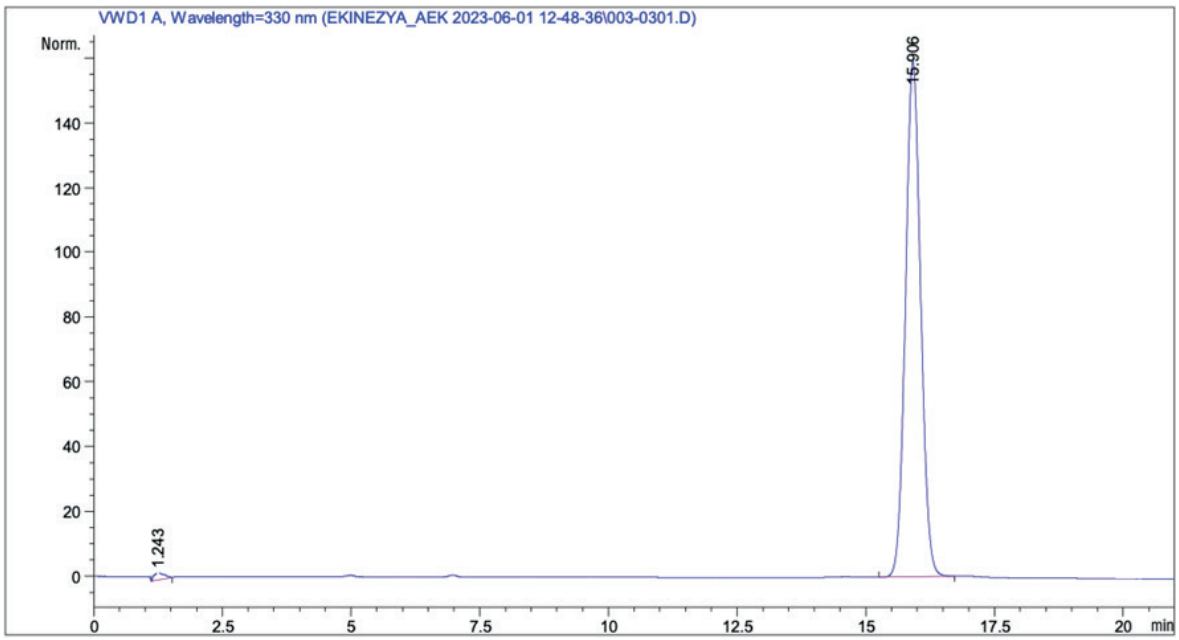

Figure 2. HPLC chromatogram of cichoric acid (tR: 15.906)

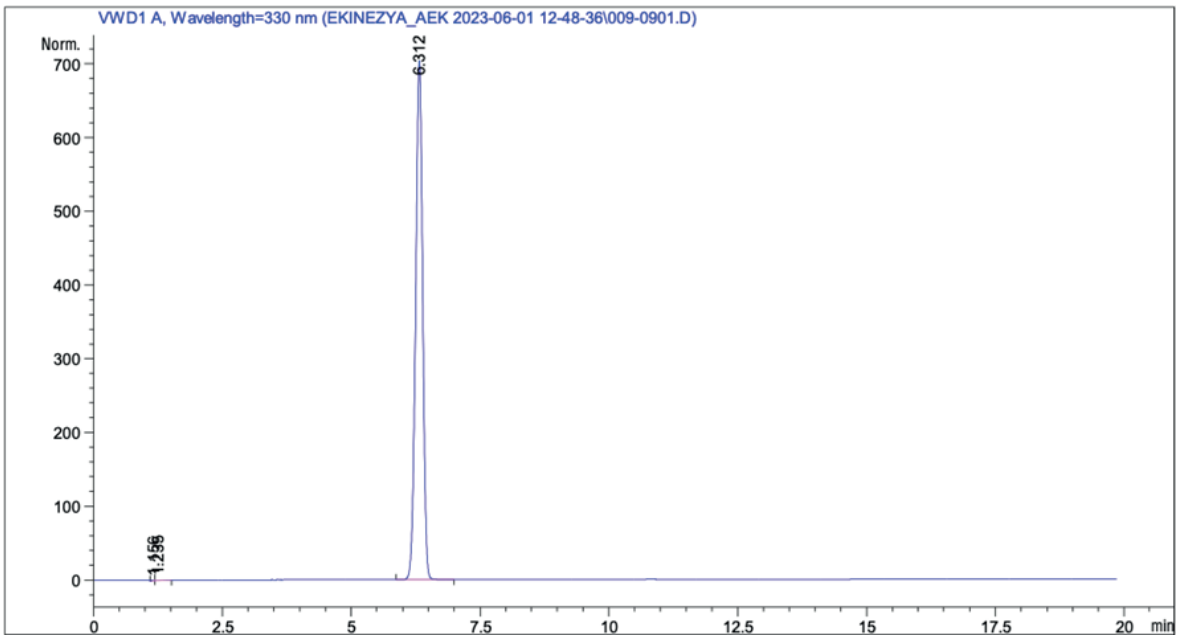

Figure 3. HPLC chromatogram of caffeic acid (tR: 6.312)

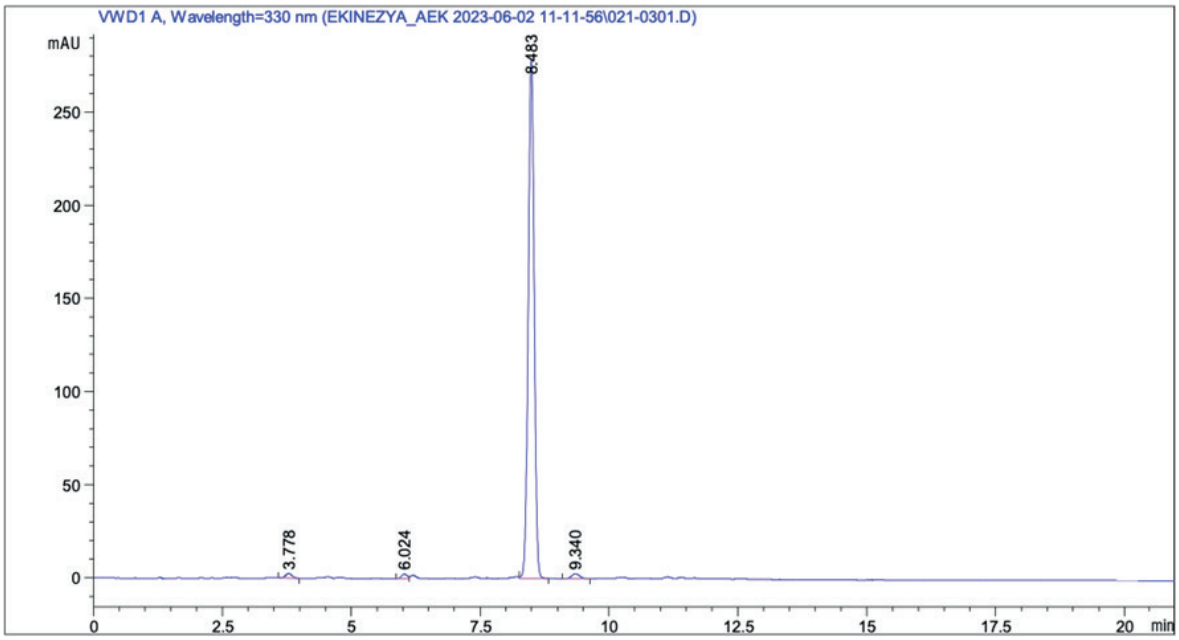

Figure 4. HPLC chromatogram of echinacoside (tR: 8.483)

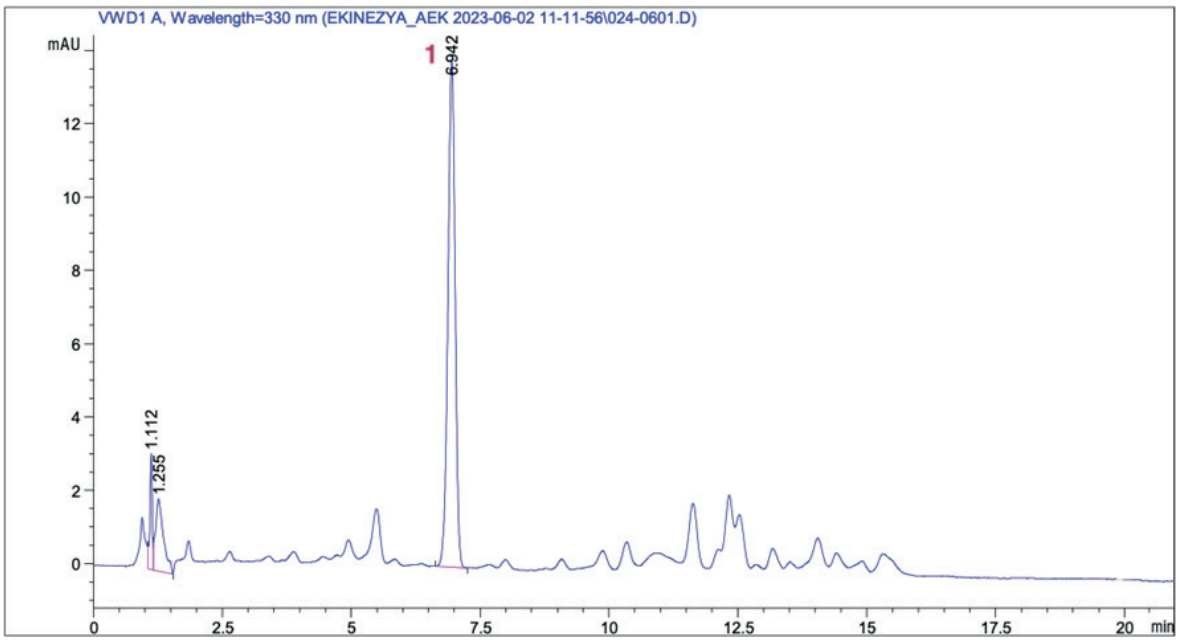

Figure 5. HPLC chromatogram of EP-1 (1. Caffeic acid tR: 6.942)

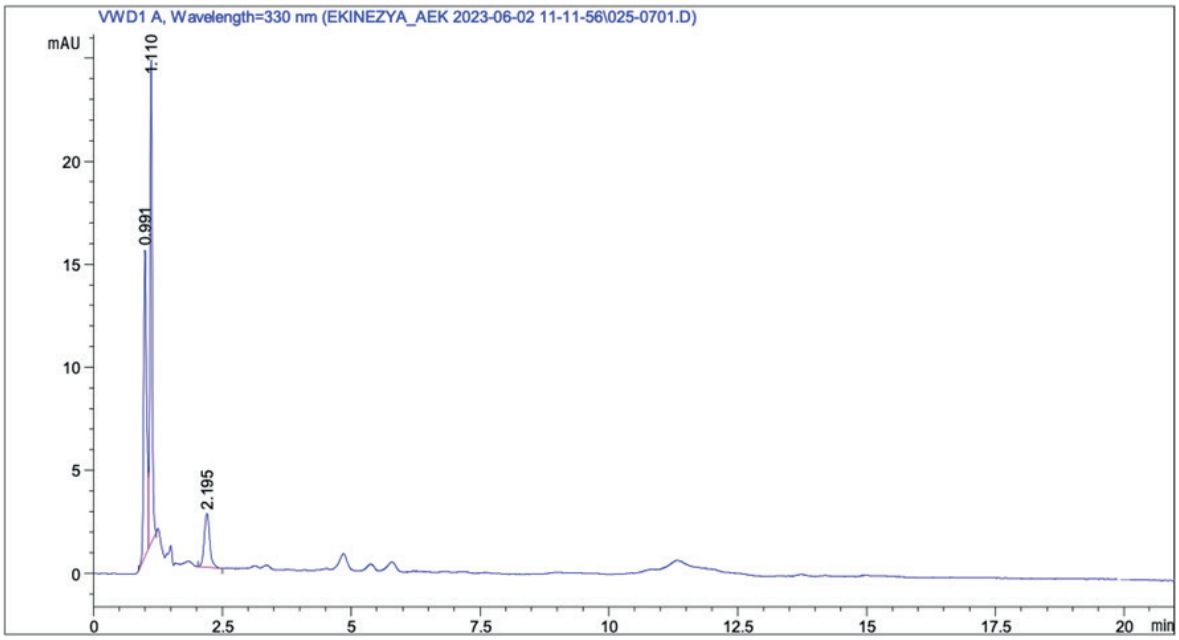

Figure 6. HPLC chromatogram of EP-2

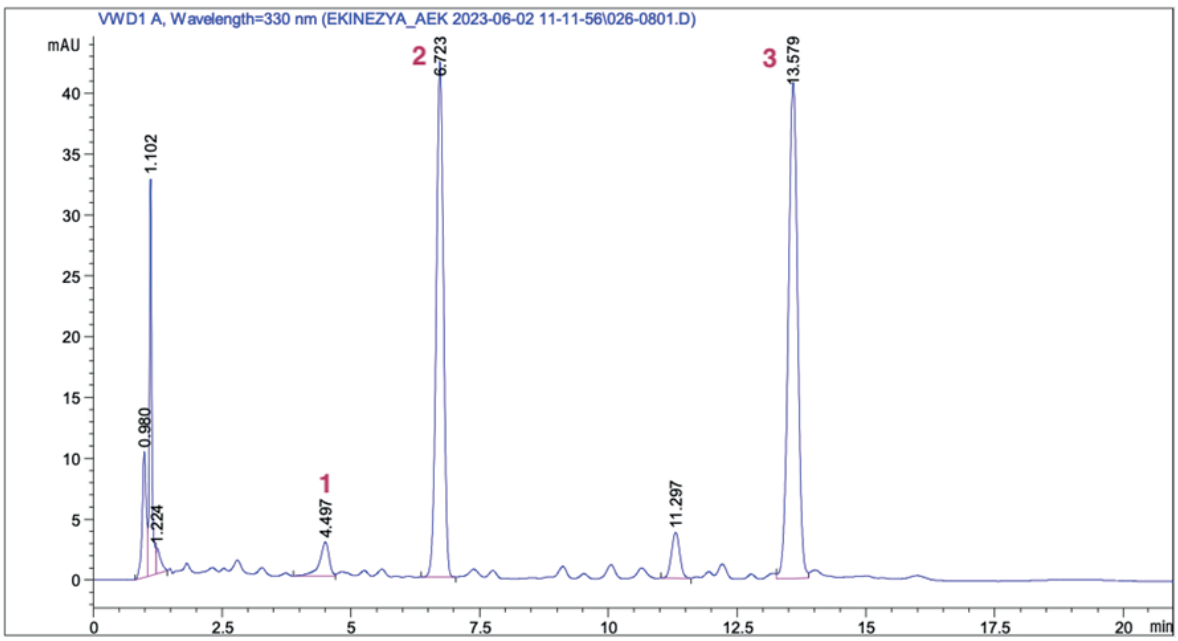

Figure 7. HPLC chromatogram of EP-3 (1. Chlorogenic acid tR: 4.497; 2. Caffeic acid tR: 6.723; 3. Cichoric acid tR: 13.579)

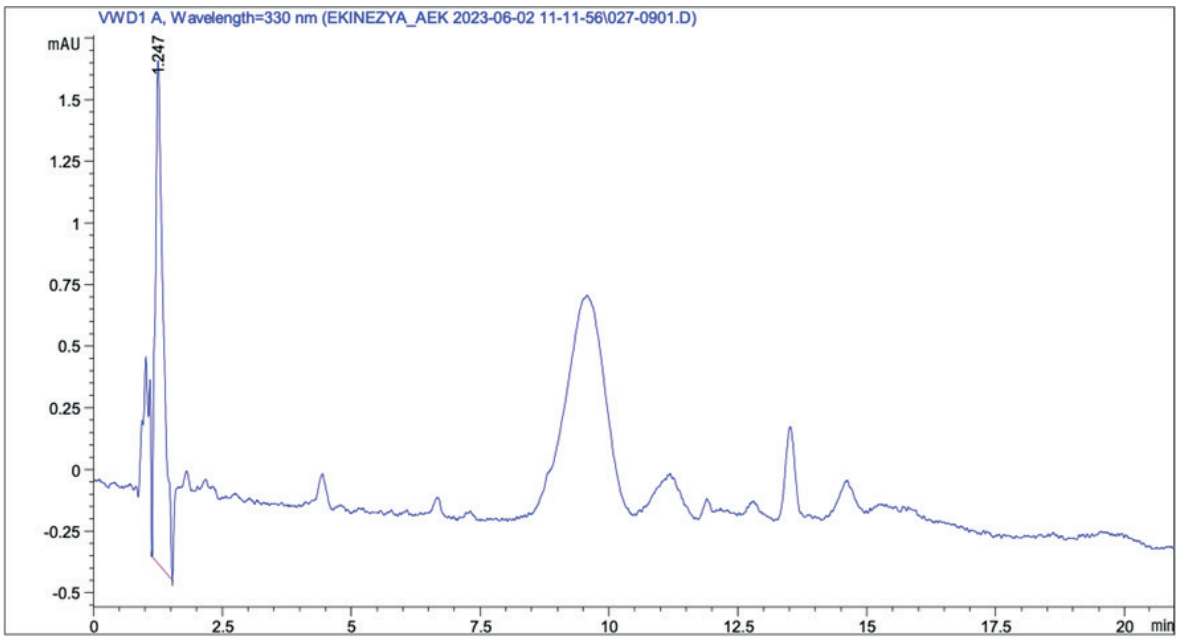

Figure 8. HPLC chromatogram of EP-4

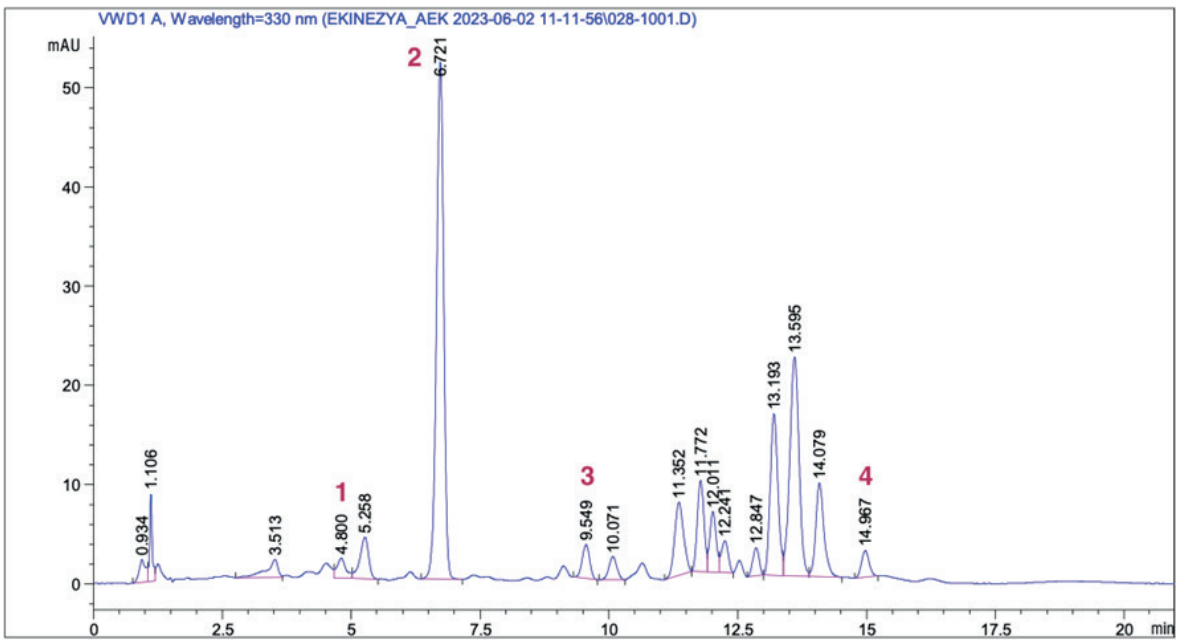

Figure 9. HPLC chromatogram of EP-5 (1. Chlorogenic acid tR: 4.800; 2. Caffeic acid tR: 6.721; 3. Echinacoside tR: 9.549; 4. Cichoric acid tR: 14.967)

According to European Pharmacopoeia, the total amount of caftaric acid and cichoric acid required for dry drugs in the roots of *E. purpurea* is at least 0.5%. It is expected to be at least 0.1% in the aerial parts. At least 0.2% echinacoside is required for *E. angustifolia* and *E. pallida*. The preparation called EP-1 used in the study, in capsule form, contains 265 mg *E. purpurea* (aerial part) powder and 65 mg *Echinacea* root extract. According to this present study, 0.1% caffeic acid was detected in EP-1. The preparation named EP-2 contains 100 mg *E. purpurea* in 2 capsules (recommended daily dose). Additionally,

it contains zinc, vitamin C, beta glucan and rosehip (*Rosa canina* L.). EP-3 preparation contains 150 mg *E. purpurea*. Additionally, it contains beta glucan, vitamin C and zinc in capsule form. EP-4 is in syrup form and contains 300 mg *Echinacea* extract and contains beta glucan, propolis, vitamin C and zinc. EP-5 is the aerial part of dried *Echinacea* sp.

When the phytochemical quality of the market preparations is evaluated according to the HPLC analysis, it was seen that the products of different companies vary greatly in quality and generally do not meet the Pharmacopoeia standards. No information can be found about the growing conditions, specific species and harvest times of *Echinacea* used in production.

Since EP-3 contains *E. purpurea*, it is not expected to contain echinacoside. Other phytochemicals (cichoric acid: 0.4%, chlorogenic acid: 0.6%, caffeic acid: 0.2%) are at a level that can provide the desired therapeutic effect from the preparation.

None of the expected phytochemicals is found in the analyses performed on EP-2 and EP-4. In EP-1, only low concentrations (%0.1) of caffeic acid were detected. These preparations, which must contain cichoric acid and caftaric acid, are not expected to give the promised immunostimulant effect or to provide results in the treatment of colds. This may be due to adulteration, the manufacturer's use of poor-quality herbal materials or inadequate production and storage conditions.

EP-5 is unprocessed, dried *Echinacea* spp. aerial part. It is not known which species it belongs to. Since it contains echinacoside (0.2%), it can be assumed that it is a medicinal species other than *E. purpurea*. It has a better profile than other preparations in terms of the phytochemicals it contains. This may be because the plant is not exposed to errors that may occur in production conditions due to the non-processing of the plant. Additionally, some preparations (EP-2,3,4) were found to contain different components. The possibility that the presence of these components may affect the results should be considered.

In 1998, a study was conducted to distinguish caffeic acid derivatives and lipophilic compounds in the tincture of the *Echinacea* species. According to the results of the previous study, it was found that *E. angustifolia* and *E. pallida* roots contain 0.3-1.7% echinacoside. It was also determined that *E. purpurea* contains cichoric acid and caftaric acid while it does not contain echinacoside. Cichoric acid is mostly found in all flowers of *Echinacea* species (1.2-3.1%) and the root of *E. purpurea* (0.6-2.1%) [19].

In a previous study, phytochemical analysis of *Echinacea*-containing products sold in Denmark was carried out. In this study, the root, leaf and flower parts of *E. purpurea* were analyzed by HPLC. According to the study results, the amount of cichoric acid was found as 24 mg/g in the roots, 42.4 mg/g in the leaves and 26.7 mg/g in the flowers, respectively. In studies conducted on the amount of alkamide, it was found as 1.20 mg/g in the root and 0.81 mg/g in the flower parts. In a study conducted on 13 different preparations (in tincture, capsule and tablet forms), it was explained that the amount of cichoric acid and alkamide was in a very variable range and even could not be found in some preparations [18].

The presence and amount of phytochemicals in plants may depend on many factors. Examples of these factors are seasonal conditions, soil type or the harvesting time of the plant. According to a previous study, the amount of phenolic acid in *E. purpurea* increases starting from spring until July. A limited increase may be seen starting from autumn. Huge differences may be encountered depending on genetic and climatic changes [20]. Factors affecting the quality include air conditions during the growing season such as rainfall, nutrition, attack by insects or microorganisms and handling of plant material during harvest and storage. Conditions during the processing of plant material are very important. Since cichoric acid is sensitive to heat, ultraviolet rays, enzymatic and oxidative degradation may occur during processing [18].

4. CONCLUSION

In conclusion, herbal products are used unconsciously without the control of a physician or pharmacist. This situation can cause major health and financial problems. As seen in the study, the contents of

herbal preparations used as food supplements for prophylactic or therapeutic purposes do not meet the desired conditions. For more reliable and healthier herbal supplements, standardization should be ensured for both raw materials and production conditions. Quality control and inspection of products must be ensured. It is important to support the results obtained in this study with a wider range of further studies in order to determine the safety levels of food supplements.

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Part of the manuscript will be presented at the International Congress on Natural Products Research (ICNPR), 13-17 July 2024, Krakow, Poland.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Conceptualization, A.E.K. and R.B.; Methodology, A.E.K., R.B. and D.K.; Software, A.E.K.; Validation, A.E.K., R.B. and D.Y.; Formal analysis, A.E.K. and R.B.; Investigation, R.B.; Resources, A.E.K.; Data curation, R.B.; Writing—original draft preparation, A.E.K. and R.B.; Writing—review and editing, A.E.K. and D.K.; Visualization, A.E.K.; Supervision, A.E.K.. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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