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Evaluating the Antioxidant Activity of Ethanol Extract from *Echinacea purpurea* (L.) Moench

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ABSTRACT

The purpose of this research was to extract simplicia of *Echinacea purpurea* at the SOHO Center of Excellence in Herbal Research Sukabumi on a laboratory scale and determine the antioxidant effect. This plant is known to grow in Europe before being cultivated in Sukabumi, Indonesia. Additionally, the specimen contains compounds with antioxidant functionalities, estimated to be useful in cell protection from oxidative stress. This property is attributed to the presence of alkamide compounds. In this research simplicia was extracted using 2 different methods, and those generated by DPPH yielded the best chicoric acid value and were further evaluated for antioxidant activities. The results showed the best results in samples isolated with stirrer maceration 1,5 hour evaporation at 70℃, compared to those plus heating at 55℃. Consequently, the yield obtained using this technique was 23.24 % grams which had 4.36% chicoric acid content and a total phenol of 13.82%. The thick extract of *Echinacea purpurea* ct demonstrated the highest the best IC50 value of 92.08 µg/mL, which was higher b than IC 50 of the other samples. This research suggests a need to improve the extraction process as an attempt to achieve optimal results.

Keywords: *Echinacea*, extraction, antioxidant, DPPH, IC50

INTRODUCTION

Free radicals are compounds with one or more unpaired electrons and are normally produced during cellular metabolism. These include reactive oxygen molecules (ROS) and reactive nitrogen molecules (RNS), which are well-known instigators of chemical changes and various chronic as well as degenerative diseases, including inflammation, cardiovascular disease, and cancer. Additionally, antioxidants are needed to avoid the negative effects of free radicals, based on the intrinsic electron-donating capacity to free radicals, which further reduces the occurrence of dangerous reactions. The predominant free radicals comprise ROS and RNS, resulting from the unstable number of electrons, which have the ability to react, and consequently cause oxidative stress. Hence, an electron-donating compound is required under this circumstance, including phenolates or polyphenols, as a class of secondary metabolites present in plants. These constituents are responsible for antioxidant, anticancer, antiviral, and anti-inflammatory activities [1].

Furthermore, *Echinacea purpurea* (L.) Moench plants contain compounds with antioxidant functions and protect against oxidative stressors responsible for the development of chronic diseases, including diabetes and heart disease. The fruits and flowers are known to contain the predominant concentration of antioxidants. Although trace amounts have been identified in leaves and roots. This plant belongs to a member of the family Asteracea and is native to North America and has

potential as a medicinal plant. Once upon a time, *E. purpurea* (L.) Moench was used by Indians to treat toothaches, snake bites and insect bites. Today, the plant is used as an antibiotic or as an immunomodulator. This efficacy is obtained from all parts of the plant [2]. A study by [3] attributed the good antioxidant activity in *Echinacea purpurea* to the chicoric acid constituent, which impedes the degradation of alkamides. This compound is particularly known to increase antioxidant activities and also repair weakened antioxidants.

E. purpurea (L.) Moench is a medicinal herbaceous perennial shrub with a height of about 60 cm, which presents with a sturdy and non-branching woody stem, characterized by coarse hairs. The plants' flowers are shaped like sunflowers with petals measuring 3-8 cm in length, and a reddish-purple, pink or lavender-like purple appearance [4]. Furthermore, other reports have identified the presence of numerous active compounds, including caffeic acid, alkamides, phenolic acids, rosmarinic acid, and others. The chemical content of oils and alkaloids can be found at the root, while at the top of the plant are found compounds such as cichoric acid and echinacoside derivatives of caffeic acid and ferulic acid. In addition, complex polysaccharides such as acidic arabinogalactan, rham-noarabinogalactans, and 4-O-methylglucurony-larabinoxylans. The characteristic compound content diversity provides various health benefits, comprising antioxidant activities [5,6].

Approximately 90% of the raw materials used by pharmaceutical industries in Indonesia are imported, including *E. purpurea*. To reduce dependency procurement from abroad, independence of procurement of raw materials traditional medicine must be done including by cultivating *E. purpurea*. In Indonesia this plant began to be studied in 1998, and based on adaptation results indicates that *E. purpurea* is able to grow good in the tropics from a height of 400-1,200 m above sea level. Optimal growth generated at an altitude of 800 m above sea level with rainfall 2,000-3,000 mm/year, soil type andosol and latosol that has good physical properties with high content of organic matter [7]. *E. purpurea* plant can grow in in *SOHO Center of Excellence in Herbal Research* (SCEHR) plantation in Sukabumi.Indonesia well with an altitude of 600 m above sea level, with rainfall 2805 mm/year [8]. Many immunostimulants come from compounds produced by medicinal plants, one of which is *E. purpurea* (L.) [9].

The method applied in this research was radical 1,1-diphenyl2-picrylhydrazyl (DPPH), based on its simplicity, speed, and inexpensive properties. This technique was used to measure the antioxidants in foods, fruits, and vegetables, to reduce free radicals [10]. Additionally, a standard or positive control was selected to identify the potential desired (antioxidant) effects, and ascorbic acid was selected for this study The purpose of this research was to extract the yield of *E. purpurea* on a laboratory scale in the SOHO Center of Excellence and consequently determine the antioxidant effect.

METHODS

This research was conducted at the Analysis Laboratory of the Research and Development Department, PT SOHO Industri Pharmasi, Pulogadung Industrial Estate, Jakarta, Indonesia. The study object was *E. purpurea* simplicia and dried over 26 hours. Furthermore, some of the tools used include Sonicator (Emasonic), UV-Vis Spectrophotometry (Thermo, Electron corporation), 20, 5, 10, 100, and 50mL volumetric flasks, Analytical balance, Sonicator, HPLC (Shimadzu Prominence UFLC - Prodigy Scientific), Beakerglass, and Rotary evaporator (Heidolph). The materials employed were *E. purpurea*, Methanol, Ethanol 70%, Ascorbic acid, Aqua purificata, and DPPH.

Extraction

Samples of 100 grams with 3,000 mL of 70% ethanol as solvent followed by maceration with a stirrer for 1.5 hours and was evaporated at 70℃, followed by maceration with. Similarly, the second method was as above, but after evaporation plus heating temperature of 55℃ for 5 minutes.

Sample preparation for measurement of total phenol

The total phenol content was measured in triplicate for all samples. This involved preparing a standard solution by weighing 100 mg of standard *Echinacea purpurea Dry Compressed* into a 100 mL volumetric flask before adding 50 mL of purified water aqua purificata. Furthermore, the mixture was ultrasonicated for 10 minutes, followed by the reintroduction of purified water to make up the

volume mark. This was then homogenized, and 5 mL of the solution was pipetted into a 50 mL volumetric flask, to which Aqua purificata was added to make up the mark, and absorption was measured using a spectrophotometer. In addition, a similar procedure was performed on the sample, the simplicia of *Echinaceae purpuea* being measured**.**

Chicoric Acid sample preparation

The chicoric acid constituent of the samples was measured three times. This involved preparing a standard solution by weighing 125 mg of *Echinacea purpurea* DC extracts characterized by 1.25% chicoric acid content into a 50 mL volumetric flask. Therefore, 30 mL of aqua purificata was added before ultrasonication for 10 minutes. Methanol was then added to the resulting solution to make up to mark before shaking to achieve homogeneity. The mixture was filtered with a 0.45-micrometre membrane filter before injecting into the HPLC. Subsequently, a similar procedure was performed on the sample/simplicial being measured.

Antioxidant Activity

The antioxidant activity was conducted using DPPH method

a. Measurement of antioxidant sample

1) Preparation of thick *Echinacea purpurea* extract
   Approximately 200 mg of the thick *Echinacea purpurea* extract was weighed into a 10 mL flask, and methanol was added to the limit mark to obtain a stock solution of 1,000 ppm. Therefore, 5, 3.3, 2.5, 2, and 0.7 mL, were respectively measured into a 5 mL flask and made up to the limit volume with methanol before homogenizing to obtain 40, 60, 80, 100, and 120 ppm concentrations, respectively. This process was performed with 2 replications.

2) Preparation of dry compressed extract
   Approximately 200 mg of the dry compressed extract was weighed into a 10 mL flask, and methanol was added to make up the limit mark. This yielded a stock solution of 1,000 ppm. Furthermore, about 2, 1.3, 1, 0.8, and 0.6 mL were respectively pipetted into a 5 mL flask and made up to the limit mark with methanol before homogenizing to obtain the required concentration. This process was conducted with 2 replications.

3) Measurement of sample solution
   Approximately 1,600 L of the standard and sample solutions were pipetted into individual cuvettes, and 400 uL of DPPH was added before homogenization. Subsequently, the mixture was incubated at room temperature and placed in a dark place for 30 minutes. The respective absorbance was read at a wavelength of 517 nm, using a UV-Vis spectrophotometer. The percent inhibition was calculated using the following formula:

$$inhibition\ \% = \frac{blank\ absorbance - \ sample\ absorbance}{blank\ absorbance\ x\ 100\%}$$

   Concentration curve (ppm) vs inhibition (%) was plotted to obtain a linear regression equation, where y = inhibition (%), a = intercept, b = slope, and x = concentration of solution

b. IC50 value determination

The sample used in this research include ascorbic acid as standard. Several studies have shown the use of vitamin C as a positive control in determining antioxidant activity . The viscous extract from lab-scale extraction, characterized by the highest chicoric acid value and the extract dry compressed as an import extract used at PT SOHO.

The IC50 value was determined by UV-Visible spectrophotometry based on the free radical reduction method. After getting the percent inhibition, the IC50 value was then calculated based on the linear regression equation where IC50 was considered as x value.

RESULTS

Extraction of *E.purpurea*

Echinacea purpurea extraction results can be seen in table 1.

Table 1. Extraction of dried *Echinacea* simplicia

No	Treatment	Simplicia Weight (grams)	Solvent (mL)	Chicoric Acid Level (%)	Total Phenol Level (%)	Yield (g)
1	Stirrer maceration 1.5 hours + Evaporation 70 ℃ + heating 55℃	100	3,000	4.36	13.82	23.24
2	Stirrer maceration at 1.5 hours + Evaporation 70 ℃	100	3,000	2.86	15.23	20.4

Antioxidant Activity Results

This test involved using ascorbic acid as a control, due to the high antioxidant activity, as evidenced by an IC50 value below 50 µg/mL. The concentrations used in this study include the blank, 2, 3, 4, 5, 6, and 7 µg/mL. Additionally, Table 2 below showed the relationship between concentration and the free radical inhibition percentage of DPPH, as well as the IC50 of the antioxidant activity.

Table 2. Percentage of Ascorbic Acid Inhibition

Concentration (µg/mL)	% Inhibition	STDEV	IC50 (µg/mL)
0	0.00	0.016	
2	21.61	0.031	
3	31.74	0.008	
4	42.68	0.008	4.72
5	52.17	0.011	
6	61.93	0.021	
7	75.77	0.027	

Subsequently, Table 3 presented the relationship between concentration (at 0, 40, 60, 80, 100,120, and 140 µg/mL) and free radical inhibition percentage, as well as the IC50 of the viscous extract of *E. purpurea*.

Table 3. Inhibition Percentage of *E. purpurea* viscous extract

Concentration (µg/mL)	% Inhibition	STDEV	IC50 (µg/mL)
0	0.00	0.011	
40	29.20	0.003	
60	41.95	0.006	
80	45.12	0.004	92.06
100	51.81	0.022	
120	60.49	0.031	
140	67.00	0.011	

The extract commonly used by industry was *E. purpurea* Dry Compressed Extract and Table 4 showed the relationship between concentration of 0, 100, 150, 200, 250, 300 and 350 µg/mL and free radical inhibition percentage.

Table 4. Inhibition Percentage of Standard *E. purpurea* Dry Compressed Extracts commonly used in industries

Concentration (µg/mL)	% Inhibition	STDEV	IC50 (µg/mL)
0	0.00	0.011	
100	29.84	0.031	
150	45.57	0.044	
200	53.07	0.010	189.36
250	62.21	0.016	
300	69.26	0.003	
350	81.28	0.009	

DISCUSSION

Marker compounds are unique compounds in plants and are reference compounds that are chemically defined as constituents of a plant. *E. purpurea* marker compounds are caftaric acid, chlorogenate acid, cynarin, echinacosid and chicoric acid, these compounds can serve as stimulants of the immune system [11]. For the pharmaceutical industry the marker compounds used are usually chicoric acid and total phenol. The content of chicoric acid varies in each part of the plant (leaves, flowers, stems or roots), plant age, harvest time, growth, drying, storage conditions and extraction methods used can affect the quality of the product [12]. *Echinacea* contains phenols in all parts of the plant above ground (aerial part) [13].

This research involved the maceration method in the extraction of *E. purpurea*, which was modified by temperature and stirrer treatment. This process aims to speed up the cell wall destruction time, and consequently speeds up maceration. In addition, phenolic compounds are known to be sensitive to heat, as more significant values have been reported in treatments with higher temperatures. Additionally, an increase in temperature also reduces solvent viscosity and enhances the penetration of matrix particles, which consequently induces extraction and produces significant yield [14].

The elevated chicoric acid levels achieved after heating at 55 ℃ was attributed to the ability for extraction temperatures to damage plant cell walls and remove chicoric acid from the tissues [15]. The antioxidativeability is clearly related to the concentration of cichoric acid [16]. Chicoric acid, caftaric acid, and echinacoside were found to be the dominant antioxidant ingredients of *Echinacea* extracts [17]. Chichoric acid was the predominant of caffeic acid derivatives compound in the extract of *E. purpurea* and exhibits good antioxidant properties. Based on the literature, *Echinacea* extract that has the highest chichoric acid value tested for antioxidant.

This research involved the use of ascorbic acid as a comparison or indicator of antioxidant activity. Previous research has shown the presence of high hydroxyl content, which increases the potential for absorption by the body due to the higher polarity, and ability to react with and neutralize free radicals. Several studies have shown the use of vitamin C as a positive control in determining antioxidant activity [18].

The IC50 is defined as the concentration of antioxidant compounds required to reduce free radical activity by 50%, where a smaller value indicates higher activity. According [19] a range lesser than 50 µg/mL denotes very strong action, 50 -100 µg/mL depicts an active level, and 101-250 µg/mL is moderate. Meanwhile, 251-500 µg/mL is considered to be weak, and values greater than 500 µg/mL demonstrated no activity.

Longer extraction time has a direct correlation with lower IC50 values and consequently higher antioxidant effects. This is because the activity is influenced by the active compound content in the extract, which tends to donate hydrogen or electrons to the free radicals present, to achieve better stability. Therefore, samples with higher bioactive components, such as flavonoids demonstrate a better outcome. The viscous extract of *E.purpurea* performed in the laboratory showed lower IC50 value compared to *E.purpurea* Dry Compressed Extracts. The extracts commonly used *E.purpurea* dry compressed extract contains fillers, thereby making it impure.

The determining factors for IC50 in this research were the length of maceration time using a stirrer, and contact with heat. However, the solvent type was also crucial, as those with higher polarity ensured the dissolution of more polar compounds and vice versa. Those capable of dissolving high-density samples enhanced the ease of further separation. This experiment showed the categorization of viscous *E.purpurea* extract as an active antioxidant while *E.purpurea* dry compressed extract was considered to be moderate antioxidant.

CONCLUSION

Extraction treatments with 1.5 hours of stirrer maceration + 55℃ heating produced 4.36% chicoric acid content, with 13.82% total phenol, and the yield weighed 23.24 grams. *E.purpurea* viscous extract performed in the laboratory has IC IC50 value at 92.08 µg/m.

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